Huntington’s disease (HD) is a severe inherited neurological disorder caused by a CAG repeat expansion in the huntingtin gene (HTT), leading to the accumulation of mutant huntingtin with polyglutamine repeats. Despite its severity, there is no cure for this debilitating disease. HTT lowering strategies, including antisense oligonucleotides (ASO) showed promising results very recently. Attempts to develop stem cell-based therapeutics have shown efficacy in preclinical HD models. Using an HD patient’s autologous cells, which have genetic defects, may hamper therapeutic efficacy due to mutant HTT. Pretreating these cells to reduce mutant HTT expression and transcription may improve the transplanted cells’ therapeutic efficacy. To investigate this, we targeted the SUPT4H1 gene that selectively supports the transcription of long trinucleotide repeats. Transplanting SUPT4H1-edited HD-induced pluripotent stem cell-derived neural precursor cells (iPSC-NPCs) into the YAC128 HD transgenic mouse model improved motor function compared to unedited HD iPSC-NPCs. Immunohistochemical analysis revealed reduced mutant HTT expression without compensating wild-type HTT expression. Further, SUPT4H1 editing increased neuronal and decreased reactive astrocyte differentiation in HD iPSC-NPCs compared to the unedited HD iPSC-NPCs. This suggests that ex vivo editing of SUPT4H1 can reduce mutant HTT expression and provide a therapeutic gene editing strategy for autologous stem cell transplantation in HD.

**INTRODUCTION**

Huntington’s disease (HD) is a progressive neurodegenerative disorder caused by the abnormal expansion of CAG repeats (>40) in the huntingtin gene (HTT) exon 1. The expanded CAG trinucleotides encode a polyglutamine stretch that can accumulate into neurotoxic proteinaceous cytoplasmic and intranuclear aggregates. HD patients display progressive brain atrophy with increases in lateral ventricle size. These changes are followed by cognitive deficits, motor control impairment, and psychological symptoms. There are currently no disease-modifying therapies for HD, consequently resulting in a significant unmet medical need. Effective neurorestorative or neurodegenerative strategies based on human stem cells are potential therapeutics. Previous studies have shown that transplanting human embryo-derived neural stem cells or mouse-induced pluripotent stem cell (iPSC)-derived neural stem cells into HD transgenic (TG) mice promoted neuronal or astrocytic differentiation with functional benefits. Patient-specific iPSCs are a potentially renewable source of autologous cells for stem cell therapy that will not induce immune rejection. However, iPSCs derived from patients with genetic diseases carry the mutation. Therefore, gene modification before transplantation may increase the therapeutic potential of autologous iPSC therapy.

HTT is an evident candidate gene to target in HD because its genetic knockout or knockdown using CRISPR/Cas9, siRNA, or antisense oligonucleotides (ASO) rescues neurotoxicity. HTT lowering strategies, including ASO were considered as a promising approach. However, these methods will likely knockdown the mutant HTT (mHTT) and normal HTT, thereby ablating the physiological role of normal HTT. Normal HTT is thought to be required for embryogenesis, since deletion of the HD gene in mice results in early embryonic lethality. Allele-specific targeting of mHTT via a single-nucleotide polymorphism (SNP) has been suggested. However, not all HD patients harbor SNPs to allow allele-specific knockdown of mHTT. Therefore, identifying a universal genetic manipulation to reduce mHTT levels without decreasing wild-type HTT levels would be useful for all HD patients.

SPT4 is a transcription elongation factor that is encoded by SUPT4H1 and regulates RNA polymerase II processivity. One case report indicated that SPT4 is required for specific expression of the trinucleotide and hexanucleotide repeat expansions in HD. Therapeutically targeting SPT4 to specifically reduce the mutant products derived from repeat expansion mutations was investigated. In the case of HD, SUPT4H1 knockdown reduced mHTT expression in Q81 or Q111 neuronal cells without compensatory increases in wild-type HTT levels. It also decreased mHTT aggregation and toxicity by inhibiting repeated trinucleotides. Furthermore, suppressing the mouse homologue of SUPT4H1, Supt4a, by administering a SUPT4H1-targeting antisense oligonucleotide in the Q175 mouse HD model decreased mHTT mRNA and protein expression. Heterozygous deletion of the Supt4a gene in R6/2 HD mice also reduced mHTT mRNA and protein expression.

