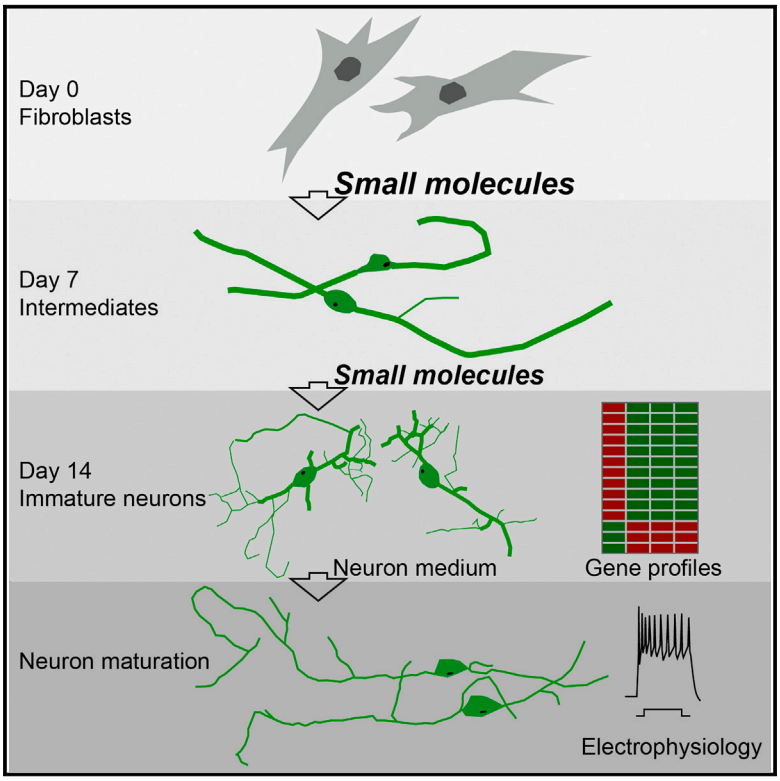
Short Article

Cell Stem Cell

Direct Conversion of Normal and Alzheimer's Disease Human Fibroblasts into Neuronal Cells by Small Molecules

Graphical Abstract



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In Brief

In this study, Pei and colleagues demonstrate that a cocktail of small molecules alone can reprogram human fibroblasts from control and Alzheimer's disease patients into functional neuronal cells. These human chemical-induced neurons resemble iPSC-derived and transcription factor-induced human neurons.

Highlights

- Human fibroblasts can be directly converted into neurons with a chemical cocktail
- Electrophysiological properties of hciNs are similar to iPSC-derived neurons and iNs
- hciNs show high neuronal but low fibroblastic gene expression profiles
- hciNs derived from FAD patient fibroblasts exhibit abnormal Aβ production

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Direct Conversion of Normal and Alzheimer's Disease Human Fibroblasts into Neuronal Cells by Small Molecules

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SUMMARY

Neuronal conversion from human fibroblasts can be induced by lineage-specific transcription factors; however, the introduction of ectopic genes limits the therapeutic applications of such induced neurons (iNs). Here, we report that human fibroblasts can be directly converted into neuronal cells by a chemical cocktail of seven small molecules, bypassing a neural progenitor stage. These human chemical-induced neuronal cells (hciNs) resembled hiPSC-derived neurons and human iNs (hiNs) with respect to morphology, gene expression profiles, and electrophysiological properties. This approach was further applied to generate hciNs from familial Alzheimer's disease patients. Taken together, our transgene-free and chemical-only approach for direct reprogramming of human fibroblasts into neurons provides an alternative strategy for modeling neurological diseases and for regenerative medicine.

INTRODUCTION

Terminally differentiated somatic cells could be reprogrammed into pluripotent stem cells by forced expression of a specific set of transcription factors (TFs), indicating that cell fate determination is reversible (Ladewig et al., 2013; Takahashi and Yamanaka, 2006). Further, different combinations of lineage-specific TFs could directly convert cardiomyocytes (Ieda et al., 2010), hepatocytes (Huang et al., 2014), or neurons (Pang et al., 2011; Vierbuchen et al., 2010) from mouse or human somatic cells, bypassing the pluripotent state and providing alternative avenues for disease modeling and regenerative medicine. Interestingly, these conversions can also be induced in vivo by the introduction of specific TFs (Guo et al., 2014; Niu et al., 2013), further implying their therapeutic potential. However, the introduction of ectopic transgenes limits their current therapeutic application. Thus, tightly controlling the expression of ectopic genes and reducing the number of TFs have been tried (Dell'Anno et al., 2014; Ladewig et al., 2012). Cell-permeable small molecules have been shown to promote cell reprogramming (Huangfu et al., 2008; Xu et al., 2008). A recent report has even shown that small molecules alone can induce mouse fibroblasts into a pluripotent state (Hou et al., 2013). Our previous report also showed that neural progenitor cells (NPCs) can be induced from mouse fibroblasts or human urinary cells with the proper chemical cocktails (Cheng et al., 2014). Moreover, chemical-promoted transdifferentiation from mouse or human fibroblasts to different cell lineages has been reported (Pennarossa et al., 2013; Sayed et al., 2015).

Considering that in vitro differentiation of either ESCs or iPSCs to generate neurons is still time-consuming and complicated (Zhang and Zhang, 2010), here we try to establish a direct conversion of human fibroblasts into neuronal cells by small molecules, bypassing the neural progenitor stage.

