Induced Pluripotent Stem Cells: A Powerful Neurodegenerative Disease Modeling Tool for Mechanism Study and Drug Discovery

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Abstract
Many neurodegenerative diseases are progressive, complex diseases without clear mechanisms or effective treatments. To study the mechanisms underlying these diseases and to develop treatment strategies, a reliable in vitro modeling system is critical. Induced pluripotent stem cells (iPSCs) have the ability to self-renew and possess the differentiation potential to become any kind of adult cell; thus, they may serve as a powerful material for disease modeling. Indeed, patient cell-derived iPSCs can differentiate into specific cell lineages that display the appropriate disease phenotypes and vulnerabilities. In this review, we highlight neuronal differentiation methods and the current development of iPSC-based neurodegenerative disease modeling tools for mechanism study and drug screening, with a discussion of the challenges and future inspiration for application.

Keywords
Neurodegenerative disease, induced pluripotent stem cells (iPSCs), disease modeling, neuronal differentiation, mechanism study, drug screening

Induced-Pluripotent-STEM-Cell-Based Therapies and Neurodegenerative Disease Modeling
Aging societies have a number of health issues, particularly regarding neurodegenerative diseases such as dementia, Alzheimer’s disease (AD), and Parkinson’s diseases (PD). During the progression of these diseases, patients may lose their memory and thinking abilities, and many can develop movement disorders. However, the majority of neurodegenerative diseases lack effective treatments. For novel drug screening, various cell lines and animals have been used as neurodegenerative disease models. After revealing specific disease mechanisms, drugs designed to target pathogenic candidates can be developed. However, differences between disease models and the actual human nervous system continue to be a risk of disease modeling.

The first mouse iPSCs were established in 2006 in Dr Shinya Yamanaka’s laboratory. Somatic cells were reprogrammed into early embryonic-like pluripotent stem cells (PSCs) via the re-expression of four transcription factors, Oct4, Sox2, Klf4, and c-Myc. These cells displayed a self-renewal ability and pluripotent differentiation...
potential (i.e. the ability to differentiate into ectoderm, mesoderm, or endoderm) in vitro. In 2007, the same research group successfully established a human iPSC line from skin fibroblasts. Subsequently, many research groups have developed methods to establish iPSCs from numerous somatic cell sources including fibroblasts, adipocyte stem cells, neural stem cells (NSCs), hematopoietic stem cells (HSCs), and peripheral blood mononuclear cells. The advantages of these approaches have expanded the application potential of pluripotent stem cells without the source limitation or ethical concerns. In the next decade, iPSC technology has attracted increasing attention worldwide, and researchers have established and banked numerous iPSC lines for developmental study, disease modeling, genetic/epigenetic studies, and transplantation therapies.

For cell transplantation therapies, iPSC and embryonic stem cell (ESC)-derived lineage-specific cells have been applied to age-related macular degeneration (AMD), PD, heart disease, spinal cord injury, blood transfusion, cancer, and arthritic disorders. Clinical trials with PSC-derived (including iPSC and ESC) cells for AMD, PD, spinal cord injury, diabetes, and myocardial infarction are under progress.

For neurodegenerative disease modeling, the greatest challenge is arguably the difficulty in obtaining disease-related tissue and cells directly from patients for pathology and physiology studies. For in vitro and in vivo modeling of neurodegenerative diseases, several cell and animal models have been developed. However, the majority of neurodegenerative disease models are based on artificial cells or animals. For example, pathogenic-gene-overexpressed models are widely used for AD, PD, amyotrophic lateral sclerosis (ALS), and spinocerebellar ataxia (SCA) studies. However, these overexpression models show different cytopathology and disease mechanisms when compared with patient brain neurons, and the differences between animal and human brain remain one of the biggest challenges of animal-based brain disease models. Furthermore, animal models of neurodegenerative diseases may take a long time to recapitulate phenotypes and are also time and resource consuming for drug screening. The iPSC modeling system allows studies to use patient cell-derived pathogenic cells to address disease phenotypes and their progression in a cell culture dish. Compared with other models, patient cell-derived iPSCs may serve as a reliable in vitro disease model of complex neuronal diseases. This model may serve as an accurate first line for drug screening and candidate exploring before animal models. Many reports have successfully established iPSC lines from patient tissues for various neurodegenerative diseases such as AD, PD, ALS, SCA, Rett syndrome, spinal muscular atrophy (SMA), Down syndrome (DS), and Huntington’s disease (HD). In some cases, patient iPSC-derived neurons recapitulate disease phenotypes, such as amyloid-β (Aβ) aggregates and neuronal function degeneration that are seen in AD and can be applied to drug screening and mechanism discovery.

**Induced-Pluripotent-Stem-Cell Establishment, Culturing, and Neuronal Differentiation**

**Induced-Pluripotent-Stem-Cell Establishment and Culturing**

The technology for establishing iPSCs is improving every day. In the beginning, retrovirus and lentivirus vectors were used for the delivery of reprogramming factors. However, the integrative property of retroviruses may be a concern for genetic stability. For an integration-free delivery system, piggyBac transposons, RNA viruses, episomal vectors, RNAs, and proteins have been used to replace integrative viruses. To improve iPSC generation efficiency, small molecules with signaling activities, as well as DNA demethylation and deacetylation, can robustly enhance iPSC colony-formation rate. Dr. Hou’s research group developed a reprogramming method with only chemical compounds. Recently, epigenetic modulation methods have been developed to generate iPSCs.

