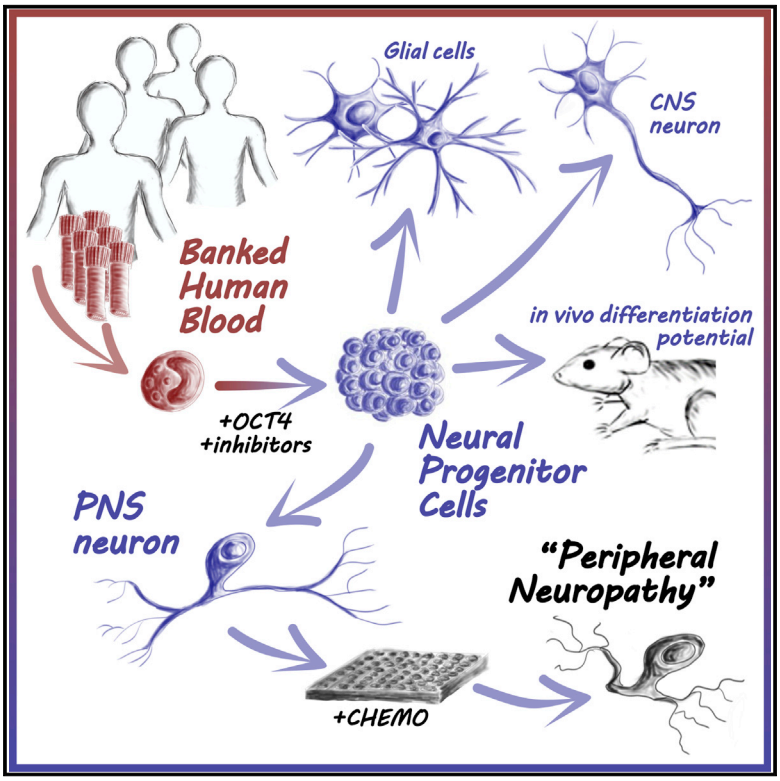
Report

Cell Reports

Single Transcription Factor Conversion of Human Blood Fate to NPCs with CNS and PNS Developmental Capacity

Graphical Abstract



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In Brief

Using OCT-4-induced direct reprogramming, Lee et al. convert human blood to neural progenitors with both CNS and PNS developmental capacity. This fate alternation is distinct from fibroblasts that are primed for neural potential. Furthermore, human sensory neurons derived from blood phenocopy chemo-induced neuropathy in formats suitable for drug screening.

Highlights

- Human blood can be directly converted to tripotent iNPCs with a single factor
- BD-iNPCs uniquely differentiate to neurons with properties of both CNS and PNS
- Conversion process toward iNPCs from blood differs from fibroblasts
- Nociceptive neurons recapitulate chemo-induced neuropathy in a screening format

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Cell Reports
Report

Single Transcription Factor Conversion of Human Blood Fate to NPCs with CNS and PNS Developmental Capacity

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SUMMARY

The clinical applicability of direct cell fate conversion depends on obtaining tissue from patients that is easy to harvest, store, and manipulate for reprogramming. Here, we generate induced neural progenitor cells (iNPCs) from neonatal and adult peripheral blood using single-factor OCT4 reprogramming. Unlike fibroblasts that share molecular hallmarks of neural crest, OCT4 reprogramming of blood was facilitated by SMAD+GSK-3 inhibition to overcome restrictions on neural fate conversion. Blood-derived (BD) iNPCs differentiate in vivo and respond to guided differentiation in vitro, producing glia (astrocytes and oligodendrocytes) and multiple neuronal subtypes, including dopaminergic (CNS related) and nociceptive neurons (peripheral nervous system [PNS]). Furthermore, nociceptive neurons phenocopy chemotherapy-induced neurotoxicity in a system suitable for high-throughput drug screening. Our findings provide an easily accessible approach for generating human NPCs that harbor extensive developmental potential, enabling the study of clinically relevant neural diseases directly from patient cohorts.

INTRODUCTION

The reprogramming of adult cells into alternative tissues holds promise for regenerative medicine and drug discovery, especially for human cell types that are difficult to procure such as neural tissue (Sancho-Martinez et al., 2012). However, significant limitations remain using current technology as it relates to human sources; thus, novel approaches that allow generation of large numbers of renewable neural cells from easily accessible tissues derived from donors are required. Complete cellular reprogramming to the pluripotent state has gone some way to realize this promise (Takahashi and Yamanaka, 2006). However, although transformative, this advance is limited by costly and time-consuming methods over several months to first derive skin fibroblasts and then generate and characterize resulting iPSCs (Stacey et al., 2013). Furthermore, resulting iPSCs acquire inefficiencies in lineage-specific differentiation from pluripotent state that limits reproducible production of specific mature cell types (Lee et al., 2014). Similarly, the use of hiPSCs in cell replacement therapy continues to precipitate barriers and concerns that require laborious measures to assure resulting cells are free from tumor-forming pluripotent cells has yet to be resolved (Cunningham et al., 2012).

More recent studies have established a paradigm whereby forced expression of lineage-specific factors allows direct reprogramming into differentiated somatic cells, including cardiomyocytes, hepatocyte-like cells, blood, and neurons without iPSC formation (Efe et al., 2011; Pang et al., 2011; Szabo et al., 2010). However, direct cell fate reprogramming of human cells is accompanied by other limitations and remains inefficient, requiring multiple transcription factors to be ectopically expressed in every cell, and is largely based on difficult to obtain human skin biopsies that are not available from historical clinical studies. Alternatively, blood cells can be readily obtained from patients, require no culture derivation prior to reprogramming, and have been stored and banked (Broxmeyer, 2010) from large cohort patient trials in the past such as those suffering from neurological disorders (http://brainbank.ucla.edu and https://www.clsa-elcv.ca/). Here, we applied our unique OCT4-induced plasticity reprogramming approach (Mitchell et al., 2014a) in combination with previously identified neural potentiating small molecules (Chambers et al., 2009; Li et al., 2011) to directly convert human blood progenitors derived from both cord blood and adult sources to neural progenitor cells (NPCs). We demonstrate that these human blood-derived (BD)-NPCs are capable of in vivo differentiation and survival as well as tripotent neural differentiation in vitro that includes neuronal differentiation toward clinically relevant CNS and peripheral nervous system (PNS) subtypes.