RESULTS

Induction of Neuronal Cells from Human Adult Fibroblasts by Small Molecules

Our previous study showed that a small chemical cocktail, VCR (V, valproic acid [VPA]; C, CHIR99021; and R, Repsox), could induce NPCs from mouse and human somatic cells (Cheng et al., 2014). We then hypothesized that VCR with the combination of chemicals known to promote neural differentiation of NPCs might facilitate conversion of human fibroblasts into neuronal cells. The initial human fibroblasts (HAFs, FS090609,

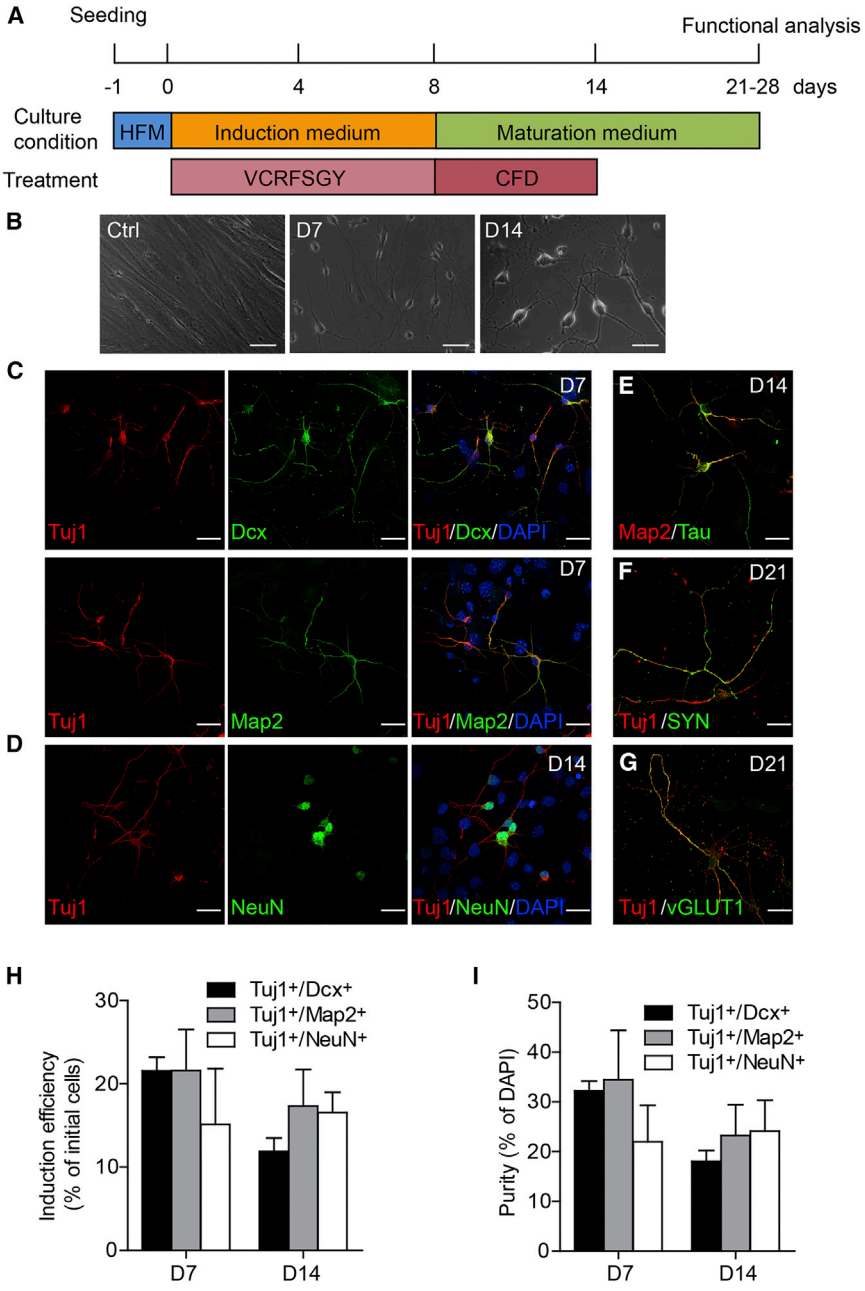

Figure 1. Induction of Human Neuronal Cells by Small Molecules

(A) Scheme of induction procedure. Initial fibroblasts were plated in DMEM and this day is termed as "Day – 1." 1 day later, cells were transferred into induction medium with chemical compounds and cultured for 8 days, and then cells were further cultured in maturation medium with supplementary chemicals and neurotrophic factors for 2–3 weeks. V, VPA; C, CHIR99021; R, Repsox; F, Forskolin; S, SP600625; G, GO6983; Y, Y-27632; D, Dorsomorphin.

(B) Phase contrast images of control HAFs (left) or hciNs at day 7 (middle) and day 14 (right). Scale bars, 100 μm.

(C) hciNs display bipolar neuronal morphologies and express Dcx (green), Tuj1 (red), and Map2 (green) at day 7. Scale bars, 20 μm.

(D–G) hciNs stained for NeuN (D), Tau (E), SYN (F), and vGLUT1 (G). Scale bars, 20 μm.

(H) hciN induction efficiencies were presented as the percentage of induced $Tuj1^+$ Dcx^+, $Tuj1^+$ $Map2^+$, or $Tuj1^+$ $NeuN^+$ neuronal cells versus initial seeding cells at the time of quantification (means ± SEM, n = 20 random selected ×20 fields from triplicate samples).

(I) The percentage of induced $Tuj1^+$ Dcx^+, $Tuj1^+$ $Map2^+$, or $Tuj1^+$ $NeuN^+$ neuronal cells in total cells at the time of quantification (means ± SEM, n = 20 random selected ×20 fields from triplicate samples).