For these reasons, we investigated whether targeted ablation of SUPT4H1 (SUPT4H1-edited) in iPSC-NPCs derived from human HD patients increases the therapeutic possibility of autologous stem cell therapy iPSC-NPCs using a mouse HD model. The transplanted SUPT4H1-edited HD iPSC-NPCs were better engrafted than the unedited HD iPSC-NPCs at 12 w post-transplantation. Moreover, differentiated neurons and astrocyte were improved similarly.

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The restoration of healthy neurons and astrocytes consequently increased the striatal intensity of NeuN-positive cells and recovered motor and cognitive functions. These results strongly suggest the potential to develop autologous cell therapy in HD patients through SUPT4H1-edited iPSCs that suppress the HTT mutation.

**RESULTS**

*SUPT4H1*-edited Q57 HD iPSC-NPCs recovered developmental defects in neurons and astrocytes

To investigate the potential therapeutic effects of *SUPT4H1* knockdown in iPSC-NPCs, we first established NPC differentiation from iPSCs derived from an HD patient with 57 CAG repeats...
(Cell line ID: ND41656, RUCDR cell line service). We used the in vitro embryoid body-based SFE8q method to differentiate these iPSCs into NPCs.

We screened and identified the optimal region of SUPT4H1 exon 1 for sgRNA targeting in NPCs using CRISPR/Cas9 (Supplementary Fig. 1a). The sgRNA-targeting noncoding region of the AAVS1 locus served as a control. Targeted deep sequencing analyses revealed efficient gene editing in the AAVS1 and SUPT4H1 loci reaching up to mean of 93.6 and 95.7% total indel efficiencies (mean of 29.5% in-frame, 64.1% out-of-frame mutation for Non-HD, and 27.9% in-frame, 67.9% out-of-frame mutation for Q57 HD iPSC-NPCs) in Non-HD and Q57 HD iPSC-NPCs subsequently (Supplementary Fig. 1a and Supplementary Table 1). To confirm SUPT4H1 mRNA knockdown following gene editing, we performed qRT-PCR analysis, which revealed robust knockdown of SUPT4H1 in both Non-HD and Q57 HD iPSC-NPCs with SUPT4H1 gene editing (Supplementary Fig. 1b). For NPC characterization, we performed immunostaining using NPC-positive markers (anti-SOX2, NESTIN, MUSASHI) and NPC-negative marker (anti-MAP2). No changes in NPC characteristics were observed after SUPT4H1 gene editing, judged by immunostaining using NPC markers (Supplementary Fig. 1c). IXMC analysis demonstrated that all treated groups showed over 90% positive staining for NPC markers (Supplementary Fig. 1d). Notably, SUPT4H1 gene editing did not produce changes in cell viability (Supplementary Fig. 1e). Furthermore, no karyotypic changes were detected after SUPT4H1 gene editing (Supplementary Fig. 2). To confirm SPT4 protein knockdown following gene editing, we performed immunostaining using anti-SPT4 and the NPC marker, anti-NESTIN. SPT4 expression in Q57 HD iPSC-NPCs increased compared to control NPCs (AAVS1- or SUPT4H1- edited control iPSC-NPCs). Conversely, SUPT4H1-edited Q57 HD iPSC-NPCs showed reduced SPT4 expression. Western blot analysis showed the same results (Supplementary Fig. 1f).

Having achieved highly efficient SUPT4H1 gene editing in HD iPSC-NPCs, we proceeded to spontaneously differentiate them into neural cells over 42 d (Fig. 1a). Before differentiation, we showed NPC characterization using immunofluorescence of NESTIN and SOX2 (Fig. 1b). After differentiation, we performed immunofluorescence and western blotting and found that SUPT4H1 gene editing reduced EM48 expression, a marker for mHTT protein, compared to AAV51 gene editing in HD cells (Fig. 1c, d). To correlate with our western blot data, we performed RT-qPCR and found reduced expression of HTT in SUPT4H1 gene-edited Q57 HD NPCs (Supplementary Fig. 3).