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RESULTS

Generation of Induced Neural Progenitor Cells from Neonatal and Adult Blood Cells Using OCT4 and SMAD+GSK-3 Inhibition

In an effort to make use of readily accessible hematopoietic cells as a starting material to generate neural derivatives, we employed OCT4-based reprogramming (Mitchell et al., 2014a, 2014b) to both cord blood and adult peripheral blood progenitors (Figure S1A). Human blood cells from both sources were negative for pluripotent markers (SSEA3, TRA1-60), early neural markers (Nestin, PAX6) as well as neural crest (NC) markers (p75, CD57) (Figure S1B), thereby excluding the presence of contaminating cells with pluripotent or NPC features within the starting blood samples. Transduction with OCT4 alone has previously been shown to induce human skin fibroblast conversion to tripotent neural progenitors (Mitchell et al., 2014a), despite reports suggesting the requirement for a chemically diverse cocktail of inhibitors in addition to OCT4 (Zhu et al., 2014). However, transduction of human blood with OCT4 alone failed to induce production of induced NPCs (iNPCs) (Figures 1A–1C). As both inhibition of SMAD and glycogen synthase kinase-3 (GSK3) signaling have been independently reported to efficiently neuralize hPSCs (Chambers et al., 2009), we examined whether dual inhibition with SMAD and GSK-3 chemical inhibitors could facilitate iNPC generation from blood coupled with OCT4-induced plasticity (Figure 1A). When human blood progenitors obtained from neonatal cord blood or adult peripheral blood expressing OCT4 were transferred to SMAD+GSK-3 inhibition conditions (SB431542, LDN-193189, Noggin, CHIR99021), iNPC-like clusters appeared within as little as 8–10 days and showed the expression of the neural stem cell marker, Nestin (Figures 1B and S1C). The addition of these same molecules to human fibroblasts had no effect on NPC generation (Mitchell et al., 2014a, 2014b) (data not shown). As SOX2 has also been implicated in direct-fate reprogramming toward the neural lineage (Ring et al., 2012), we tested whether SOX2 transduction alone or in combination with OCT4 enhanced iNPC-like cluster formation. We found no detectable iNPC-like clusters upon expression of SOX2 alone, as well as reduced iNPC formation when used in combination with OCT4 (Figure 1C). The use of OCT4 expression combined with chemical inhibitors was a highly efficient process, and up to 12 putative iNPC-like colonies could be generated from as few as 50,000 (50K) human blood progenitors (Figure 1C). OCT4-dependent generation of human iNPC colonies could not be established from more mature blood cells devoid of CD34 expression ($CD34^{-}$) and was restricted to the hematopoietic progenitor-containing compartment (Figure 1D). Furthermore, individual iNPC-like colonies demonstrated robust survival that allowed subsequent collection and re-culturing to promote cell proliferation and expansion into primary neurospheres using suspension culture (Ring et al., 2012) conditions known to support human NPCs (Figure 1E). The absence of pluripotent markers (TRA1-60 and SSEA3) demonstrated that OCT4-induced iNPCs were not products of intermediate pluripotent states (Figure S1D), which was further supported by the failure to give rise to teratomas when transplanted into immunodeficient mice (Figure S1E). The complete absence of a pluripotent cell from human blood derived OCT4-induced iNPC also removes safety concerns regarding potential future use of BD-iNPCs. BD-iNPCs derived from either neonatal cord blood or adult peripheral blood consistently expressed neural stem cell-associated markers, including PAX6, NESTIN, SOX2, and CD133 similar to control human NPCs (Figures 1F, 1G, S1F, and S1G). Moreover, cultured BD-iNPCs contained ki67 expressing proliferative cells (Figure S1F) that enabled serial passaging without the loss of NPC marker expression, neural transcriptional programs, or genomic integrity (Figures S1H–S1J, S2A, and S2B). As a testament to their robust practical utility, with an average of as few as 12 iNPC colonies consistently generated from 50K human blood progenitors, we have determined that we can generate as many as 100-million progenitor cells over ten passages (Figure 1H) using this direct-conversion approach from human blood samples.

SMAD+GSK-3 Inhibition Facilitates NPC Generation from Human Blood

In order to gain a better understanding for the requirement of dual SMAD+GSK-3 inhibition during OCT4-mediated conversion of human blood cells to iNPCs, we assembled molecular profiles of blood cells expressing OCT4 that were either treated or not treated with inhibitors and compared them with profiles of recently described Fibs-$iNPC^{OCT4}$ that were derived in the same fashion. To evaluate the molecular profiles, we performed hierarchal cluster analysis of global gene expression profiles and included SOX2 expressing primary neural stem/progenitor cells isolated from human brain tissue as a base of reference (Figure 2A). As expected, Fib-iNPCs were highly related to primary human NPCs regardless of inhibitor addition (Mitchell et al., 2014b), whereas BD-iNPCs required SMAD+GSK-3 inhibition in order to cluster together with primary NPCs (Figure 2A). Investigation of differential gene regulation between ± inhibitor-treated fibroblasts and blood cells during generation of NPCs displayed minimal changes in fibroblast transcriptome compared with blood cells, suggesting a unique role for SMAD+GSK-3 inhibition during blood based OCT4 reprogramming (Figure 2B). In order to classify the gene programs that were specifically regulated in blood cells undergoing OCT4 iNPC reprogramming, we performed gene set enrichment analysis on blood cells ± inhibitor treatment during derivation of NPCs. The addition of SMAD+GSK-3 inhibition resulted in the enrichment of multiple neural-related gene sets that were otherwise not activated in the presence of OCT4 expression alone (Figure 2C). Furthermore, filtering of both upregulated and downregulated genes using the Tissue Expression analysis tool on DAVID Bioinformatics Resource revealed enrichment of downregulated genes within hematopoietic programs and upregulated genes within neural programs (Figure S2). In order to validate the trends from our molecular profiling studies, we performed candidate quantitative PCR (qPCR) on BD-iNPCs for potent hematopoietic and neural progenitor regulatory genes, which confirmed a successful molecular switch from blood to neural progenitors (Figure 2D). These detailed analyses indicate the processes involved in conversion of human skin fibroblasts to NPCs versus blood derived NPCs are also molecularly distinct and reveal a complete conversion of human

