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Restoration of Progranulin Expression Rescues Cortical Neuron Generation in an Induced Pluripotent Stem Cell Model of Frontotemporal Dementia

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SUMMARY

To understand how haploinsufficiency of progranulin (PGRN) causes frontotemporal dementia (FTD), we created induced pluripotent stem cells (iPSCs) from patients carrying the *GRN*$^{IVS1+5G > C}$ mutation (FTD-iPSCs). FTD-iPSCs were fated to cortical neurons, the cells most affected in FTD. Although generation of neuroprogenitors was unaffected, their further differentiation into CTIP2-, FOXP2-, or TBR1-TUJ1 double-positive cortical neurons, but not motorneurons, was significantly decreased in FTD-neural progeny. Zinc finger nuclease-mediated introduction of *GRN* cDNA into the *AAVS1* locus corrected defects in cortical neurogenesis, demonstrating that PGRN haploinsufficiency causes inefficient cortical neuron generation. RNA sequencing analysis confirmed reversal of the altered gene expression profile following genetic correction. We identified the Wnt signaling pathway as one of the top defective pathways in FTD-iPSC-derived neurons, which was reversed following genetic correction. Differentiation of FTD-iPSCs in the presence of a WNT inhibitor mitigated defective corticogenesis. Therefore, we demonstrate that PGRN haploinsufficiency hampers corticogenesis in vitro.

INTRODUCTION

Frontotemporal dementia (FTD) accounts for ~50% of dementia cases before the age of 60. Up to 40% of FTD patients have a familial history (Goldman et al., 2005; van Swieten and Heutink, 2008) due to mutations in the microtubule-associated protein tau gene (*MAPT*), progranulin gene (*GRN*), or *C9orf72* gene (Baker et al., 2006; Cruts et al., 2006; DeJesus-Hernandez et al., 2011; Hutton et al., 1998; Renton et al., 2011). The majority of FTD-causing mutations in *GRN* are predicted to result in functional null alleles, causing haploinsufficiency. Progranulin (PGRN) has neurotrophic function in vitro and in vivo. Although PGRN$^{-/-}$ mice are viable, they do not recapitulate all the features of FTD (Kayasuga et al., 2007).

Human somatic cell reprogramming to a pluripotent state (induced pluripotent stem cells; iPSCs)(Takahashi et al., 2007a) can create human disease models in vitro using patient-derived iPSCs (Kim, 2014), including neurodegenerative diseases (Qiang et al., 2013) and, specifically, FTD (Almeida et al., 2012). Unlike in the published FTD-iPSC model that differentiated iPSCs to a mixture of neuronal cells, we evaluated cortical neuron development from FTD-patient-derived iPSCs, as FTD is characterized by selective neurodegeneration of the frontal and/or temporal cortex (Neary et al., 2005). We demonstrate that FTD-iPSCs carrying a *GRN*$^{IVS1+5G > C}$ mutation differ in their ability to generate cortical neurons from control lines (iPSCs and human embryonic stem cells; hESCs) and that genetic correction restores this differentiation defect.

RESULTS

FTD-iPSCs Differentiation into Neuroprogenitors Is Normal

To study the effect of PGRN haploinsufficiency in human neurogenesis, iPSC lines were generated from three

different patients carrying the $GRN^{IVS1+5G > C}$ mutation (Figure S1A available online) as previously described (Takahashi et al., 2007b). The human embryonic stem cell line, H9 (H9-ESC), and iPSCs from normal donor fibroblasts (CTRL-iPSCs) were used as control lines (Figures S1B–S1E). Transcript and protein levels of PGRN in FTD-iPSC lines were reduced, approximately 30% and 50%, respectively, compared to H9-hESCs and CTRL-iPSCs (Figures S1F and S1G). We next induced cortical differentiation (Espuny-Camacho et al., 2013), which induced an increase in transcripts for the neuroprogenitor genes *SOX1*, *PAX6*, and *FABP7* (*BLBP*), with concomitant decrease in *OCT4* expression in day (d)24 FTD-iPSCs as well as CTRL-iPSC and H9-hESC progeny (Figure S2A). Immunostaining confirmed that d24 neuroprogenitors did not express OCT4. Nearly 100% of the progeny of all lines were positive for the neuroectoderm-specific NESTIN marker, with a PAX6-positive dorsal fate, and stained positive for BLBP and OTX1-2 (Figure S2B). Thus, neuroprogenitor formation from FTD-iPSCs appeared normal.