The traditional PSC culture, including those of ESCs and iPSCs, consists of a coculture with fibroblast feeder cells. For cell viability, avoiding single-cell dissociation is a common approach when passing PSCs. However, the feeder cell coculture system can become a challenge for cell property analysis, and dissociated cell death restricts cell clonal
purification. Recently, many feeder-free and xeno-free culture systems have been reported to support the long-term growth of PSCs. Commercialized medium including mTeSR, Essential 8, PSGro, L7, and StemFit have been combined with coating matrix Matrigel, Geltrex, vitronectin, synthemax, laminin 521, and laminin E8. These culture systems have eliminated the contamination of feeder cells and animal serum. In addition, it has been discovered that the Rho/ROCK signaling pathway plays a major role in dissociation-induced cell bleeding in PSCs. This finding provides the possibility for single-cell dissociation and has expanded the PSC application aspects to genome editing, clonal isolation, and single-cell characterization.

**Neural Differentiation**

For neurodegenerative disease modeling, the differentiation of PSCs into candidate neural lineages is the key factor to recapitulating disease phenotypes. The differentiation protocol from PSCs to NSCs is dependent on human embryonic development (Fig. 2). Neuronal cells primarily come from a neuroectodermal lineage. To specifically differentiate PSCs into NSCs, the dual inhibition of the SMAD signaling pathway via the bone morphogenetic protein (BMP) and transforming growth factor beta 1 (TGF-β1) antagonists lead to robust neuroepithelial generation via inhibition of mesendoderm formation. The dual SMAD inhibition protocol converts PSCs into high purity forebrain NSCs with expression of Pax6, FOXG1, and Otx2 to form the cerebral cortex. For naïve cell fate, most NSCs induced via the dual SMAD inhibition method convert into forebrain cortical neurons. For other neural-type patterns, patterning factors are needed. In a previous study, researchers demonstrated that the combination of basic fibroblast growth factor (bFGF or FGF2), a TGF-β1 inhibitor, and a Wnt agonist promotes high efficiency NSC differentiation.

**Specific Neural Lineage Patterning**

During early embryonic neurogenesis, neuroepithelial cells form the neural plate and neural tube for the central nervous system (CNS). NSCs in the neural tube are patterned by ‘morphogens,’ which are dose-dependent developmental signaling factors, to generate and form the whole CNS. During early embryonic neurogenesis, neuroepithelial cells into a dorsal lineage. For rostral-caudal patterning, Wnt signaling, BMP signaling, and retinoic acid (RA) signaling, all participate in the neural fate decision. Thus, for in vitro CNS neuronal differentiation, morphogens or their agonists/antagonists are applied for specific neuron patterning. For midbrain dopaminergic neuron (DA neuron) differentiation, Shh and fibroblast growth factor 8b (FGF8b) are applied to pattern NSCs to ventral midbrain–hindbrain boundary (MHB) neurons, which is the primary localization of DA neurons. FGF8b is highly expressed in MHB neurons during embryonic neural tube development. However, DA neuron patterning efficiency in the FGF8b/Shh method is not good enough at only 10–30% overall. To improve DA neuronal patterning efficiency, the GSK3β inhibitor CHIR99021 is applied, which has been shown to greatly improve DA neuron patterning efficiency. CHIR99021 is the first small molecule shown to dose-dependently inhibit GSK3β activation, a Wnt signaling downstream protein. This small molecule has benefited many PSC in vitro differentiation studies that required different Wnt activation levels. Combining low dosage CHIR99021 with the patterning factors Shh and FGF8b enhances DA progenitor-specific markers, including FOXA2, Lmx1a, Nurr1, and Pitx3. The addition of CHIR99021 can also improve DA neuron generation efficiency up by more than 80% with the DA-specific markers TH, DAT, and dopamine secretion. In the previous DA neuron differentiation two-step methods, PSCs are induced into NSCs and then they start the patterning process. A novel DA neuron differentiation process that induces neural differentiation and DA neuron patterning simultaneously has been shown to enhance DA neuron generation efficiency. For DA neuron purity, cell surface specific markers are another key factor for isolating DA progenitors. The ventral neural tube-specific surface protein Corin and the midbrain-specific surface marker LRTM1 can also be applied for fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) for high purity DA progenitor isolation.

Motor neuron (MN) differentiation is a three-step protocol, with dual SMAD inhibition to convert PSCs to NSCs, activate RA signaling for caudalization, and activate Shh signaling for ventralization. For better MN generation efficiency, various protocols with different small compounds, treatment combinations, and time periods have been applied for MN patterning. One breakthrough in MN patterning was the evaluation of the GSK3β inhibitor CHIR99021 on spinal cord neural lineage patterning. Maury et al. and Du et al. discovered that the addition of CHIR99021 to the traditional MN induction protocol greatly enhances the spinal cord specific markers CDX1, CDX2, HOXA4, and HOXA5. MN-specific marker expression enhances by 90% depending on the CHIR99021 dosage, including Oligo2, Islet1, and HB9. Subsequently, various studies have demonstrated that GSK3β inhibition plays a key role in spinal cord MN differentiation; thus CHIR99021-based methods have become widely used.