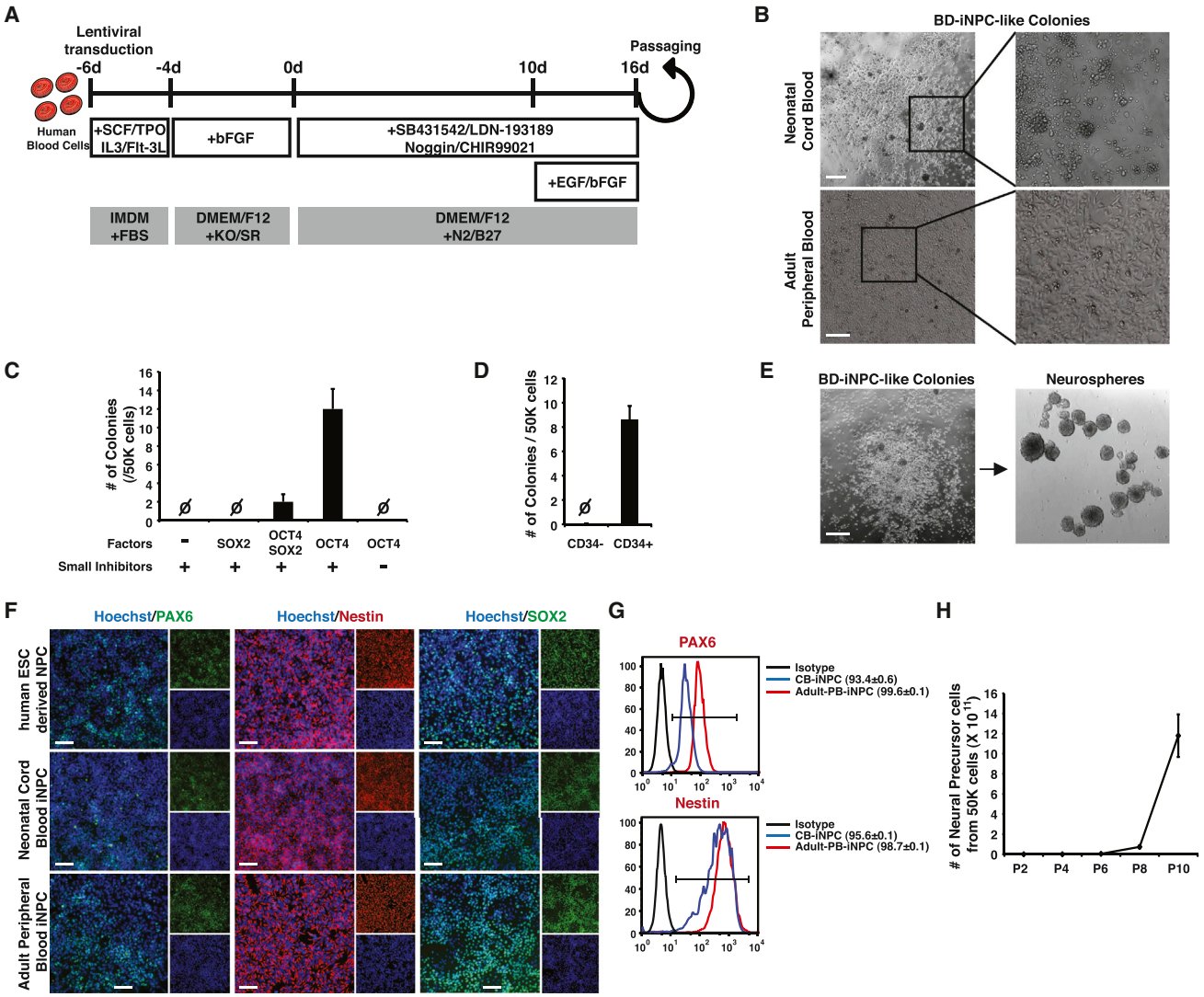

Figure 1. Generation of iNPC from Neonatal and Adult Blood Cells

(A) Schematic for deriving iNPCs from lineage-depleted $CD34^+CD45^+$ blood.

(B) Phase-contrast images of iNPCs. Scale bar represents 300 μm.

(C) iNPC colony numeration. Scale bar represents SD.

(D) iNPC colony numeration from $CD34^+$ or $CD34^-$ cells. Scale bar represents SD.

(E) Phase-contrast image of iNPC spheres. Scale bar represent 300 μm.

(F) Immunofluorescence of iNPCs for PAX6, Nestin, and SOX2. Scale bar represents 100 μm.

(G) FACS for PAX6 and NESTIN, from iNPCs (n = 4).

(H) Predicted iNPCs from 50K human blood progenitors. Scale bar represents SD.

blood progenitors to NPC fate that is not limited to phenotypic alternations alone.

BD-iNPCs Expand and Functionally Respond to In Vivo and Directed In Vitro Differentiation Cues

Having established the role for SMAD+GSK-3 inhibition during the initial generation of BD-iNPCs from human blood progenitors, we next examined the direct effects on proliferative expansion and developmental potential of the resulting BD-iNPCs. SMAD+GSK-3 inhibition resulted in enhanced proliferation of BD-iNPCs compared with inhibitor-withdrawn cultures (Figure S3A). However, enhanced proliferation came at the expense of differentiation, as BD-iNPCs maintained in the presence of SMAD+GSK-3 that were transferred to culture conditions conducive for neuronal differentiation (Figure S3B), displayed continued PAX6 expression but failed to upregulate Tuj1 compared with BD-iNPCs where inhibitors were withdrawn (Figures S3C and S3D). Despite a clear indication that inhibitor treatment imposed differentiation block, this phenomena was rapidly reversed within one round of passaging in the absence