See also Figure S1, Table S2, and Table S3.

derived from the foreskin of a 28-year-old male) showed no contamination of neuronal cells (Figure S1A). We treated the fibroblasts with VCR in a neuronal induction medium, consistent with our previous results, and we did not detect any neuron-like cells (Figure S1B). We then treated HAFs with VCR plus chemicals shown to promote neural differentiation of NPCs (Table S1). We found that VCRF (VCR+Forskolin) treatment induced a bipolar neuron-like cell morphology. Some of these bipolar neuron-like cells even expressed the neuronal maker Tuj1 at day 7 following VCRF treatment (Figure S1B). However, the round and prominent cell bodies are unlike a typical neuron morphology, suggesting a partial and inefficient conversion (Liu et al., 2013; Pang et al., 2011). Therefore we added other reported neural-promoting chemicals to improve the neuronal conversion. We found that the small molecules SP600125 (JNK inhibitor, S), GO6983 (PKC inhibitor, G) and Y-27632 (ROCK inhibitor, Y) could facilitate the neuronal conversion by VCRF and resulted in the generation of Tuj1-positive cells with neuron-like morphology (Figure S1B). This VCRFSGY treatment showed the highest potency in the induction of neuronal cells from HAFs. 7 days after VCRFSGY treatment, we found that a significant fraction of the living cells exhibited typical neuronal morphology and expressed neuronal makers including Dcx, Tuj1, and Map2 (Figures 1A–1C).

Most of the induced neuronal (iN) cells survived until day 10–12 and stopped developing into more mature neurons. We then screened for additional chemicals to promote neuron survival and maturation. Recent reports showed that CHIR99021 (C), Forskolin (F), and Dorsomorphin (D) are beneficial for neuron survival and maturation (Ladewig et al., 2012; Liu et al., 2013). Indeed, when we replaced the induction medium with the neuron maturation medium supplemented with CFD and extra neurotropic factors (BDNF, GDNF, and NT3), neuronal cell survival and maturation were significantly improved. In 2 or 3 weeks, the iN cells were stained positive for mature neuronal markers

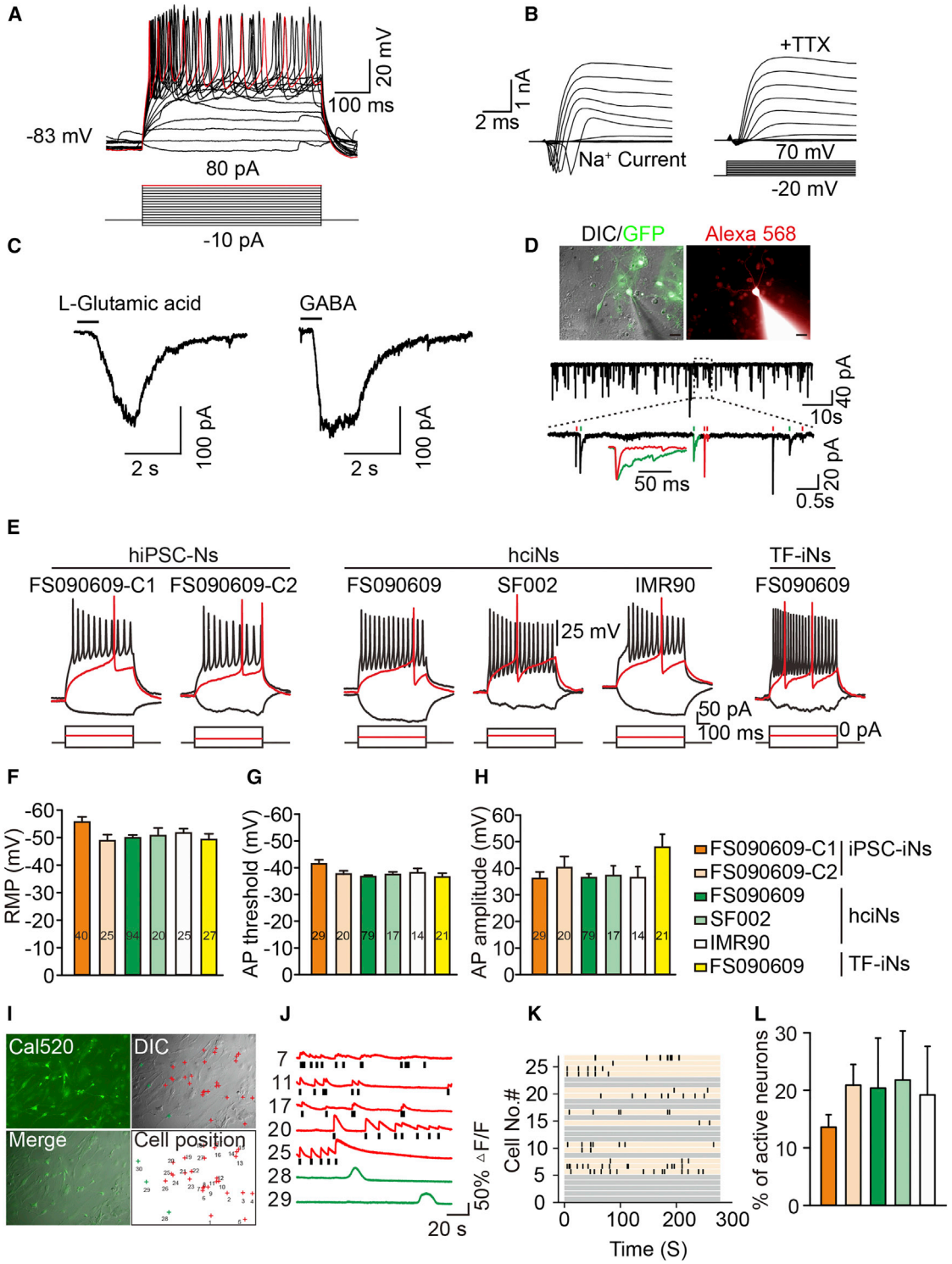

Figure 2. hciNs Have Electrophysiological Properties of Functional Neurons

(A) Current-clamp recordings of hciNs generated from HAFs showing a representative train of action potentials (top panel). Step currents were injected from −10 pA to 80 pA in 6 pA increments (bottom panel).