For other specific neural lineage patterning, protocols have been established for gamma amino-butyric acid (GABA)-ergic interneurons, serotonin neurons, and glutamate neurons, by following the basic neural tube development protocol and Wnt signaling level.
A very special neuron lineage with a high patterning challenge is cerebellum Purkinje cells, which represent the major relevant neurons related to SCA pathology. Purkinje cells are located at the cerebellar plate of dorsal MHB neurons. However, there is no efficient protocol to generate Purkinje cells from PSCs according to the neural tube

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**Fig. 2.** Protocols of neural differentiation from pluripotent stem cells follow the mammalian central nerve system developmental process. (A) The relationship of neuron types, morphogens and positions at early neural tube development. (B) The major signaling involved in NSC differentiation and specific types of neuron patterning.

BMP: bone morphogenetic protein; BMPi: bone morphogenetic protein induced; Shh: sonic hedgehog; PSC: pluripotent stem cell; TGFβi: transforming growth factor beta induced; FGF: fibroblast growth factor; GABA: gamma-amino butyric acid; TUJ1: neuron-specific class III beta-tubulin; TH: tyrosine hydroxylase; DAPI: 4',6-diamidino-2-phenylindole; NF200: neurofilament 200; RA: retinoic acid; NSC: neural stem cell.
development principle. Muguruma et al.\textsuperscript{81} discovered that endogenous MHB patterning factors are more important compared with exogenous factors such as cycloamine (a small compound Shh antagonist) or FGF8b. In mouse PSCs, the collective effect of bFGF and insulin can dramatically enhance MHB marker expression, including that of Wnt1, FGF8, EN2, and Gbx2. Furthermore, bFGF/insulin can also increase the early Purkinje cell markers Corl2, Neph3, and Ptf1a. After neuron maturation, Purkinje cells express the specific marker L7 and have classical Purkinje cell morphology and neuroelectric response.\textsuperscript{81} This differentiation method can also be applied to human PSCs. After bFGF/insulin treatment, about 20\% of PSCs can become Purkinje cell progenitors. After Purkinje progenitor cell purification using specific surface markers and granule cell coculture (or brain slice coculture), mature Purkinje cells display classical neuronal function.\textsuperscript{81}

Methods have also been developed for another important CNS cell lineage, glial cell differentiation. Two major kinds of CNS glia are astrocytes and oligodendrocytes. Astrocytes are multi-function cells that benefit neuron survival and function, including nutrition exchange, metabolism control, and immune regulation. Oligodendrocytes can myelinate neurites to protect CNS neurons. For astrocyte differentiation, PSC-derived NSCs are primarily induced with BMP, ciliary neurotrophic factor (CNTF), bFGF, and fetal bovine serum or serum-free medium with Activin A, heregulin 1, ciliary neurotrophic factor (CNTF), bFGF, and fetal bovine serum or serum-free medium with Activin A, heregulin 1, bFGF, and insulin-like growth factor 1 (IGF-1).\textsuperscript{83} For astrocyte purification, repeating trypsinizing passages serve to eliminate the majority of other neuronal cells.\textsuperscript{84} Afterward, the purified astrocytes can be applied to disease modeling, neuron coculture, or neuron–astrocyte interaction studies.

Another key glial cell type is oligodendrocytes, and their differentiation is much more complex and challenging. In this process, there are six steps that take more than 180 days to obtain functional oligodendrocytes from PSCs.\textsuperscript{85} The first step is to transfer PSCs into NSCs. Afterward, the Shh agonists, RA and bFGF help NPCs to become oligo2+/Nkx2.2+ oligodendrocyte progenitor cells (OPCs). For myelogenic OPC differentiation, combined growth factor treatment for 120 days is needed. Finally, OPCs are transplanted into animals for terminal maturation. The resulting O4+ OPC purity is typically 4.1–11.9\%.

Microglia are the macrophages of the CNS, where they play an important role in the CNS immune response. During early embryonic development, there are two origins of microglia, the early yolk sac and mesoderm-derived HSCs. Muffat et al.\textsuperscript{86} developed a novel chemically defined method to differentiate PSCs into microglia via the yolk sac route. However, this protocol is complex for routine use. To address this issue, Pandya et al.\textsuperscript{87} provided a concise two-step protocol to generate microglia. The first step consists of converting PSCs into HSCs with CD34 and CD43 expression. The second step involves the coculture of PSC-derived HSCs with astrocytes. After coculture, CD39+ microglia are sorted for use in subsequent experiments.

Overall, patterning methods that induce NSCs to differentiate into various types of specific neural lineages have greatly benefited PSC-based neurodegenerative disease modeling systems. However, some special kinds of neurons and glial cells present with unique technical difficulties for routine generation and application to disease modeling.