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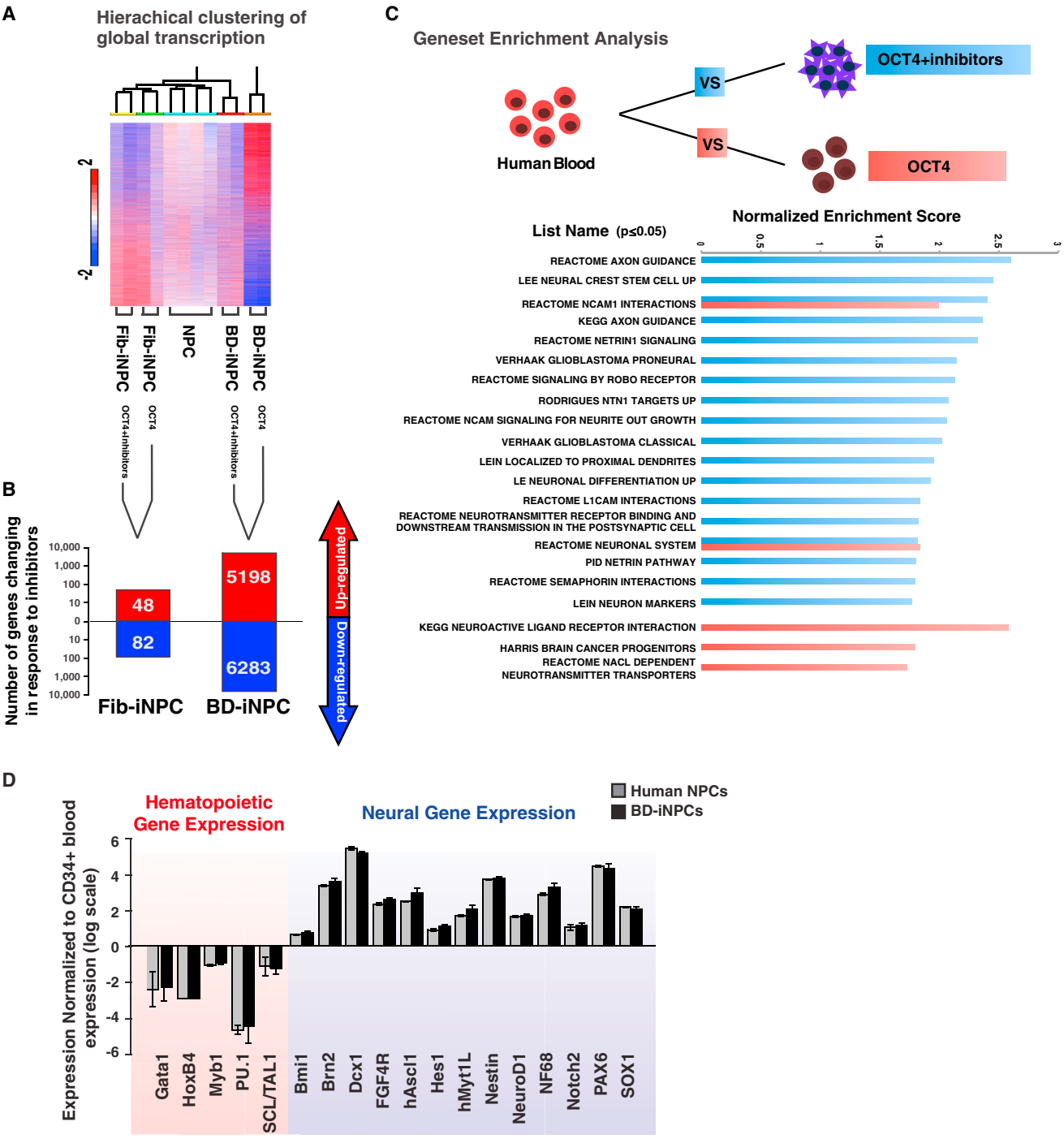

Figure 2. Molecular Profiling of OCT4 BD-iNPC Generation

(A) Hierarchal cluster analysis on global gene expression of Fib-iNPC and BD-iNPC ± inhibitors with primary human NPC.

(B) Number of genes changing in response to inhibitors in Fib-iNPC versus hBD-iNPCOCT4 (false discovery rate [FDR] $p \leq 0.05$, fold change ≥ 1.5).

(C) GSEA on iNPC ± inhibitors.

(D) Expression of hematopoietic or neural specific genes in BD-iNPCs and control NPCs derived from hPSCs. Scale bar represents SD.

of inhibitors, indicative of successful maintenance of differentiation potential throughout proliferative cycles (Figures S3E and S3F). Interestingly, quantified levels of PAX6 in long-term cultures revealed that the expression of the NPC marker was higher in the presence of SMAD+GSK-3 inhibitors, although the frequencies of positive cells were comparable (Figures S3G and

S3H). These results reveal that modulation of SMAD+GSK-3 signaling plays an important role in the regulation of proliferation and differentiation potential of BD-iNPCs.

We next set out to evaluate the developmental potential of OCT4 induced BD-iNPCs by assessing their ability to functionally differentiate in vivo toward the three main neural lineages. BD-iNPCs were transduced with a GFP expressing lentiviral vector and then injected into the brains of p2–p4 mouse pups and allowed to engraft for 3 weeks (Zhu et al., 2014). Analysis of GFP signal from sectioned brain tissue as a surrogate of human engraftment revealed multiple sites containing intact human cells (Figure 3A). Investigation for differentiated BD-iNPC progeny revealed populations of GFP-positive cells that co-expressed both TUJ1 and MAP2 with clear neuronal morphology (Figure 3B). Moreover, we identified GFP-positive human cells that also co-expressed glial fibrillary acidic protein (GFAP), consistent with the presence of astrocytes (Figure 3B). Despite confirming in vivo differentiation potential toward both neurons and astrocytes, we did not find evidence of in vivo differentiation toward oligodendrocytes, a finding not unlike that of other human iNPC studies that have relied on murine xenograft assays (Zhu et al., 2014).