(B) Representative traces of tetrodotoxin-sensitive fast inward currents recorded in voltage-clamp mode from hciNs at day 14. Tetrodotoxin (1 μM) treatment inhibited voltage-dependent sodium currents (right panel). Cells were depolarized from −20 mV to 70 mV in 10 mV increments.

(C) Focal application of 10 μM L-glutamic acid (left panel) or 10 μM GABA (right panel) induces inward membrane currents.

(D) Representative trace of spontaneous postsynaptic currents in hciNs generated from GFP-labeled HAFs. GFP-labeled HAFs were treated with indicated chemicals for 1 week and then replated in high density on pure astrocytes. Two to three weeks after replating, GFP-labeled hciNs with complex neuron

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Tau, NeuN, and synapsin (SYN) (Figures 1D–1I). We referred to them as hciN cells (hciNs). We optimized the dosages of each small molecule (Table S3) and a cocktail with 0.5 mM VPA, 3 μM CHIR99021, 1 μM Repsox, 10 μM Forskolin, 10 μM SP600125, 5 μM GO6983, and 5 μM Y-27632 was able to induce ~5% Tuj1-positive hciNs (Table S3). Further, the time course of chemical treatment has been optimized. VCRFSGY treatment for 8 days resulted in the highest induction efficiency (Figure S1C). More than 80% of Tuj1-positve cells expressed vGLUT1, whereas GABAergic, cholinergic, or dopaminergic neurons were merely detected (Figures 1G and S1D). We applied the VCRFSGY induction protocol to fibroblasts obtained from eight human individuals with different genders, ages, and passages (Table S2). The conversion efficiency of each individual fibroblast line was comparable as shown in Figure S1E and Table S2. These results indicate that our chemical induction protocol could reproducibly induce neuronal cells from human fibroblasts.

We next tested whether induction of hciNs from HAFs using our chemical induction protocol bypassed the neural progenitor stage. RNA expression profiling by qRT-PCR and immunostaining analysis showed that the neural progenitor genes Sox2, Pax6, FoxG1, or Nestin were never expressed during the hciNs induction procedure (Figures S1F and S1G). Interestingly, we found that the proliferation marker Ki67 was not detectable after day 3 (Figure S1H), suggesting that our chemical induction protocol might trigger a fast cell cycle exit of human fibroblasts and that the conversion of human fibroblasts into hciNs using this chemical strategy is probably direct.

Physiological Properties of hciNs

To test whether the hciNs from HAFs possess basic electrophysiological properties of neurons, such as the ability to fire action potentials (APs) and the induction of membrane current, we performed whole-cell patch-clamp recording of hciNs with complex neuron morphology at day 14 after chemical cocktail treatment. 88% of recorded hciNs (n = 89) generated repetitive trains of APs when we depolarizing the membrane in current-clamp mode (Figure 2A and Figure S2C). Depolarizing voltage steps in voltage-clamp mode elicited fast inward sodium currents (Figure 2B; 93%, n = 15). Moreover, the fast inward currents were completely blocked by the sodium channel blocker tetrodotoxin (TTX). To test whether the hciNs have functional glutamate and GABA receptors, we puffed exogenous L-glutamic acid (Figure 2C, left panel; 50%, n = 10) and GABA (Figure 2C, right panel; 67%, n = 9) onto the hciNs and found that inward currents were induced, indicating that functional glutamate and GABA receptors were present in hciNs. Co-culturing neuronal cells with astrocytes was reported to facilitate neuron maturation and synapse formation by providing neurotropic factors (Chanda et al., 2014). Thus we infected HAFs with lentivirus encoding GFP and seeded GFP-positive hciNs (6 days after VCRFSGY treatment) to a monolayer astrocyte culture. Two weeks after plating, we carried out whole-cell patch-clamp recording on GFP-positive hciNs with mature neuron morphology (Figure 2D, top panel). Spontaneous postsynaptic currents (sPSCs) was recorded (Figure 2D; 31%, n = 26), indicating that hciNs could form functional synapses. Moreover, analysis of the kinetics of sPSCs demonstrated that hciNs possessed fast-decay spontaneous excitatory postsynaptic currents (sEPSCs) and slow-decay spontaneous inhibitory postsynaptic currents (sIPSCs) (Figures 2D and S2A). Application of bicuculline selectively blocked the slow-decay sIPSCs (Figure S2B), whereas NBQX eliminated the fast-decay sEPSCs (data not shown), indicating the presence of GABAergic and glutamatergic synapses in the hciNs. Together, these results show that hciNs induced by small molecules are functional neurons.

To further test the reliability of our chemical induction protocol, we recorded more hciN cell lines and compared the electrophysiological properties of hciNs with those of human induced pluripotent stem cell (hiPSC)-derived neurons and TF-iNs (Figures 2E–2H). The physiological properties of AP amplitude, AP threshold, and resting membrane potential (RMP) were not significantly different. Although there were some variations between cell lines, the overall electrophysiological properties of hciNs resembled those of hiPSC-derived neurons and TF-iNs.