Interestingly, we observed that AAV51-edited Q57 HD iPSC-derived MAP2- or GFAP-positive cells exhibited different morphology compared to the control or SUPT4H1-edited group (Fig. 1e, h). MAP2-positive cell maturation was first identified by western blotting (Fig. 1f). Low molecular weight (LMW) MAP2 isoforms are the immature forms that result from the exclusion of the sequence encoded by exons E7-E9. They are downregulated after the early stages of neuronal development when E7-E9 exons-including high molecular weight (HMW) isoforms are favored. Although HMW-MAP2 expression was observed in the AAV51-edited group, the SUPT4H1-edited group showed significantly increased HMW-MAP2 expression compared to the AAV51-edited group.

We next analyzed MAP2-positive cell morphology using MetaX-pose for more reliable evidence. We found that the SUPT4H1-edited Q57 HD iPSC-derived MAP2-positive cells exhibited significantly more total outgrowths and increased maximum outgrowth length compared to the AAV51-edited Q57 HD iPSC-derived MAP2-positive cells (Fig. 1g). Furthermore, western blotting revealed expression of GFAP and the potassium channel Kir4.1, functional astrocyte markers. The SUPT4H1-edited group showed increased GFAP and Kir4.1 expression compared to the AAV51-edited group (Fig. 1i). To identify differentiated neurons and astrocytes, we examined mRNA expression of GABA and DARPP-32 for mature neurons, and N-cadherin (CDH2) and S100β for radial glial cell and mature astrocytes, respectively. The SUPT4H1-edited group showed significant increases in GABA and DARPP-32 mRNA expression, compared to the AAV51-edited group (Supplementary Fig. 4). In addition, the SUPT4H1-edited groups showed significant increases in CDH2 and S100β mRNA compared to the AAV51-edited group (Fig. 1j). Therefore, the SUPT4H1-edited Q57 HD iPSC-NPCs with reduced mHTT expression underwent normal neuronal differentiation similar to the control iPSC-derived NPCs.

Although knocking down HTT using gene editing reduces mHTT, it also reduces normal wild-type HTT levels, which play a role in neuronal development. To investigate this, we performed HTT gene editing using sgRNAs targeting the start codon of the HTT gene (Supplementary Fig. 5a). We achieved a similar gene-editing efficiency between AAV51- and SUPT4H1-targeting sgRNAs. The HTT-edited Q57 HD iPSC-NPCs showed an incomplete differentiation compared to the SUPT4H1-edited group. Regarding MAP2-positive cell morphology, the number of total neurites and maximum neurite lengths were significantly reduced in the HTT-edited group compared to the control group or AAV51- or SUPT4H1-edited groups (Supplementary Fig. 5b, c). Interestingly, the MAP2-positive cells of the HTT-edited group did not exhibit increased maturation compared to the AAV51-edited group. As HTT-edited Q57 HD iPSC-NPCs showed defective neuronal development, we chose to focus on SUPT4H1-edited Q57 HD iPSC-NPCs.

Functional improvement and neuroprotective effects of SUPT4H1-edited Q57 HD iPSC-NPC transplantation in YAC128 HD mice

To compare the therapeutic potential of SUPT4H1-edited and unedited Q57 HD iPSC-NPCs, we transplanted these cells into YAC128 mice harboring mHTT with 128 CAG repeats. We investigated whether SUPT4H1-edited HD iPSC-NPCs rescued motor deficits on the rotarod test in the YAC128 mice at 3 m following transplantation (Fig. 2a). We found that mice injected with unedited...
YAC128 mice transplanted with motor control and grip strength. Therefore, we further observed that Q57 HD iPSC-NPCs (neurons was quantified using the NeuN antibody and the intensity of NeuN-positive sections from neuronal survival in the brain of YAC128 mice. Three striatal sections from five mice per group were stained with neuronal nuclei (NeuN) antibody and the intensity of NeuN-positive sections from those transplanted with control iPSC-NPCs (Supplementary Fig. 6).