### Current Neurodegenerative Disease Modeling and Drug Screening Using Induced-Pluripotent-Stem-Cell Models

The most serious neurodegenerative diseases, including frontotemporal dementia (FTD), AD, and PD, represent the major targets of iPSC-based disease modeling. iPSC-based in vitro models have also been applied to some rare diseases such as DS, ALS, SMA, HD, and SCA. The major publications resulting from these studies are listed in Table 1.

### Induced-Pluripotent-Stem-Cell-Based Modeling for Alzheimer’s Disease

Amyloid accumulation and Tau protein abnormalities are the two major cytopathies observed in AD patient brain neurons. To recapitulate AD cytopathies in an in vitro modeling system, DS and AD iPSC-derived cortical neurons have been applied to mimic AD for mechanism studies and drug screening.\textsuperscript{11,12,25,29,30,34,37,41–44} DS neurons express some classical AD cytopathies, including Aβ42 aggregations, Aβ40 over-secretion, Tau protein overexpression, hyperphosphorylation, and redistribution.\textsuperscript{41} Moreover, DS forebrain neurons have reduced synaptic activities and show vulnerability to oxidative stress.\textsuperscript{43} Interestingly, studies have also found that DS astroglia cannot support neurogenesis.\textsuperscript{12} Because of this strong phenotype expression, DS models are suitable for AD drug screening. In our previous study, we found that the herbal compound N-butylidenephthalide decreases Aβ and Tau protein cytopathies.\textsuperscript{11} However, the genetic backgrounds of DS and AD are largely diverse, suggesting that the accuracy of DS-based AD modeling may be another challenge. Cortical neurons derived from familial and sporadic AD iPSCs have amyloid and Tau protein abnormalities, including Aβ accumulation, Aβ42/Aβ40 ratio dysregulation, and Tau protein hyperphosphorylation.\textsuperscript{25,29,30,34,37,42,44} In some reports, increased reactive oxygen species and apoptosis signaling have been observed in AD neurons.\textsuperscript{29} Interestingly, Kondo et al.\textsuperscript{29} demonstrated that early AD phenotypes within different kinds of familial and sporadic AD neurons are varied. One candidate AD compound, docosahexaenoic acid (DHA), was shown to only rescue some types of AD neurons and showed no effect on others. This study demonstrated that separating AD subpopulations may be helpful for selecting the right drug for targeting the specific AD types for effective personal therapy. The same research group also established an AD platform for systemic drug screening. In this study, they found that a set of combined anti-Aβ cocktails may inhibit
Table 1. Lists of typical publications of induced pluripotent stem cell-based neurodegenerative disease modeling.

| Disease | Related gene | Phenotype | Cell type | Other | Reference |
|---------|--------------|-----------|-----------|-------|-----------|
| PD      | LRRK2 and SP | α-synuclein accumulation; increased DA neuron degeneration; increased immature DA neurons; deficient competence for autophagic clearance; deficient competence for autophagic clearance | DA neuron | | 38 |
| PD      | SNCA         | Increased α-synuclein protein | DA neuron | | 16 |
| PD      | LRRK2        | Mitochondrial DNA damage | DA neuron and DA neuron | | 39 |
| PD      | PINK1 and Parkin | Increased dendrite degeneration; decreased tyrosine hydroxylase expression; enlarged mitochondria and multilamellar inclusions | DA neuron | Progerin induced aging | 32 |
| PD      | PINK1 and LRRK2 | Mitochondrial dysfunction | DA neuron | Coenzyme Q10, rapamycin and GW5074 | 15 |
| AD      | APP and PSEN1 | Aβ accumulation; Tau hyperphosphorylation | FB neuron | | 37 |
| AD      | PSEN1        | Aβ40 and Aβ42 accumulation; increased Aβ42/Aβ40 ratio; Aβ oligomer accumulation; ROS increase | FB neuron | Anti-Aβ cocktail | 30 |
| AD      | APP and SP   | Aβ40 and Aβ42 accumulation; increased Aβ42/Aβ40 ratio; Aβ oligomer accumulation; ROS increase | FB neuron | DHA | 29 |
| AD      | PS1 and PS2  | Aβ42 accumulation | FB neuron | | 44 |
| AD      | APP and SP   | Aβ40 accumulation; increased p-TAU; active GSK3β; large early endosomes accumulation. | FB neuron | | 25 |
| AD      | PSEN1        | Increased Aβ42/40 ratio; 14 genes differentially regulated | FB neuron | | 42 |
| AD      | APP          | Increased APP; Aβ accumulation; increased total and p-TAU | FB neuron | | 34 |
| ALS     | TDP43        | Shorter neurites; increased mutant TDP-43; TDP-43 aggregates; MN death | MN | Anacardic acid | 18 |
| ALS     | VAPB         | Reduced VAPB | MN | | 33 |
| ALS     | SP           | Decreased mitochondrial gene expression | MN | | 8 |
| ALS     | SOD1, TDP43, C9ORF72 and SP | MN degeneration; autophagy dysregulation | MN | Src/c-Abl pathway | 23 |
| ALS     | FUS          | FUS mislocalization; increased stress granules; cellular vulnerability | MN | | 22 |
| ALS     | SOD1         | SOD1 aggregates; neurofilament dysregulation | MN | | 13 |
| ALS     | SOD1         | Increased oxidative stress; mitochondrial dysfunction; increased ER stress; increased UPR pathways | MN | | 27 |
| ALS     | SP           | TDP-43 aggregations | MN | | 10 |
| ALS     | C9ORF72      | Nucleocytoplasmic transport defects | MN | | 46 |
| ALS     | TDP43        | Increased mutant TDP-43; TDP-43 mislocalization; cell death | Astrocyte | | 40 |
| ALS     | SOD1         | MN death | Oligodendrocyte | | 19 |
| ALS     | FUS          | FUS mislocalization; hypoexcitability; axonal transport defects | MN | HDAC6 inhibitor | 21 |
| DS      | Trisomy 21   | Aβ peptide accumulation; Aβ aggregates; increased p-Tau and total Tau; Tau redistribution | FB neuron | | 41 |
| DS      | Trisomy 21   | Aβ peptide accumulation; Aβ aggregates; increased p-Tau and total Tau; Tau redistribution | FB neuron | F127-Bdph | 11 |
| DS      | Trisomy 21   | Reduced synaptic activity; affecting excitatory and inhibitory synapses | FB neuron | | 43 |
| DS      | Trisomy 21   | Higher ROS; decreased synaptogenic molecules; abnormal gene expression profiles; decreased neurogenesis NSCs | Astrocyte | Minocycline | 12 |
| SCA3    | ATXN3        | Decreased autophagy | Neuron | | 36 |
| SCA3    | ATXN3        | ATXN3 aggregates | Neuron | | 28 |
| SCA6    | CACNA1A      | Increased CaV2.1; decreased α1ACT fragment; TH depletion-dependent degeneration | Purkinje cell | TRH and riluzole | 24 |
| HD      | HTT          | Proteasome inhibition; HD pathology | GABA neurons | DARPP-32 | 26 |
| HD      | HTT          | Cadherin, TGF-β, BDNF decrease, and caspase activate | DARPP-32 neurons | | 9 |