Patch-clamp analysis only qualified a small fraction of cells. To monitor the activity of large numbers of cells at once, we loaded hciNs (co-cultured with monolayer astrocytes) with Cal520, a sensitive Ca^{2+} indicator (Figure 2I). The hciNs exhibited robust spontaneous calcium transients (Figures 2J and 2K), suggesting that the hciN neural network was rather active. The percentage of hciNs firing spontaneous calcium transients was not significantly different from that of iPSC-derived neurons (Figure 2L).

hciNs Possess a Neuronal Gene Expression Signature

To further characterize the hciN cell phenotype, we quantitatively analyzed the expression of 25 neuronal genes and 7

morphology were recorded. Recording configurations were shown (top panel). The insert shows two distinct types of sPSCs: fast-decay sEPSCs (indicated with red vertical bars) and slow-decay sIPSCs (indicated with green vertical bars). The enlarged sEPSC (red line) and sIPSC (green line) were normalized. Scale bars, 20 μm.

(E) Representative APs from six different cell lines (FS090609-C1 and FS090609-C2 for hiPSCs; FS090609, SF002, and IMR90 for hciNs; FS090609 for TF-iNs).

(F–H) Statistics of intrinsic electrophysiological properties of hiPSCs (two cell lines: FS090609-C1 and FS090609-C2), hciNs (three cell lines: FS090609, SF002, and IMR90) and TF-iNs (FS090609). F, AP amplitude; G, AP threshold; H, resting membrane potential (RMP).

(I) Fluorescence and DIC images of hciNs stained with calcium indicator Cal520. Neurons, red crosses; the number of cells is indicated (1–27). Astrocytes, green crosses; the number of cells is 28–30.

(J) Representative traces of spontaneous calcium transients in Figure 2I. Black vertical bars indicate the initiate time of calcium transients. The rise phase of astrocytes calcium transients (green lines) is much slower than that of neurons (red lines).

(K) Raster plots of spontaneous calcium transients in Figure 2I.

(L) Quantification of the percentage of neurons exhibiting spontaneous calcium transients. In total, 22 movies were acquired and 1,249 putative neurons were analyzed (FS090609-C1 hiPSCs, 138 cells from 3 movies; FS090609-C2 hiPSCs, 650 cells from 3 movies; FS090609 hciNs, 108 cells from 5 movies; SF002 hciNs, 170 cells from 5 movies; IMR90 hciNs, 183 cells from 6 movies). See also Figure S2.

Data of at least three independent experiments are shown as means ± SEM.