We next investigated whether transplanted SUPT4H1-edited Q57 HD iPSC-NPCs (Q57/SUPT4H1) provide a protective effect on neuronal survival in the brain of YAC128 mice. Three striatal sections from five mice per group were stained with neuronal nuclei (NeuN) antibody and the intensity of NeuN-positive neurons was quantified using ImageJ software 3 m after media or Q57 HD iPSC-NPC transplantation. The YAC128 mice exhibited significantly reduced NeuN-positive density compared to WT. However, the YAC128 mice transplanted with SUPT4H1-edited Q57 HD iPSC-NPCs showed significantly increased NeuN-positive density (Fig. 2c, d).

**SUPT4H1-edited Q57 HD iPSC-NPCs engrailed better than unedited cells in YAC128 mice**

As SUPT4H1-edited Q57 HD iPSC-NPCs showed therapeutic efficacy compared to unedited cells, we next investigated the fate of these cells following transplantation. Immunofluorescence analysis for human-specific NESTIN (hNESTIN) confirmed the absence of SPT4 expression in transplanted SUPT4H1-edited Q57 HD iPSC-NPCs, indicative of transplanted human cells. However, notable SPT4 expression was found in nodified cells 1 week following transplantation (Fig. 3a). We counted the pixel numbers of merged hNESTIN and SPT4 in the immunofluorescence images using colocalization analysis of Zen black software (Fig. 3b).

Human nuclei (hNu)-positive cells were observed in the striatum of SUPT4H1-edited and unedited cell transplantation groups 3 m after transplantation (Fig. 3c), indicating that the transplanted iPSC-NPCs survived in the striatum of YAC128 mice. However, the total number of hNu-positive cells in the striatum of the unedited cell transplantation group was substantially less than in the SUPT4H1-edited cell transplantation group (54,280 ± 823 or 128,676 ± 4356 in the striatum, corresponding to approximately 13.5 or 32.2% of the total number of transplantected cells; i.e., 4 × 10^6) (Fig. 3d). More GFAP-positive cells, co-localized with hNu-positive cells, were observed in YAC128 mice transplanted with the unedited Q57 HD iPSC-NPCs (Fig. 5a) compared to hMAP2-positive cells (Fig. 4c). In contrast, more hMAP2-positive neurons were detected in YAC128 mice transplanted with the SUPT4H1-edited Q57 HD iPSC-NPCs (Supplementary Fig. 7).

**Neurons differentiated from SUPT4H1-edited Q57 HD iPSC-NPCs showed improved morphology in YAC128 mice**

As more cells survived, we further investigated whether transplanted SUPT4H1-edited Q57 HD iPSC-NPCs were likely to show improved differentiation, as shown by the increased hMAP2-positive neurons (Fig. 5a) compared to hMAP2-positive cells (Fig. 4c). In contrast, more hMAP2-positive neurons were detected in YAC128 mice transplanted with the SUPT4H1-edited Q57 HD iPSC-NPCs (Supplementary Fig. 7).
differentiate into medium spiny neurons (MSNs) or show other morphology. For this purpose, we double stained slices with hNu and DARPP-32 antibodies (Fig. 4a). The merged hNu/DARPP-32-positive cells in YAC128 mice transplanted with unedited Q57 HD iPSC-NPCs exhibited an unhealthy morphology, such as the defective morphology of DARPP-32-positive neurons, compared to the SUPT4H1-edited group (Fig. 4a). The cells in the unedited group had EM48 aggregates that were not present in the SUPT4H1-edited group (Fig. 4b). To identify the neuronal maturation morphology, like in the in vitro study, we performed immunofluorescence and MetaXpress software analysis using a human-specific MAP2 (hMAP2) antibody. Interestingly, we found that hMAP2-positive cells in YAC128 mice transplanted with unedited Q57 HD iPSC-NPCs had shorter dendrites and no straight morphology compared to the SUPT4H1-edited group (Fig. 4c). The MetaXpress software analysis revealed that the outgrowth numbers and maximum length of hMAP2-positive cells in the SUPT4H1-edited group were significantly increased compared to the unedited group (Fig. 4d).