Although in vivo xenograft studies are considered to be the gold standard for many assays of human biology that can otherwise not be measured, in vitro differentiation allows for the directed production of specific cell types that will likely be useful in near term personalized medicine applications of drug screening/testing rather than cellular transplantation. Despite limited detection of oligodendrocytes in our in vivo tests, BD-iNPCs possessed astrocyte and oligodendrocyte differentiation potential in vitro, as evidenced by GFAP and O4 expression, respectively, with characteristic morphology similar to differentiated cells from human PSCs (Figures 3C, 3D, and S3I). Furthermore, culture conditions for the specification for neuronal development resulted in mature neurons expressing canonical markers TUJ1 and MAP2 (Figures 3E and S3J), with the majority expressing high levels of glutamate, consistent with excitatory glutamatergic neurons. Importantly, prior to differentiation BD-iNPCs express OCT4 transgene at observable levels, however, similar to previous reports (Mitchell et al., 2014b), OCT4 expression is silenced upon complete differentiation toward mature functional cells types (Figure S3K). Using specific conditions for GABAergic neurons, we successfully generated GABA-positive inhibitory neurons (Figure 3E), suggesting BD-iNPCs harbored broad neuronal developmental potential. Moreover, BD-iNPC derived neurons also exhibited a punctate pattern of synapsin expression, suggesting the development of synapses (Figure 3F), which was confirmed using electrophysiological analysis (Figures 3G and 3I). Specifically, upon positive current injection, spontaneous repetitive action potential firing was induced (Figure 3G), and voltage-dependent transient Na^+ and sustained K^+ currents were detected (Figure S3L). Application of tetrodotoxin (TTX) blocked rapidly activating and inactivating inward currents, further demonstrating that the differentiated neurons expressed voltage-activated sodium channels associated with primary neurons (Figure 3I). Thus, neurons derived from iNPCs appear to exhibit the functional membrane properties and activities of mature neurons.

Having observed robust functional neuronal differentiation activity, we investigated whether BD-iNPCs neuronal differentiation capacity could be expanded into more specialized neurons, such as dopaminergic (DA) neurons, in response to specific instructions. We found that treatment with Sonic Hedgehog (SHH) and FGF8b (Li et al., 2011) further differentiated BD-iNPCs into neurons expressing tyrosine hydrolase (TH), the rate-limiting enzyme in the synthesis of DA (Figure 3J). These neurons also expressed the nuclear receptor NURR1 (also known as NR4A2), a key regulator of the DA system (Figure 3J). Moreover, the detection of secreted DA in culture medium further supported the presence of functional DA neurons in vitro (Figure 3K).

Taken together, these results confirm that BD-iNPCs are capable of robust expansion without sacrificing their broad developmental potential and thereby exhibit the most critical features of bona fide human neural progenitors.

BD-iNPC Generate Functional Nociceptors that Model Chemotherapy-Induced Neuropathy

Based on the broad neuronal developmental potential of BD-iNPCs, we further analyzed the transcriptome of BD-iNPCs. These analyses revealed an enrichment of neural crest cell-related gene activity compared with that found in blood progenitors (Figure 2C). Recent work has demonstrated the conversion of human fibroblasts to both putative neural crest (Kim et al., 2014), as well as sensory neurons (neural crest-derived peripheral neurons) using typical lineage specifying transcription factor reprogramming strategies (Blanchard et al., 2015; Wainger et al., 2015). Despite a lack of neural crest or sensory neuron functional activity in starting populations of fibroblasts, these human fibroblasts were found to be enriched for neural crest-related genes compared with that of human blood cells, suggesting a transcriptionally primed state of neural potential for conversion toward the neural lineage within skin fibroblast cultures not seen in blood progenitors (Figure 3L). Therefore, in contrast to skin fibroblasts, BD-iNPC conversion involves de novo acquisition of neural crest-related gene expression (Figure 3M). Based on this observation, we hypothesized that their developmental potential may extend to the peripheral nervous system derivatives, such as sensory neurons.

Recent work has demonstrated that combined small-molecule inhibition (SU5402, DAPT, and CHIR99021) converts human pluripotent cells into sensory neurons (nociceptors) (Chambers et al., 2012). Based on previous reports (Chambers et al., 2012; Guo et al., 2013), we made modifications to this procedure (Figure S4A) and tested whether these small-molecule inhibitors could induce generation of nociceptive sensory neurons from directly converted human neonatal and adult BD-iNPCs. We found that the canonical sensory neuronal markers ISL1 and BRN3A were expressed within differentiated neuron preparations from cord blood and adult peripheral blood BD-iNPCs, in a similar fashion as human embryonic stem cell (hESC)-derived cells shown previously (Figure 4A). In addition, sensory culture-derived neurons expressed glutamate, consistent with an excitatory glutamatergic neuronal phenotype (Figure S4B), and demonstrated transcript-level expression of sensory neuron-related genes such as NTRK1, 2, and 3 receptors, neurofilamin heavy chain peptide (NEFH), and calcitonin-related peptide