(continued)
Table 1. (continued)

| Disease | Related gene | Phenotype | Cell type | Other | Reference |
|---------|--------------|-----------|-----------|-------|-----------|
| HD      | HTT          | Mutant Htt aggregates; increased lysosomes/autophagosomes; increased nuclear indentations; neuronal death | GABA neuron |       | 35        |
| HD      | HTT          | Disease-associated changes in electrophysiology, metabolism, cell adhesion; neuronal death; stress vulnerability | NSCs and GABA neuron |       | 14        |
| SMA     | SMN          | Decreased SMNs; neurite degeneration; excitability dysfunction | MN | VPA and tobramycin | 17        |
| SMA     | SMN          | Decreased MN; fewer pre-synaptic maturation | MN | VPA and PMOs | 20        |
| SMA     | SMN          | Decreased UBA1; UBA1 mislocalization; decreased neurodevelopment and differentiation | MN |     | 45        |
| SMA     | SMN          | Impaired AChR | MN |     |           |

Note: ACT: C-terminal of CaV2.1; AChR: acetylcholine receptor; AD: Alzheimer’s disease; ALS: amyotrophic lateral sclerosis; Aβ: amyloid beta; APP: amyloid precursor protein; BDNF: brain-derived neurotrophic factor; Bdp: N-butyldeneophthalide; CaV2.1: gene product of CACNA1A; DA: dopaminergic; DHA: docosahexaenoic acid; SN: Down syndrome; ER: endoplasmic reticulum; FB: forebrain; FUS: fused in sarcoma gene; GSK3β: glycogen synthase kinase 3 beta; HTT: Huntingtin; HD: Huntington’s disease; HDAC6: histone deacetylase 6; Htt: Huntingtin; MN: motor neuron; NSCs: neural stem cells; PD: Parkinson’s disease; PMOs: phosphorodiamidate morpholino oligonucleotides; p-Tau: phosphorylated Tau protein; ROS: reactive oxygen species; SCA: spinocerebellar ataxia; SMA: spinal muscular atrophy; SMN: survival motor neuron; SP: sporadic; TGF-b: transforming growth factor beta; TH: thyroid hormone; TRH: thyrotropin-releasing hormone; UBA1: ubiquitin-like modifier activating enzyme 1; UPR: unfolded protein response; VAPB: vesicle-associated membrane protein-associated protein B; VPA: valproic acid.

cytotoxic Aβ accumulation in both familial and sporadic AD neurons.

Induced-Pluripotent-Stem-Cell-Based Modeling for Parkinson’s Disease

PD is another serious neurodegenerative disease that affects patients all around the world. The number of DA neurons decrease 5–10% per decade due to the natural aging process and are majorly induced by reactive oxygen species (ROS) stress and metabolism alterations. However, the DA neurons are largely dead in the PD patient’s brain within a few years and cause serious movement disorders. For PD in vitro modeling, familial (including LRRK2, SNCA, PINK1, and PARK2 mutation) and idiopathic PD iPSCs have been differentiated into DA neurons and applied to cytopathic studies. In addition, α-synuclein accumulations have been observed in LRRK2 mutations and SNCA triplication DA neurons. In PINK1 and LRRK2 mutant DA neurons, mitochondrial dysfunction and DNA damage were both observed. For idiopathic PD modeling, DA neuron degeneration, Lewy-body accumulation, mitochondrial deficiency, and autophagy dysregulation were also found in sporadic and progerin-induced aging DA neurons. Taken together, both familial and idiopathic PD iPSC-derived DA neurons had late disease phenotypes such as neurite degeneration and cell apoptosis. However, the expression of specific early cytopathies such as α-synuclein accumulations were restricted to familial PD with appointed genetic mutations.