Astrocytes differentiated from SUPT4H1-edited Q57 HD iPSC-NPCs reduced reactive astrocytes in YAC128 mice

We next examined whether transplanted SUPT4H1-edited-Q57 HD iPSC-NPCs had the potential to differentiate into astrocytes (GFAP). Slices were double stained with hNu and GFAP antibodies (Fig. 5a). The hNu/GFAP-positive cells in the YAC128 mice transplanted with unedited Q57 HD iPSC-NPCs had shorter dendrites and no straight morphology compared to the SUPT4H1-edited group (Fig. 4c). The MetaXpress software analysis revealed that the outgrowth numbers and maximum length of hMAP2-positive cells in the SUPT4H1-edited group were significantly increased compared to the unedited group (Fig. 4d).

**DISCUSSION**

This study demonstrated that SUPT4H1-edited Q57 HD iPSC-NPC transplantation stabilized motor function and improved neuroprotection in the damaged brain of YAC128 mice. We show that SUPT4H1 gene editing in HD iPSC-NPCs can facilitate normal neural differentiation and functional recovery while avoiding the risks of immunosuppression associated with autologous stem cell therapy. For genetic diseases like HD, ex vivo approaches that combine gene and cell therapy will be highly useful for developing autologous stem cell therapies.

Experimental and preclinical studies supported the commencement of the first neural transplantation clinical trial in HD patients in...
the 1990s\textsuperscript{23}. From human fetal tissue to porcine striatal cells, several cell sources have been used for clinical trials in HD patients\textsuperscript{24–28}. However, the results have been highly variable and the clinical benefits were modest for motor\textsuperscript{26} and neuropsychological outcomes\textsuperscript{27}. Graft survival and neuronal differentiation have been studied in patients using magnetic resonance imaging \textsuperscript{29}. By contrast, iPSCs may produce an unlimited source of patient’s autologous cells that can be used in regenerative medicine\textsuperscript{30}. However, mutated genes in autologous cells may cause abnormal neural differentiation and disrupt normal physiological functions, necessitating cell editing for stem cell therapy in HD. In this study, we showed that ablating SPT4 can contribute to the improvement of neurological defects associated with m\textit{HTT} in YAC128 mice.

**Fig. 4** Transplanted unedited or \textit{SUPT4H1}-edited Q57 HD iPSC-NPCs have different neuronal differentiation potential in YAC128 mice. \textbf{a} Double staining for the transplanted cell marker (hNu) and MSN marker (DARPP-32) revealed that transplanted unedited or \textit{SUPT4H1}-edited Q57 HD iPSC-NPCs differentiated into MSNs (scale bar: 20 μm). \textbf{b} Double staining for the human-specific MSN marker (hDARPP-32) and HD phenotype marker (EM48) in the striatum demonstrated decreased EM48 expression in transplanted \textit{SUPT4H1}-edited Q57 HD iPSC-derived MSNs (scale bar: 20 μm). \textbf{c} Immunostaining to identify hMAP2-positive cells exhibited neuronal dendrite degeneration in the transplanted unedited Q57 HD iPSC-NPCs-derived neurons (scale bar: 20 μm). \textbf{d} Quantification of neurite numbers and max neurite length indicated more neuronal maturation in transplanted \textit{SUPT4H1}-edited Q57 HD iPSC-NPCs-derived MAP2-positive cells than the unedited cells. (Photograph: same color is one cell for analysis, scale bar: 100 μm, for two regions in 6–7 brain sections of each group, \textit{***} \textit{p} < 0.001). Data were analyzed using two-way ANOVAs followed by Tukey’s post hoc tests or Student’s t tests with GraphPad Prism. The error bars on the bar charts represent the standard deviation.
be a focus of gene therapy in HD. However, normal and expanded alleles of the endogenous HTT gene are not readily distinguished by siRNA or antisense oligonucleotides. HTT loss and mutation in mouse embryonic stem cells impairs progenitor specification and maturation and targeted genetic modification results in fewer neuronal cells and decreased neurite length. The current study similarly edited the HTT gene, which is present in normal and expanded alleles. Our results showed that HTT-edited Q57 HD iPSC-NPCs reduced neuronal differentiation and neurite length, indicating that HTT gene ablation reduced neural differentiation and the maintenance of neuronal survival in vitro. This might be because our CRISPR/Cas9 HTT strategy did not distinguish between the wild-type and mutant alleles. In addition, complete HTT knockout might negatively affect neuronal development. Allele-specific targeting of mHTT with CRISPR/Cas9 based on SNP associations has recently been developed. However, this approach is likely to be limited by the various associative patterns between SNPs and mHTT in patients. In addition, one base pair