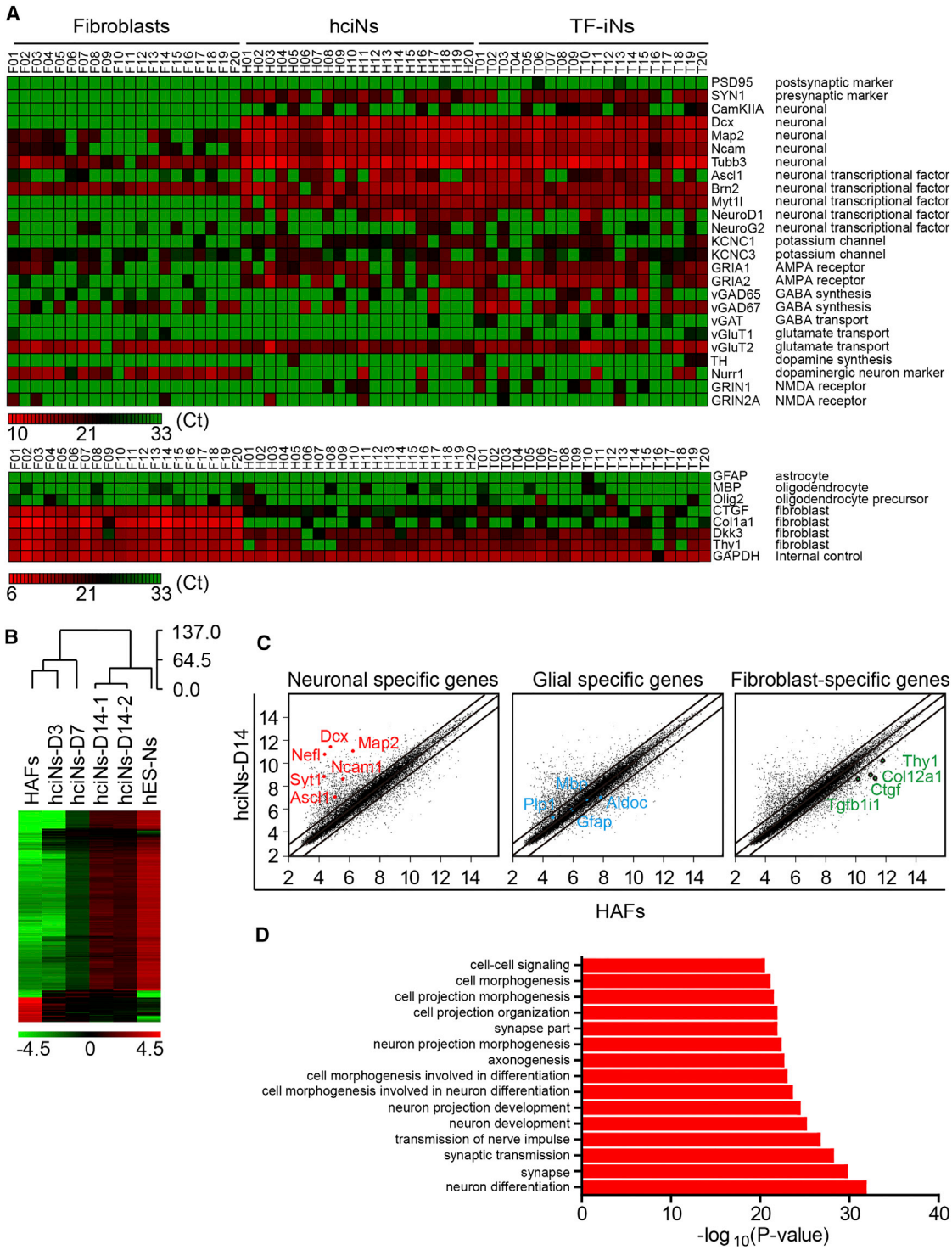

Figure 3. hciN Cells' Expression Profiling during Chemical Reprogramming

(A) Gene expression of single cells as ascertained by the Fluidigm Biomark platform. hciNs were collected at day 21 after chemical treatment and purified using FACS. Expression levels (expressed as Ct values) are color-coded as shown at the bottom (color scales). H1-20 indicates hciN samples, F1-20 indicates fibroblast samples, and T1-20 represents TF-iNs samples.

(B) Heatmap and hierarchical clustering of genes with significance from microarray data. Samples of HAFs, hciNs (day 3, day 7, day 14-1, and day 14-2) and control neurons (derived from an hES cell line) were compared. Shown are probes selected on fold change ≥10 in hciNs (day 14) versus HAFs. In the heatmap, green indicates decreased expression whereas red means increased expression as compared to that in HAFs.

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non-neuronal genes at a single-cell level using the Fluidigm Biomark platform. TF-iNs were prepared as reported previously (Ladewig et al., 2012) and were used as a positive control (Figure S3A). hciN and TF-iN cell cultures at day 21 were purified using fluorescence-activated cell sorting (FACS, Figures S3B and S3C). The heatmap analysis showed that hciNs consistently expressed major neuronal genes, channel genes, and synaptic genes, but not glial-specific genes or fibroblast-specific genes (Figure 3A). The expression pattern of hciNs was similar to that of TF-iNs but distinguished from that of initial fibroblasts.

We further compared the global gene expression patterns of human embryonic stem cell (hESC)-derived neurons (control neurons), HAFs, pre-hciNs (VCRFSGY treated HAFs at days 3 and 7), and hciNs (induced with VCRFSGY for 14 days) by microarray analysis. The global genome heatmap with hierarchical cluster analysis showed a significant difference between hciNs and their parental HAFs and a high similarity between hciNs and control neurons (Figure 3B). It is of note that many representative neuronal-specific genes including Ncam1, Dcx, Map2, Ascl1, and Nefl were strongly upregulated (Figure 3C, left panel, and Figure S3D), whereas glial genes were almost unaltered in hciNs (Figure 3C, middle panel). Interestingly, the upregulation of some neuronal genes was also observed in VCRFSGY-treated HAFs as early as on days 3 and 7 (Figure S3E). Moreover, fibroblast-specific genes including Col12A1, Tgfb1i1, Thy1, and Ctgf were dramatically downregulated during neuronal conversion (Figure 3C, right panel, and Figure S3F) (Kim et al., 2010). Genes that show a more than 2-fold alteration were subjected to further gene ontology (GO) function enrichment analysis. We found that the shared expression core sets of hciNs from different batches and control neurons were mainly related to neuron differentiation, synapse, and synaptic transmission (Figure 3D). On the other hand, the expression of genes related to the extracellular region, the extracellular matrix, and the regulation of cell motion were significantly downregulated GO items in hciNs versus those in HAFs (Figure S3G).

We further detected the effect of each molecule on downstream-molecule events in our induction protocol, as shown in Figure S4A. We found that some small molecules alone could elevate neuronal gene expression and downregulate fibroblast-specific expression of some genes such as Col1a1, Dkk3, Thy1, and Ctgf. Further, we removed each small molecule from our chemical cocktails, and we found that removing CHIR99021 or SP600125 from the chemical cocktails led to no induction of neuronal gene expression, but the downregulation of fibroblast gene expression can still be observed, indicating that CHIR99021 and SP600125 are critical for the upregulation of neuronal gene expression (Figure S4B). These results further support our hypothesis that each molecule of the cocktail should operate coordinately for a successful neuronal conversion (Figure S3H).

Generation of hciNs from FAD Patient Fibroblasts

To test whether our chemical induction protocol can be applied to generate hciNs for modeling of neurological diseases, we induced neuronal conversion of human skin fibroblasts derived from patients with familial Alzheimer's disease (FAD) carrying mutations in APP (V717I) or presenilin 1 (I167del or A434T or S169del) (Guo et al., 2010; Jiao et al., 2014). The hciNs derived from FAD fibroblasts displayed similar neuronal characteristics to those of hciNs derived from normal fibroblasts (Figure 4A). The induction efficiency of hciNs from these fibroblasts was comparable to that of normal fibroblasts (Figure S1E).