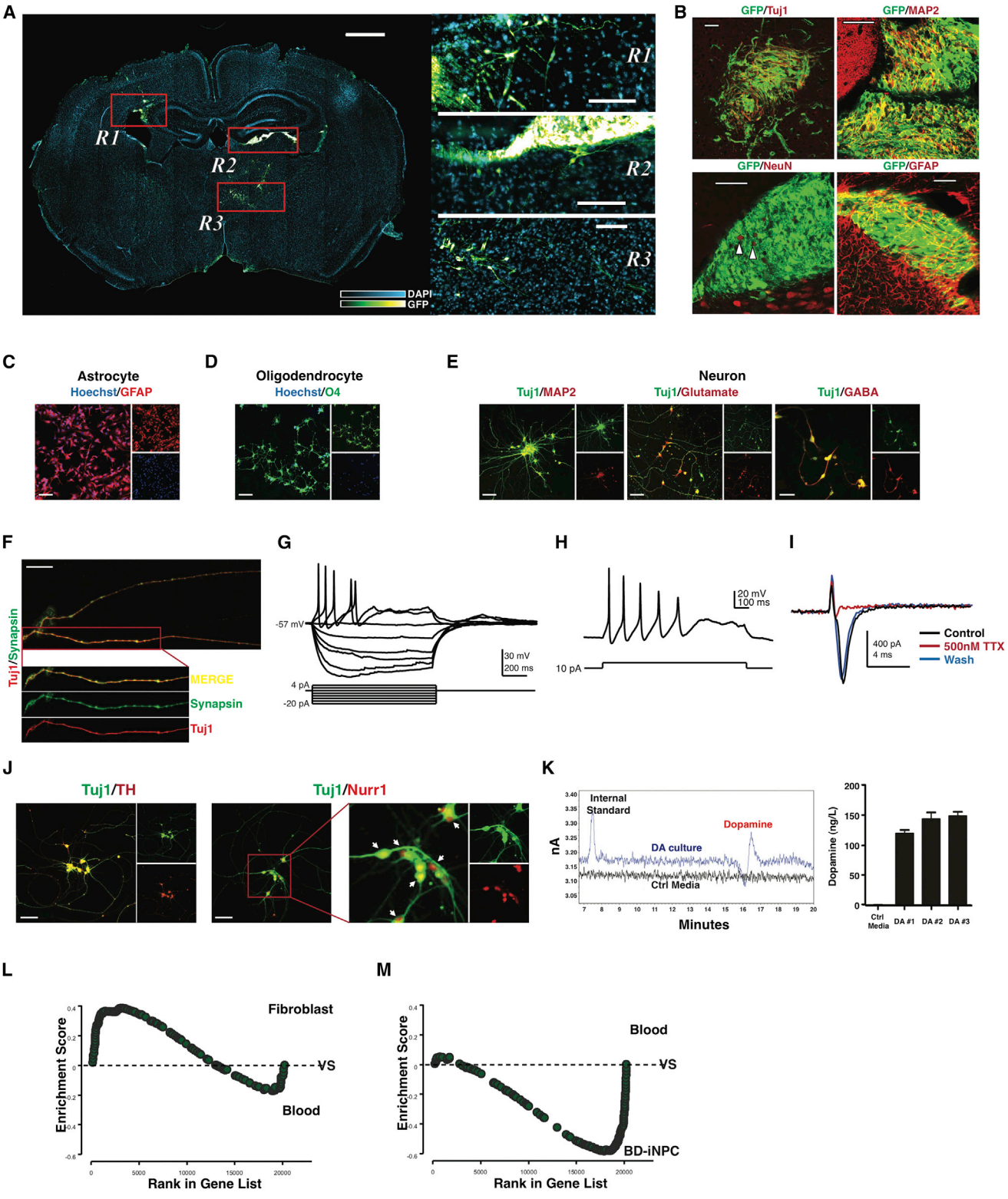

Figure 3. In Vivo and In Vitro Differentiation Potential of BD-iNPCs

(A) Montage of individual images from sectioned brain tissue 3 weeks after injection of BD-iNPCs expressing GFP ("R" - zoomed images have been manually aligned for visual continuity).

(B) In vivo differentiation of BD-iNPCs into neurons (expression of Tuj1, MAP2, and NeuN) and astrocytes (expression of GFAP).

(legend continued on next page)

alpha (CALCA) (Figure S4C). These results confirmed that upon treatment with appropriate culture conditions, BD-iNPCs were capable of generating putative sensory neurons; supportive of our idea that BD-iNPC developmental potential extends to PNS-related progeny.

Given strong clinical interest for furthering our understanding of neurological pain and neuropathy conditions (Bennett and Woods, 2014; NCI, 2010), combined with the notion that nociceptive neurons (NTRK1 expressing) can be functionally assayed (Blanchard et al., 2015; Wainger et al., 2015), we decided to focus our efforts on nociceptive (NTRK1) neuron generation from BD-iNPCs for further characterization and optimization for use. Analysis of NTRK1 expression at the protein level revealed approximately 50% of differentiated cell positivity of putative nociceptors (Figure 4B). Over 14 days, analysis of ISL1, BRN3A, and NTRK1 expression indicated that putative nociceptive sensory neurons could be sustained and continually generated from differentiating BD-iNPCs over time (Figure 4C). Induced neurons were often organized into ganglia-like structures in long-term culture and expressed Substance P (TAC1), indicating the presence of peptidergic nociceptors (Figure 4D). Moreover, the expression of nociceptor-specific channels and receptors was upregulated during sensory neural induction (Figure 4E). Expression of the purinergic receptor, P2RX3, considered a unique phenotype of human sensory neurons (Jarvis et al., 2002), was confirmed by immunofluorescence analyses (Figure 4F).

Functionally, human cord blood and adult BD-iNPC-differentiated neurons were evaluated using calcium flux in response to α,β-methylene-ATP (α,β-meATP), a selective agonist of $P2X_3$ (Figures 4G and 4H) (Jarvis et al., 2002). Although neurons at day 7 post-induction showed expression of putative sensory neuron markers (Figure 4C), only a minimal response to α,β-methylene-ATP was detectable, whereas continued culture to day 14 allowed a robust response to manifest (Figures 4I, 4J, and S4D). This indicates that the development of phenotype alone does not suggest functional capacity in terms of ligand responsiveness—an important caution in pragmatic use of converted human neuronal cell types. Furthermore, both the TRPV1 vanilloid receptor agonist capsaicin and $P2X_3$ agonist α,β-meATP could evoke calcium transients in BD-iNPC derived neurons (Caterina et al., 1997), demonstrating functional activity of nociceptive sensory neurons (Figures 4I, 4J, and S4D). Importantly, A-317491, a selective $P2X_3$ inhibitor, significantly decreased this response (Figure 4K), providing evidence that the α,β-meATP mode of action was indeed through activation of $P2X_3$ receptors (Figure 4K). Our findings demonstrate the differentiation potential of BD-iNPCs into nociceptive sensory neurons, which when combined with their expansion capacity, raises the possibility of generating sufficient quantities of these specialized cells for drug discovery, toxicity, and screening applications. We have estimated that a single round of reprogramming could support the generation of as many as 100-million sensory neurons (Figure 4L) from 50K human blood cells.

Approximately 30% to 40% of cancer patients experience the cancer treatment complication of chemotherapy-induced peripheral neuropathy (CIPN) (NCI, 2010) through the direct impact of the drug on nerve fibers causing nerve degeneration and axon dieback (Boyette-Davis et al., 2011). We tested whether BD-iNPC-derived sensory neurons showed a similar response to chemotherapy treatment in vitro. Forty-eight hours after treatment with Taxol, neurites of sensory neurons generated from human blood were quantified and showed a dose-dependent reduction in length without concomitant loss of viability (Figure 4M). This miniaturized and automated approach illustrates the potential utility of these converted cells as an in vitro model of human axonopathy for drug discovery and form the basis for future development to expand to other PNS and CNS disorders.