Inefficient Cortical Neuron Formation from FTD-iPSCs

We next allowed the neuroprogenitors to mature into cortical neurons. *GRN* mRNA levels in FTD cells during differentiation were approximately 50% compared to control lines (Figure 1A). D40 progeny from CTRL- and FTD-iPSCs contained functional neurons based on whole-cell current-clamp analysis. FTD-iPSC neurons consistently fired action potentials in response to depolarizing current injections, similar to neurons from control cell lines (Figures S2CI–S2CII). Whole-cell voltage-clamp recordings revealed time- and voltage-dependent currents during depolarizing voltage steps, consistent with functional voltage-gated Na^+ and K^+ channels (Figure S2CIII). The cortical neurotransmitter GABA induced transmembrane currents in FTD-iPSC-derived neurons, exhibiting the typical features of ionotropic $GABA_A$ receptors (Figure S2CIV). We also observed spontaneous action potential firing in FTD-iPSC neurons (Figure S2CV). Thus, FTD-iPSC neuroprogenitors were able to differentiate into functional, excitable neurons.

Between d24 and d40 of differentiation, transcript levels of *REELIN*, *CTIP2*, *FOXG1*, *FOXP2*, and *TBR1* progressively increased in neural progeny from FTD- and CTRL-iPSC lines. However, on d40, *CTIP2* and *FOXG1* mRNA levels were significantly lower in FTD-iPSC than in CTRL-iPSC progeny (Figures 1B and S2D). Also, mature TUJ1-positive neurons coexpressed the cortical markers TBR1, FOXP2, and CTIP2. However, compared to CTRL-iPSC and H9-hESC progeny, only a small fraction of FTD-iPSC progeny was positive for TUJ1 (CTRL-iPSCs, 20.7% ± 3.1%; FTD-iPSCs, 4.0% ± 0.69%) (Figures 1C and 1D). In both CTRL-iPSC and FTD-iPSC progeny, a proportion of undifferentiated NESTIN-positive neuroprogenitors persisted till d40 (Figure 1E). Thus, using a cortical neuron differentiation protocol, we demonstrate significantly decreased corticogenesis from FTD-iPSCs.

To test if the neurogenesis defect was specific for cortical neuron generation, FTD-iPSCs and hESCs were differentiated to motor neurons (Hu and Zhang, 2009). Immunostaining for the mature motor neuron markers *HB9* and *ISLET1* (Figure 1F) demonstrated that FTD3-iPSCs generated motor neurons in vitro. Thus, in contrast to what we observed during cortical neuron differentiation, motor neuron generation from FTD-iPSCs was not affected.

We stained cortical neuron progeny for activated caspase-3 but found no significant differences in the number of apoptotic cells between FTD and CTRL lines (Figure S2E). As *GRN* mutations in humans lead to accumulation of TDP-43-positive inclusions, we performed TDP-43 staining, which did not identify TDP-43 aggregates, and TDP-43 displayed a nuclear staining in all cells (Figure S2F).

Genetic Correction of FTD-iPSCs Restores PGRN Levels

To study the relationship between PGRN haploinsufficiency and the phenotype observed, we introduced *GRN* cDNA by homologous recombination with zinc finger nucleases (ZFNs) in the *AAVS1* locus of FTD3#6-iPSCs (Figure 2A). To identify correct targeting and absence of random integrations, we performed genotyping based on PCR and Southern blot analysis (Figures 2B and 2C). One correctly homozygously targeted clone (#9) derived from the FTD3#6 line (hereinafter referred to as FTD3#6-PGRN) was chosen for complete characterization. As an additional control, we recombined the *GRN* cDNA into the *AAVS1* locus of H9-hESCs (H9-hESC-PGRN) (Figure S3A).

GRN transcript levels in FTD3#6-PGRN and H9-hESC-PGRN cells were not significantly different from that in H9-hESCs (Figure 2D). FTD3#6-PGRN cells expressed the pluripotency markers at levels comparable to that of H9-hESCs (Figures 2E and 2F) and formed teratomas (Figure 2G). Genome integrity of FTD3#6-PGRN, assessed by array comparative genomic hybridization, revealed no significant acquired genetic abnormalities after gene editing, compared to the original line.

Genetic Correction of FTD-iPSCs Restores Cortical Neuron Formation

We differentiated FTD3#6-iPSC, FTD3#6-PGRN, H9-hESC, and H9-hESC-PGRN lines to cortical neurons. Patch clamp recording confirmed the functional maturity of FTD3#6-PGRN and H9-hESC-PGRN neurons (data not shown). Differentiation toward neuroprogenitors until d24 was similar for the FTD3#6, FTD3#6-PGRN, H9-ESC, and H9-PGRN lines, as shown by immunostaining for neuroprogenitor markers (Figure S3B) and quantitative RT-PCR (Figure S3C).