Muratore et al. (2014) show that iPSC-derived neurons harboring the APPV717I mutation have elevated Aβ42 and Aβ38 levels and Aβ42/40 ratio, but the Aβ40 levels are not significantly increased as compared with those of normal control cells. Higher total Tau levels and p-Tau levels are detected, but only p-S262 (12E8) versus total Tau levels at a very late stage of differentiation (day 100) are upregulated. Terwel et al. (2008) demonstrated that GSK-3 activities in APPV717I mouse brains were higher than those of age-matched wild-type control brains, but GSK-3 activities in human neurons with the mutation were still difficult to predict without further exploration. We measured the levels of extracellular Aβ40 and the Aβ42 level of the hciNs derived from these FAD fibroblasts and compared those with those of TF-iNs. Similar to previous reports, the extracellular Aβ42 level and the Aβ42/Aβ40 ratio were increased in hciNs derived from FAD109 (APPV717I) fibroblasts compared to those of hciNs derived from healthy persons (Figures 4B–4D). In FAD hciNs from another patient, in which newly reported mutations in presenilin 1 (I167del or S169del) were carried (Jiao et al., 2014), the extracellular Aβ42 levels were also elevated (Figure 4C). However, we did not detect obvious alternation of Aβ levels in FAD132 (PS1 A434T) fibroblasts. The Aβ level in fibroblasts was much lower than that in iN cells. We found that the levels of phosphorylated Tau and total Tau in original fibroblasts were very low (Figure S1I). However, the levels of phosphorylated Tau and total Tau of FAD109 hciNs were higher than that of controls, whereas the other three FAD hciNs (FAD131, FAD132, and FADA16) carrying the presenilin 1 mutation did not show obvious change in p-Tau (Thr 231) and total Tau levels (Figure 4E). Surprisingly, the amount of active GSK-3β in all four FAD hciNs and TF-iNs was similar to that of normal hciNs and TF-iNs (Figure 4E). These results indicate that the chemical induction protocol might be a feasible way to generate patient-specific neuronal cells that might be useful for neurological disease modeling, related mechanism studying, and drug screening.

DISCUSSION

This is a proof-of-principle study demonstrating that the direct lineage-specific conversion of neuronal cells could be achieved by small molecules without the introduction of ectopic genes. Our previous study showed that a chemical cocktail, VCR, could induce NPCs from mouse embryonic fibroblasts and human urinary cells under a hypoxic condition. But we found the

(C) Pair-wise gene expression comparisons show that most neuronal markers (left panel) are increased, whereas glial markers (middle panel) are not activated and some fibroblast-specific genes (right panel) are silenced. Black lines indicate 2-fold changes between the sample pairs.
(D) Gene ontology (GO) analysis of the overlapping genes whose expression changes are ≥2-fold identified in hciNs (day 14-1 and day 14-2) and control neurons compared to that of HAFs. See also Figures S3 and S4.

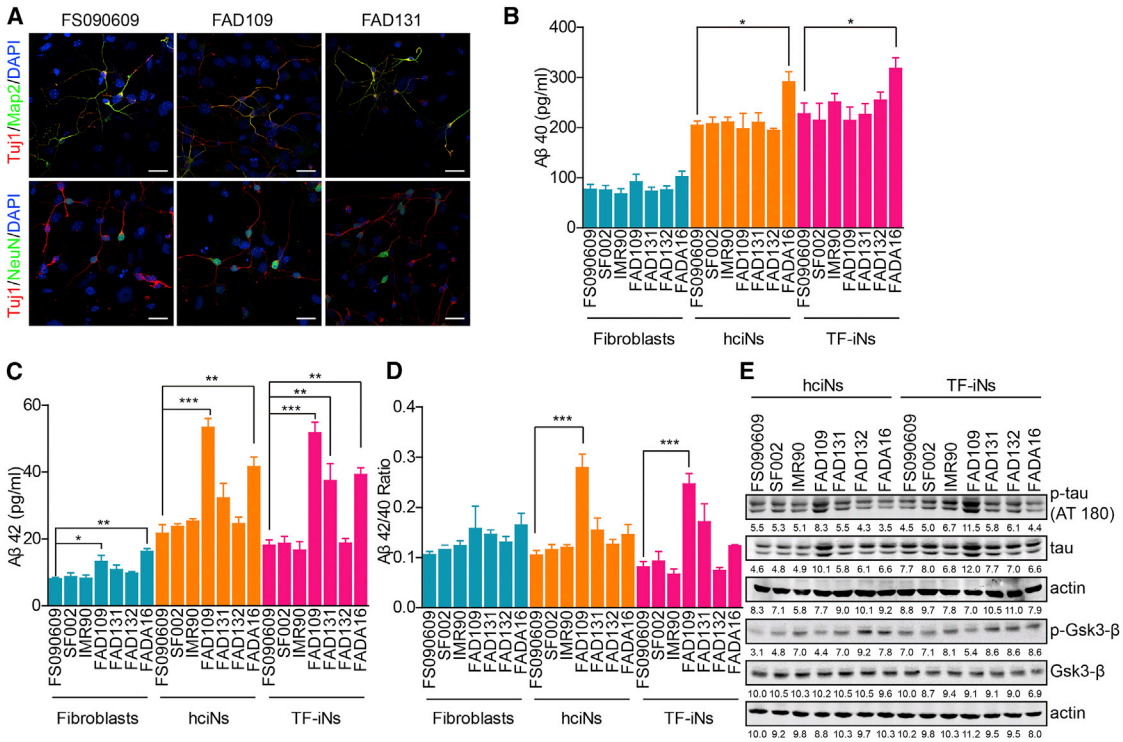

Figure 4. Generation of hciNs from FAD Fibroblasts

(A) Characterization of hciNs generated from FAD fibroblasts (FAD109 and FAD131).

(B–D) Aβ levels in FAD hciN and TF-iN cell cultures compared to those of WT hciN cultures.

(E) The level of phosphorylated Tau and total Tau of FAD109 hciNs and TF-iN cells.