Induced-Pluripotent-Stem-Cell-Based Modeling for Amyotrophic Lateral Sclerosis

ALS is arguably the most concerning, rare neurodegenerative disease. For ALS modeling, patient iPSCs have been differentiated into MNs, astrocytes, and oligodendrocytes for modeling. Sporadic and HDAC6 mutant MNs show cytopathies, including cytosolic TDP-43 aggregations, neurite degeneration, and MN death. In addition, SOD1 gene mutation MNs have been shown to express SOD1 protein aggregates and neurofilament dysregulation phenotypes. Disrupted nucleocytoplasmic transport has been identified in c9orf72-mutant MNs. In FUS-gene-mutant MNs, FUS protein mislocalization, cellular vulnerability, and axonal transport defects were also observed. Inclusions in HD NSC and GABAergic neurons. In SMA MNs, decreased functional SMN protein leads to neurite...
Organoid and Coculture Systems Improve the Integrity of In Vitro Modeling

iPSC-derived neurons from patients may be the closest model for diseases. However, single-cell type in vitro models may be not enough to reproduce complex diseases such as neurodegenerative diseases. Indeed, specific interactions within many cell types such as neurons, glial cells, microglia, and connective tissue may underlie the progression of neurodegenerative diseases. Therefore, many coculture and organoid methods have been developed for whole-map analysis of neurodegenerative diseases.

Eiraku et al.92 first demonstrated that three-dimensional (3D) cultures of neuronal embryoid bodies (EBs) can form primitive neural-tube-like structures in 2008. In 2011, the same research group differentiated human ESCs with the EB method to establish eye-cup-like organoids.93 Later, Kadoshima et al.94 discovered the forebrain-like development and structure in neural EBs. The neuroepithelial cells formed a lumen similar to the ventricular and the NSCs that expressed N-cadherin and CD133 were surrounded by the ventricular. This finding is similar to the NSCs in the subventricular zone (SVZ) of the brain. Lancaster et al.95 developed a simple 3D culture system to enlarge brain organoids in 2013. They embedded neural EBs in Matrigel droplets and used a spin culture system to force EB growth to become a well-organized brain organoid. These brain organoids could form multi-layer cortical-like structures after months of culture. Moreover, the neural division and migration in the organoid followed that of brain development, such as interkinetic nuclear migration. After applying brain organoids to disease research, authors mimicked inherited microcephaly and found reductions in NSC division in the SVZ-like zone of patient organoids. For further application, brain organoids have also been used for epidemic disease studies. iPSC-derived brain organoids were used to study Zika virus infection, and it was found that the Zika virus induced premature differentiation of neural progenitors, followed by microcephaly.96 This novel system has allowed investigators to mimic neural development and migration in the human brain and to realize the actual situation of brain diseases. Subsequently, several publications have demonstrated organoids of cortical brain,97 dorso medial telencephalic tissue,98 midbrain,99,100 cerebellum,82, and neural tube101. However, whether these organoids can be applied to developmental and disease modeling still needs evaluation.

For neurodegenerative diseases, the cytopathies may not lie only within neurons. Indeed, surrounding glial cells such as astrocytes, oligodendrocytes, and even immune cells may aggravate the progress of these diseases. Therefore, several publications have suggested that astrocytes derived from the iPSCs of patients with DS and ALS revealed deficiencies in their ability to support neuronal maintenance and even displayed toxicity to healthy neurons.12,40. ALS patient iPSC-derived oligodendrocytes have also been reported to hurt MNs in vitro.19 Although researchers have developed methods to directly differentiate PSCs into microglia, microglia differentiation remains a formidable challenge for disease modeling. For some neurodegenerative diseases, such as ALS and SMA, muscle cells that form neuromuscular junctions are important to recapitulate disease phenotypes in vitro.45

Combining Genome Editing Technology with Induced Pluripotent Stem Cells for Disease Modeling