Fig. 5 Transplanted unedited or SUPT4H1-edited Q57 HD iPSC-NPCs have different astrocytic differentiation potential in YAC128 mice. a Double staining for the transplanted cell marker (hNu) and astrocyte marker (GFAP) revealed that transplanted unedited or SUPT4H1-edited Q57 HD iPSC-NPCs differentiated into astrocytes (scale bar: 20 μm). b Double staining for the human-specific astrocyte marker (hGFAP) and HD phenotype marker (EM48) in the striatum revealed decreased EM48 expression in transplanted SUPT4H1-edited Q57 HD iPSC-derived astrocytes (scale bar: 20 μm). c Immunostaining to identify C3 expression revealed reactive astrocytes in transplanted unedited Q57 HD iPSC-NPCs-derived astrocytes (scale bar: 20 μm). d Quantification of the GFAP-positive area and intensity indicated more activation in the transplanted SUPT4H1-edited Q57 HD iPSC-NPCs-derived astrocytes than the unedited cells. (Photograph: same color is one cell for analysis, scale bar: 100 μm, for two regions in 6–7 brain sections of each group, ***p < 0.001). Data were analyzed using two-way ANOVAs followed by Tukey’s post hoc tests or Student’s t tests using GraphPad Prism. The error bars on the bar charts represent standard deviation.
difference in target sites for CRISPR/Cas9 would likely result in quantitative, but not qualitative, preference in cleavage, increasing the chances of residual cleavage on wild-type HTT alleles.

Therefore, we considered that SPT4, a transcription elongation factor, would be the ideal candidate gene for editing HD iPSC-NPCs. Yeast and animal cells with defective SPT4 show reduced synthesis of proteins containing long polyQ stretches, such as mHTT. In contrast, SPT4 gene defects do not alter cellular mRNA synthesis. Furthermore, HD Q175 mice injected with SPT4 antisense 2′-O-methoxyethyl oligonucleotide and SPT4-one-copy-deleted R6/2 mice showed selective decreases in mHTT mRNA and protein expression. Furthermore, SPT4-one-copy-deleted R6/2 mice showed a prolonged lifespan and delayed motor impairments.17,12

For these reasons, we hypothesized that SPT4 knockout or knockdown would be the best approach to selectively reduce mHTT. The present study investigated the in vivo and in vitro functions of Supt4H14-edited Q57 HD iPSC-NPCs. Our first result demonstrated that Supt4H1-edited Q57 HD iPSC-NPCs showed decreased SPT4 protein levels. Compared to dual HTT-edited Q57 HD iPSC-NPCs, the Supt4H1-edited cells were more likely to differentiate into neuronal cells with increased neurite length and decreased expression of mHTT protein.

Second, our results showed that the unedited Q57 HD iPSC-NPCs were more likely to differentiate into astrocytes than neurons. It has been reported that HD Q140 knock-in NSCs or human HD iPSC (60, 109, and 180Q)-NPCs resulted in fewer NPCs, fewer mature neurons, and more astrocytes due to defects in BDNF expression, which modulates neuronal maturation.34 Our transplantation results demonstrated that unedited Q57 HD iPSC-NPCs were more likely to differentiate into astrocytes, whereas Supt4H1-edited Q57 HD iPSC-NPCs were more likely to differentiate into mature neurons. Therefore, although further study is required, these results suggest that reducing mHTT via Supt4H1 gene editing may enhance neuronal differentiation following in vivo transplantation.

Third, we found that the Supt4H1-edited Q57 HD iPSC-NPCs improved astrocytic function, which is as important as neuronal replacement. HD patients′ brains contain reactive astrocytes with thicker processes, a larger soma, and abnormal function that is associated with reduced glutamate transporter.35 In addition, abnormal astrocytes are implicated in HD pathophysiology.36-37 Our results demonstrated that the Supt4H1-edited Q57 HD iPSC-NPCs formed less reactive astrocytes due to reduced mHTT expression. Therefore, Supt4H1-edited Q57 HD iPSC-derived astrocytes may improve the HD brain environment.