DISCUSSION

We provide evidence that small molecule inhibitors targeting SMAD+GSK3 enable ectopic expression of OCT4 to directly convert human blood progenitors into proliferative, non-tumorigenic neural precursors with unique multipotent developmental properties that includes generation of both DA and sensory neurons. Unlike skin fibroblasts with hallmarks of neural lineages, purified $CD34^+CD45^+$ blood is devoid of ectoderm derived cells, and as such, BD-iNPCs represent evidence for epigenetic conversion of cell fate state from one developmentally distinct cell type to another (Rieske et al., 2005). Within the context of fibroblast reprogramming, expression of OCT4 and the addition of basal neural progenitor culture conditions are sufficient to support conversion toward iNPCs (Mitchell et al., 2014b), whereas generation of BD-iNPCs shown here is highly dependent on the usage of SMAD+GSK-3 inhibition. As inhibition of these pathways has already been established as a means of specifying neural identity within cells undergoing cell fate changes (Chambers et al., 2009; Li et al., 2011), we hypothesize OCT4-induced plasticity within hematopoietic cells similarly responds to these cues in order to support direct conversion toward the neural lineage. Previous attempts to convert human hematopoietic tissue toward the neural lineage were restricted to the use of neonatal cord blood-derived MSCs (Yu et al., 2015) or have resulted in the production of neuronal restricted progenitors with limited

(C–E) In vitro differentiation of BD-iNPCs in to GFAP-positive astrocytes (C) and O4-positive oligodendrocytes (D). (E) Tuj1 and MAP2-positive neurons and glutamatergic and GABAergic neuronal subtypes. Scale bar represents 100 μm.
(F) Expression of Synapsin. Scale bar represents 50 μm.
(G) Raw traces of membrane potential changes to stepwise current injection of equal increment.
(H) Repetitive action potential firing was induced upon depolarizing current injection.
(I) TTX-sensitive fast inward currents on depolarization.
(J) TH and Nurr1-positive DA neurons derived from BD-iNPCs. Scale bar represents 100 μm.
(K) HPLC for dopamine (left) and levels in multiple DA cultures (right). Scale bar represents SD.
(L) Gene set enrichment analysis for LEE_NEURAL_CREST_STEM_CELL gene list between human fibroblasts and human blood.
(M) Gene set enrichment analysis for LEE_NEURAL_CREST_STEM_CELL gene list between human blood and BD-iNPCs.

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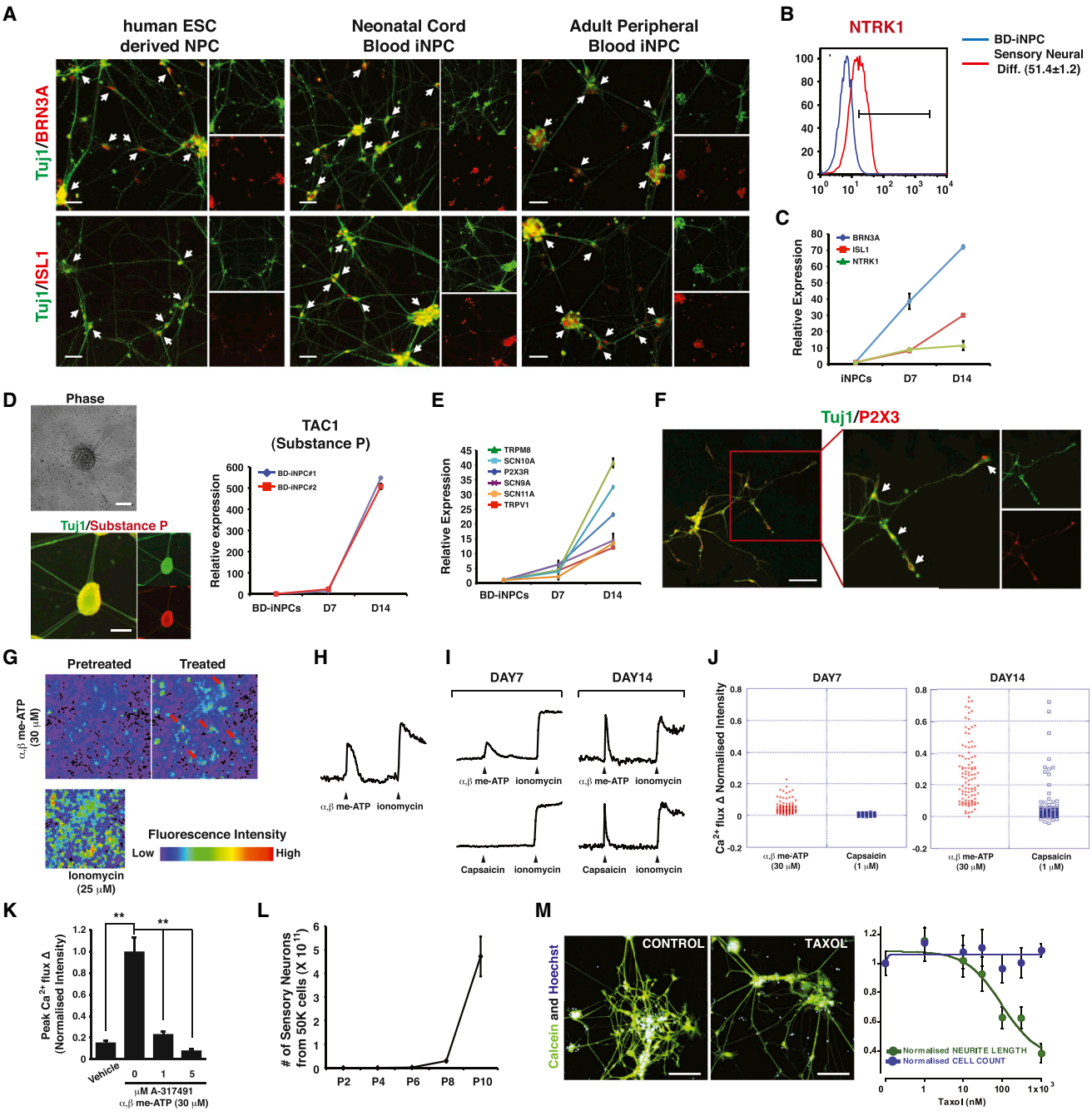

Figure 4. Generation of Functional Nociceptive Neurons that Model Chemotherapy-Induced Neuropathy

(A) Tuj1$^+$ neurons express BRN3A and ISL1. Scale bar represents 50 μm.

(B) Expression of NTRK1 by FACS.

(C) Transcript expression of BRN3A, ISL1, and NTRK1 during differentiation from iNPCs toward sensory neurons.