Isogenic control is extremely important for accurate neurodegenerative disease modeling. The generation and correction of single-site DNA mutations benefit studies of genetic function and novel single nucleotide polymorphism (SNP) discovery. Most common genetic modification strategies include the use of enzymes to cut a nick at a specific DNA site; for DNA repair, cells would start non-homologous end joining or homologous recombination to generate DNA-point mutations or increase foreign DNA integration ratio for correction. To recognize specific sites on chromosomes, zinc finger nuclease (ZFN)104, transcription-activator-like effector nucleases (TALENs)105–107, and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-1-associated system 9 (Cas9)108–110 have been applied for site-specific genome editing. Compared with DNA interactive protein (ZFN and TALEN)-based genome editing tools, RNA-based DNA pairing CRISPR/Cas9 is much easier to handle and has quickly become the most popular genome editing tool. Site-specific corrections have been widely used in AD, PD, ALS, HD, and other neurodegenerative diseases’ iPSC lines to have isogenic controls for research studies and large-scale drug screening. Genome editing
technology is well established for generating site-specific knock-outs, mutant site modifications, polyglutamine (PolyQ) corrections, and large fragment deletions and insertions\textsuperscript{112,113} in iPSC lines. For large DNA fragment modifications, a study was provided by Jiang et al.\textsuperscript{114}. This research group introduced an inducible X-inactive specific transcript (XIST) gene into the DYRK1A locus of chromosome 21 and successfully silenced one copy of trisomy chromosome 21 on DS iPSCs to rebalance genetic expression level. Until now, CRISPR/Cas systems had only been applied to nicking DNAs or RNAs, but now they are being used for epigenetic regulations. After disrupting the DNA nuclease function, DNA methyltransferases (DNMTs) and methylcytosine dioxygenase have been fused to the catalytically inactive Cas9 (dCas) protein, which targeted the promoter regions to transiently turn on/off specific genes\textsuperscript{115}. Combining these powerful tools, researchers are able to explore in more detail the mechanisms of neurodegenerative diseases.

**Challenges of Induced-Pluripotent-Stem-Cell-Based Neurodegenerative Disease Models**

Personal PSC-derived neurons have huge potential for modeling pathologies in a given patient’s brain. However, there are still many challenges that remain. Although iPSC technology was established over a decade ago, mimicking neurodegenerative diseases via iPSC-based models is still in infancy.

Until now, most models have been based on known genetic mutations. However, most high prevalence neurodegenerative diseases involve genetic mutations that are unknown or independent. For AD and ALS, less than 10% of patients have amyloid precursor protein (APP), secretase, ApoE, SOD1, TDP-43, or c9orf72 genetic mutations. Previous studies have also demonstrated that sporadic AD iPSC-derived cortical neurons do not show Tau-protein-related pathology. In some other cases, of neurodegenerative disease modeling with iPSCs, similar phenomena occur. These studies suggest the challenges to recapitulating disease phenotype with iPSC models. Therefore, large-scale input of familial and idiopathic disease iPSC lines for studies may provide improved data and experimental results to move the state of the science forward.

Aging is another serious issue for iPSC-based disease models. When reprogramming, most aging-related genes are turned off and the cells become original young cells. To accelerate disease phenotype expression, various treatments are added to the differentiated cells to increase oxidative stress, endoplasmic reticulum stress, mitochondria stress, ion channel stress, nutrition depletion, or autophagy inhibition. However, these relevant factors may be different with natural aging, thus decreasing the accuracy of iPSC-based disease models. Furthermore, how to discover the real relevance of pathogenic cells is still unclear. Progerin, a truncated form of lamin A protein that is involved in Hutchinson–Gilford progeria syndrome (HGPS), a premature aging disease, is found to accelerate cellular aging. Miller et al.\textsuperscript{32} demonstrated that Progerin overexpression enhances aging and pathology of PD iPSC-derived midbrain DA neurons, including neurite degeneration and Lewy-body-like inclusions. This study may bring new insights for recapitulating the aging process of neurodegenerative diseases with iPSC models.

Another big challenge is the identification of the correlation between early abnormal phenomenon observed from iPSC-derived pathogenic neurons and real neuronal degeneration in the patient brain. Several reports have demonstrated an early pathologic-like phenomenon of iPSC-based neuronal disease models, such as Aβ42 and Tau abnormal in AD, TDP-43 distribution, neurofilament dysregulation, and nucleocytoplasmic transport disruption in ALS. However, it remains difficult to identify these early cytopathies as highly correlated with neural dysfunction, apoptosis, and cell death. Therefore, how to uncover detailed mechanisms that connect these early cytopathies with late-stage neuronal loss in culture and the patient brain may be the next step to overcome.

The neural differentiation process always takes at least a couple of months to obtain functional neurons for pathology studies, thus delaying the efficiency of iPSC-based drug screening. To accelerate neural differentiation and apply these findings to large-scale drug screening, inducible neurogenin 2, islet 1, and LIM Homeobox 3 have been introduced into iPSCs, which largely shorten the differentiation time of cortical neurons and MNs (less than 15 days to obtain functional neurons)\textsuperscript{23,30}.

Furthermore, it still takes time to develop mature organoid and coculture systems to imitate complex nervous system tissue for detailed studies instead of neuron-only studies.

**Future Aspects for Application of Induced Pluripotent Stem Cells in Neurodegenerative Disease Modeling**

Besides stem cell techniques, big data, genome sequencing, and microarray technologies have been growing in leaps and bounds in recent years. By combining these novel technologies with iPSC disease models, unprecedented inspiration may also be freed (Fig. 3).

Next-generation sequencing (NGS) provides efficient and low-cost full genome and transcriptome sequencing. Combining NGS with big data and iPSC models, researchers can share and compare sequencing and in vitro cytopathic data to reveal correlations between SNPs, transcriptomes, and disease phenotypes. This inspiration quickly brings genetics and cytopathies together to benefit disease studies.