In addition, we demonstrated interesting iPSC characterization results. Several studies have demonstrated that HD patients′ iPSCs or their derived NPCs function normally without mHTT expression, based on ubiquitin-protein system activity.36-40 For example, human embryonic stem cells with mHTT exhibited the associated pathology approximately 2 m following differentiation.41 Patient-derived iPSCs from juveniles and adults with HD showed mHTT expression in neurons after 6 m of culture or transplantation.42 The ubiquitin-protein system loses its normal function over the course of neural differentiation.43 In the present study, YAC128 mice transplanted with unedited Q57 HD iPSC-NPCs exhibited motor recovery at 1 m post-transplantation. However, these mice also had mHTT, which was associated with reduced neural differentiation. Therefore, our results demonstrate that mHTT in the unedited Q57 HD iPSC-derived neurons or astrocytes of YAC128 mice may hamper iPSC-NPCs from engrafting and differentiating.

Stem cell therapy is an important therapeutic strategy for several diseases. Numerous clinical and non-clinical studies are actively underway to achieve more effective outcomes. Specifically, some research groups are attempting gene editing to develop stem cells that will not undergo immune rejection. A sufficient supply of autologous stem cells could produce the best treatment if they do not suffer from immune rejection. However, patients with gene mutations, such as in HD, should only have their iPSCs transplanted after gene correction or editing. An easier and more convenient “universal” gene editing approach would be to target the regulatory gene instead of the specific gene in question, which would require more laborious procedures, like gene correction. In the case of HD, we suggest Supt4H1 gene editing as an appropriate and efficient strategy to develop autologous stem cell treatment. Furthermore, ex vivo approaches based on combining Supt4H1 gene editing and cell therapy may warrant further study for autologous stem cell therapy in other repeat expansion mutation-associated diseases, such as C9FTD/ALS.44

METHODS

Lead contact and material availability

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Jihwan Song (jsong873@gmail.com). This study did not generate new unique reagents.

iPSC cell lines

The HD patient iPSC line is the Q57 HD iPSC line (Cell line ID: ND41656) purchased from the RUCDR cell line service. The control iPSC line used in this study was CHA001-A, which was established from the frozen cord blood of a healthy donor using the episomal method.45

Cell culture and NPC induction

The control and Q57 HD iPSC lines were maintained in Stemfit Basic02 media (Ajinomoto, Japan) supplemented with 100 ng/ml of basic fibroblast growth factor (bFGF, Peprotech) and 10 μM Y27632 (ROCK inhibitor, Peprotech) for approximately 7 d before they were treated with TrypLE solution (GIBCO) for 5 min at 37 °C in a CO2 incubator. The dissociated cells were cultured in SBEFq media consisting of DMEM/F12 (Invitrogen) supplemented with 1% antimycotic-antibiotics, 1% nonessential amino acids (NEAA, GIBCO), 0.1% beta-MeOH, 20% Knockout36 serum replacement, 10 μM SB431542, 100 nM LDN193189, and 3x ROCK inhibitor at 37 °C in a CO2 incubator for neural induction. The cells were maintained in SBEFq media for 8 days. Embryoid bodies were dissociated in NPC media consisting of DMEM/F12 supplemented with 1:100 antimitic-antibiotics (Wegene), 1:100 NEAA, sodium pyruvate (GIBCO), d-glucose (Sigma-Aldrich), L-glutamine (Wegene), 1:1000 beta-MeOH, 1:50 B-27 (without vitamin A, GIBCO), and 20 ng/ml bFGF in a dish coated with poly-L-ornithine (Sigma-Aldrich) and laminin (Sigma-Aldrich). Accutase (Stem Cell Technologies) was used to split the cells.

Preparation of sgRNA

sgRNAs were generated by in vitro transcription using T7 polymerase (New England Biolabs) according to the manufacturer’s protocol. sgRNAs used in this study are listed in Table 1.