Data from three independent experiments are shown as means ± SEM.

Scale bars, 20 μm. *p < 0.05; **p < 0.01; ***p < 0.001; versus control hciNs.

VCRFSGY-treated fibroblasts quickly exited the cell cycle as early as day 3 (Figure S1H), suggesting that the chemical induction of neuronal cells from human fibroblasts might bypass a proliferative intermediate.

The conversion process induced by our chemical strategy is accompanied by the downregulation of fibroblast-specific genes and the increased expression of endogenous neuronal transcriptional factors. It is consistent with the previous studies that the forced expression of certain neuronal TFs could convert mouse or human somatic cells into neuronal cells (Caiazzo et al., 2011; Pang et al., 2011). Consistently, our microarray analysis showed that the gene expression patterns of related signaling pathways in hciNs were more similar to those of control neurons than those of HAFs. Thus, by coordinating multiple signaling pathways, these seven small molecules modulate neuronal TF gene expression and thereby promote the neuronal cell transition.

A proper fine-tuning of multiple signaling pathways is critical for a successful neuronal conversion from human fibroblasts by the chemical induction protocol. VPA induces cells into a more amenable state for cell-fate transition through epigenetic modification (Huangfu et al., 2008). Inhibition of TGF-β and GSK-3 improves the neuronal conversion of stably transduced human fibroblasts by Ascl1 and Ngn2 (Ladewig et al., 2012). Forskolin enables Ngn2 to convert human fibroblasts into cholinergic neurons (Liu et al., 2013). SP600125 could facilitate the neural reprogramming of AHDF transduced with OCT4 alone (Zhu et al., 2014). PKC inhibition improves naive human pluripotent stem cells and induces neuritogenesis of Neuro-2a cells (Gafni et al., 2013; Miñana et al., 1990). Y-27632 assists in the maintenance of pluripotent stem cells and neuron survival (Lamas et al., 2014; Xi et al., 2013). Therefore, the chemical cocktail VCRFSGY erases fibroblast-specific gene expression of the initial cells, specifically upregulating neuronal gene expression and facilitating the neuronal conversion of HAFs (Figure S4). However, the precise regulatory mechanisms of this chemical cocktail remain to be investigated.

Most of the hciNs are glutamatergic neurons in our induction system. Previous reports showed that different combinations of small molecules and TFs may induce certain subtypes of neurons (Caiazzo et al., 2011; Liu et al., 2013). Thus, it should be possible to generate different neuronal subtypes with similar chemical approaches but with slightly modified chemical cocktails. Moreover, it also needs to be explored in the future whether functional neurons could be induced by chemical cocktails in vivo against neurological diseases or injury. Together, our study presents another strategy for the generation of patient-specific neuronal cells, thus providing desirable neuron resourses for personalized disease modeling or even potential cell replacement therapy of neurological disorders.

EXPERIMENTAL PROCEDURES

Generation of hciNs

Initial fibroblasts were seeded onto gelatin-coated culture plates (10,000-15,000 cells/well in 24-well plates) and cultured in fibroblast medium for 1 day. Then, the cells were transferred into neuronal induction medium (DMEM/F12: Neurobasal [1:1] with 0.5% N-2, 1% B-27, 100 μM cAMP, and 20 ng/ml bFGF) with indicated chemicals. Medium containing indicated chemicals was changed every 4 days. After 8 days, cells were switched to neuronal maturation medium (DMEM/F12: Neurobasal [1:1] with 0.5% N-2, 1% B-27, 100 μM cAMP, 20 ng/ml bFGF, 20 ng/ml BDNF, 20 ng/ml GDNF, and 20 ng/ml NT3) with indicated chemicals. After 2 weeks, iN cells were cultured in neuronal maturation medium without bFGF and chemicals. Neuronal maturation medium was half-changed every other day. The concentrations of chemicals used were as follows: VPA, 0.5 mM; CHIR99021, 3 μM; Repsox, 1 μM; Forskolin, 10 μM; SP600125, 10 μM; GO6983, 5 μM; Y-27632, 5 μM; Dorsomorphin, 1 μM. Stepwise induction protocols as well as detailed information about the medium used are provided in Supplemental Experimental Procedures. Information about all chemicals applied in this study are in Table S1. The detailed information of human fibroblasts used is listed in Table S2. Human adult fibroblasts were derived from human foreskin or skin biopsies of healthy and patient individuals with approval for collection and use of human samples by institutional ethical committees.

Statistical Analysis

All quantified data were statistically analyzed and presented as means ± SEM. One-way ANOVA and Bonferroni's multiple comparison test were used to calculate statistical significance with p values, unless otherwise stated.

ACCESSION NUMBERS

The accession number for the microarray data reported in this paper is GEO: GSE69480.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2015.07.006.

AUTHOR CONTRIBUTIONS

G.P. supervised and controlled research conception and design and interpreted data. J.Z. controlled research conception, designed the experiments, interpreted data, and drafted and revised the manuscript critically for intellectual content. Y.Y. designed the electrophysiological analysis experiments, interpreted the correlated data, and revised the manuscript. W.H., B.Q., and W.G. conducted the overall experiments and analyzed the data. W.H. and B.Q. collected and analyzed data, organized figures, and drafted the manuscript. Q.W., L.G., and W.L. contributed to the hciN induction and iPSC differentiation. Y.H. and G.X. carried out single-cell PCR and heatmap analysis. M.W. contributed to transplantation experiments. H.Z., Y.J., B.T., and L.S. provided human fibroblast lines and commented on the manuscript. All authors contributed to the figures and the manuscript preparation and approved the version that was submitted.

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