(D) Neuronal clustering (top left) and Substance P expression (low left). Scale bar represents 100 μm. (Right) RT-PCR for TAC1 (Substance P).

(E) Expression of channels, channel subunits, and receptors, specific to nociceptors.

(F) Immunocytochemistry for $P2X_3$. Scale bar represents 100 μm.

(G and H) Calcium flux in response to 30 μM α,β-meATP treatment of day 14 sensory neurons derived from adult PB-iNPCs.

(I and J) Calcium trace (I) and distribution of cells (J) responsive to 30 μM α,β-meATP, and 1 μM capsaicin.

(K) $P2X_3$ antagonist A-317491 significantly inhibited the calcium-response to α,β-meATP.

(L) Predicted number of nociceptors from 50K human blood progenitors.

(M) In vitro response of BD-iNPC-derived sensory neurons 48-hr post-Taxol treatment (left) and normalized dose-dependent neurite length and cell count (right). Where applicable, scale bar represents SD.

proliferative potential (Castaño et al., 2014). To the best of our knowledge, our current study defining BD-iNPCs are the first example of trilineage neural progenitor cells produced from direct conversion of adult human blood.

Our report uniquely provides a practical and simple approach for generating neural progenitor cells capable of nociceptive neuron differentiation. Although recent work using fibroblasts has demonstrated successful conversion toward pain sensing neurons, these studies require a multifactor trans-differentiation strategy that bypasses the neural progenitor state (Blanchard et al., 2015; Wainger et al., 2015). As such, each resulting cell is unique from one another given the heterogeneity of fibroblast populations and complex multivector integration. BD-iNPCs could aid in realizing goals of better understanding the peripheral-neuropathy component of pain associated with complex disorders such as diabetes and chemotherapy, as well as primary pain that often precedes motor dysfunction in Parkinson's patients by several years (Tesfaye et al., 2013). For the purpose of drug screening or replacement therapy, prospective isolation or generating pure populations from single progenitor clones will be critical to moving forward. Moreover, it will be critical to understand how application of selectable inducible expression systems that have been widely used during mouse direct reprogramming strategies may elucidate the required epigenetic transformations (Wapinski et al., 2013) that take place during human direct conversion reprogramming. Thus, further studies are required in order to develop appropriate scale-up protocols to allow BD-iNPCs to better serve as a personalized neurological model system, where the conversion process detailed here provides the foundation for patient specific analyses.

EXPERIMENTAL PROCEDURES

Cell Culture and Derivation of iNPCs

To derive iNPCs, purified $CD34^+$ cells from cord blood or adult mobilized peripheral blood were transduced OCT4 lentivirus in the presence of SCF, Flt-3L, IL3, and TPO cytokines (R&D System). After 48 hr, $CD34^+$ blood cells were cultured on Matrigel (BD Biosciences) or irradiated MEFs with reprogramming media and basic fibroblast growth factor (bFGF) (R&D System) for 5 days. Cells were then switched to basal NPC media (DMEM/F12, 1 × N2, 1 × B27) (Invitrogen) supplemented with SB431542, LDN-193189 (Stemgent), Noggin (R&D System), and CHIR99021 (Stemgent). After 10–14 days, neural precursors-like colonies were manually picked and transferred to Polyornithine/Laminin (POL)-coated culture plates for propagation with neural induction medium supplemented with bFGF and epidermal growth factor (EGF) (R&D System). Primary neurosphere culture was used to further enrich iNPCs. Experiments using adult peripheral blood derived iNPCs are shown in Figures 1B, 1F, 1G, 4A, 4G, 4H, and S3.

iNPC Differentiation

For neuronal differentiation, basal media were supplemented with retinoic acid (Sigma), forskolin (Stemgent), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) (R&D System), and ascorbic acid (Sigma). Please see Supplemental Information for specific neuronal differentiation conditions. For astrocyte differentiation, media were supplemented with 5% fetal bovine serum (FBS). For oligodendrocyte differentiation, basal media were supplemented with SHH C25II, basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) (R&D System) for 7 days. Afterward, PDGF and bFGF were replaced by 3,3,5-triiodothyronine (T3) hormone (Sigma), Noggin, IGF1, NT3, and forskolin adapted from Lujan et al. (2012) and Najm et al. (2013).

Generation of Neuronal Subtypes from iNPC

For GABA neuron induction, we adapted Barberi et al. (2003) and Ma et al. (2012); iNPCs were cultivated in basal medium supplemented with SHH C25II without EGF. After 7 days, media were supplemented with VPA, NT4, BDNF, GDNF, IGF1, and forskolin for 21 days. For DA neuron induction, we adapted Kriks et al. (2011) and Li et al. (2011); iNPCs were cultured in basal medium supplemented with SHH C25II and FGF8 (R&D System) without bFGF/epidermal growth factor (EGF). After 7 days, media were supplemented with, BDNF, GDNF, transforming growth factor-β3 (TGF-β3), ascorbic acid, forskolin, and N-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester (DAPT) (Sigma) for 21 days. For nociceptive sensory neurons, we adapted Chambers et al. (2012), Guo et al. (2013), and Lee et al. (2012). Briefly, iNPCs were cultured in basal medium supplemented with SU5402, DAPT, and CHIR99021. After 4 days, media were supplemented with BDNF, GDNF, nerve growth factor (NGF), NT3 (R&D System), ascorbic acid, and forskolin for 7–14 days until the desired maturation stage for a given experiment.

Statistical Methods

Unless otherwise noted, SD was used in performing a Student's t test (two tailed) where $^*p = 0.05$ and $^{**}p = 0.01$.

All animal procedures received the approval of the animal ethics board at McMaster University.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.04.056.

AUTHOR CONTRIBUTIONS

J.-H.L. and R.R.M. designed the study, performed experiments, and co-wrote the paper. J.D.M. provided support for calcium imaging. Z.S. provided support for microarray analysis. S.L. and B.T. provided assistance with PCR. C.M. performed immunofluorescence on xenografted brain slices. F.C. provided assistance for high-powered liquid chromatography (HPLC). A.F.-C. provided technical assistance for all animal work. T.J.C. designed experiments and assisted with calcium imaging and wrote the paper. K.K.S. designed and assisted during in vivo xenotransplantation experiments. M.B. designed and oversaw the entire study and co-wrote the paper.

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