Gene editing of iPSC-NPCs

For gene editing, ribonucleoprotein (RNP) complex-mediated electroporation was performed as previously described.46 Briefly, a Neon electroporator (Thermo Fisher Scientific) was used to transfect 1 × 105 iPSC-NPCs with RNP complexes comprised of 1 μg of sgRNA and 4 μg of Cas9 protein (ToolGen). For targeted deep sequencing, genomic DNA (gDNA) was collected from cells 72 h after transfection.

Table 1. sgRNAs used in this study.

| Target site | sgRNA # | Target sequence - PAM (5′ to 3′) |
|-------------|---------|----------------------------------|
| Human Supt4H1 | sgRNA 1 | CGCAAGATGCGGCAAGTTCCCTT-GGG |
| Human AAV51 | sgRNA 1 | ATGGAGCCAGAGAGATCTCT-GAG |
| Human Htt | sgRNA 1 | GGAGACCGCATGGCGACCC-TGG |
| Human Htt | sgRNA 2 | CAGCCTTTTCCAGGGTCGCA-TGG |
end deep sequencing using Mi-Seq (Illumina). The deep sequencing data
England Biolabs). The resulting PCR amplicons were then subjected to paired-
the gDNA extracted from transfected cells using Phusion polymerase (New
Cas9-induced mutations. The primers used in this study are listed in Table 2.
fi
B27 supplement without vitamin A (GIBCO). The cells were
(GIBCO, Waltham, USA) and composed of 1× Glutamax (GIBCO) and 1%
The NDM was based on a 1:1 mixed medium of DMEM F12: Neurobasal
immunocytochemistry and western blot 6 w following differentiation.
using the SYBRTM Green system (Thermo Fisher Scienti
used as a template for RT reaction. Quantitative RT-PCR was performed
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All experiments were performed in YAC128 mice maintained on a FVB/N
transplanted brains (Supplementary Fig. 8). Both cell-transplanted and
Quantification of hNu-positive cells and striatal density
Unbiased stereological estimation of the total number of hNu-, DARPP-32-, and Iba-1 positive cells, as well as double stained GFP/hNu-positive cells in the striatum was made using a ImageXpress microconfocal high-content imaging system (Molecular device). The sections used for counting covered
Table 4. TaqMan probe Accession numbers.

| Primer-F (5’ to 3’) | Primer-R (5’ to 3’) |
|---------------------|---------------------|
| SUPT4H1             | TaqMan probe Accession #: Hs01051404_g1 |
| HTT                 | TaqMan probe Accession #: Hs00918174_m1 |
| GAPDH               | TaqMan probe Accession #: Hs02786624_g1 |

Table 5. Primers used in this study.

| Primer-F (5’ to 3’) | Primer-R (5’ to 3’) |
|---------------------|---------------------|
| Sox2                | GCTGCGAAAGAGAACACCAACCTTCTGCAAAGGCTCCTACC |
| MUSASHI             | AAGGGCTTAGCTGGAAGACTCCACAGATGTCCTACTCTA |
| GABA                | AGAGGGTATGCATGGGATGGGATGATTGATGTGGTGG |
| DARPP-32            | CCTGAAGGCTACGAGCGAGGTCGCCACTTGGGTGTCCTCA |
| GAPDH               | GCTCAATACGGAAATGACCTGACAGTCCATGCACATC |

The number of merged positive cells was measured using the cell sorting module of MetaXpress software (Molecular Devices).

Cell viability analysis

Upon gene editing, cells were washed with PBS and detached by TrypLE Select (ThermoFisher). Cells were then centrifuged for 5 mins at 1500 rpm. These were then stained with 0.4% Tryphan Blue and viabilities were measured using automated cell counter FACSCSCOPE B (Curiosis).

Karyotype analysis

Karyotyping was performed using a GTG-banding analysis (Korea Research of Animal Chromosomes, Korea).

Statistical analysis

Data were analyzed using two-way ANOVAs followed by Tukey’s post hoc tests or Student’s t tests, using SPSS software (version 10.0; Chicago, IL) or GraphPad Prism (version 5.0; San Diego). Significance was accepted at the 95% probability level. Data are presented as mean ± SEM.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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