Neurodegenerative diseases are so complex that detailed disease processes and mechanisms may be largely different between patients. Therefore, extensive drug screening with iPSC in vitro models may help us realize the relationship
between candidate drugs and disease phenotypes, and indicate potential drug cocktails to treat specific groups of patients. Another potential solution is to establish personal iPSCs and select candidate compounds to these personal in vitro models before treatment. According to this idea, some drugs that have failed in clinical trials previously may still have potential if we can select suitable patients via in vitro pre-tests. After that, personal precise medicine may be applied for choosing the right drugs to fit the right patient.

In conclusion, although we still remain in the initial stages of understanding neurodegenerative diseases, the robust novel techniques described here may furnish us with the potential to better understand these complex and challenging conditions and develop appropriate treatment methods.

Authors' Note
Chia-Yu Chang and Hsiao-Chien Ting are co-first authors.

Authors' Contributions
SZL and HJH initiated this project. CYC edited, organized and wrote the article. HCT drew figures and tables. CAL and HCT wrote the text body. HLS and TWC organized and proofed the article. All authors reviewed this manuscript.

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References
1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–676.
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–872.
3. Hester ME, Song S, Miranda CJ, Eagle A, Schwartz PH, Kaspar BK. Two factor reprogramming of human neural stem cells into pluripotency. PLoS One. 2009;4(9):e7044.
4. Liu T, Zou G, Gao Y, Zhao X, Wang H, Huang Q, Jiang L, Guo L, Cheng W. High efficiency of reprogramming CD34(+) cells derived from human amniotic fluid into induced pluripotent stem cells with Oct4. Stem Cells Dev. 2012;21(12):2322–2332.
5. Loh YH, Hartung O, Li H, Guo C, Sahalie JM, Manos PD, Urbach A, Heffner GC, Grskovic M, Vigneault F, Lensch MW, Park IH, Agarwal S, Church GM, Collins JJ, Daley GQ. Reprogramming of T cells from human peripheral blood. Cell Stem Cell. 2010;7(1):15–19.
6. Umegaki-Arao N, Pasmooij AM, Itoh M, Cerise JE, Guo Z, Levy B, Gostynski A, Rothman LR, Jonkman MF, Christiano AM. Induced pluripotent stem cells from human revertant keratinocytes for the treatment of epidermolysis bullosa. Sci Transl Med. 2014;6(264):264ra164.
7. Chang CY, Ting HC, Su HL, Jeng JR. Combining induced pluripotent stem cells and genome editing technologies for clinical applications. Cell Transplant. Epub ahead of print 17 February 2017. DOI: 10.3727/096368917X695119
8. Alves CJ, Dariolli R, Jorge FM, Monteiro MR, Maximino JR, Martins RS, Strauss BE, Krieger JE, Callegaro D, Chadi G. Gene expression profiling for human iP3S-derived motor neurons from sporadic ALS patients reveals a strong association between mitochondrial functions and neurodegeneration. Front Cell Neurosci. 2015;9:289.
9. An MC, Zhang N, Scott G, Montoro D, Wittkop T, Mooney S, Melov S, Ellerby LM. Genetic correction of Huntington's
disease phenotypes in induced pluripotent stem cells. Cell Stem Cell. 2012;11(2):253–263.

10. Burkhardt MF, Martinez FJ, Wright S, Ramos C, Volfson D, Mason M, Games J, Dang V, Lievers J, Shoukat-Muntaz U, Martinez R, Gai H, Blake R, Vaisberg E, Griskovic M, Johnson C, Iriom S, Bright J, Cooper B, Nguyen L, Griswold-Prenner I, Jawaherian A. A cellular model for sporadic ALS using patient-derived induced pluripotent stem cells. Mol Cell Neurosci. 2013;56:355–364.

11. Chang CY, Chen SM, Lu HE, Lai SM, Lai PS, Shen PW, Chen PY, Shen CI, Harn HJ, Lin SZ, Hwang S-M, Su H-L. N-butylidenephthalide attenuates Alzheimer’s disease-like cytopathy in Down syndrome induced pluripotent stem cell-derived neurons. Sci Rep. 2015;5:8744.

12. Chen C, Jiang P, Xue H, Peterson SE, Tran HT, McCann AE, Parasit MM, Li S, Pleasure DE, Laurent LC, Loring J, Liu Y, Deng W. Role of astroglia in Down’s syndrome revealed by patient-derived human-induced pluripotent stem cells. Nat Commun. 2014;5:4430.

13. Chen H, Qian K, Du Z, Cao J, Petersen A, Liu H, Blackbourn LWt, Huang CL, Errigo A, Yin Y, Lu J, Ayala M, Zhang SC. Modeling ALS with iPSCs reveals that mutant SOD1 misregulates neurofilament balance in motor neurons. Cell Stem Cell. 2014;14(6):796–809.

14. Consortium HDi. Induced pluripotent stem cells from patients with Huntington’s disease show CAG-repeat-expansion-associated phenotypes. Cell Stem Cell. 2012;11(2):264–278.

15. Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T, et al. Pharmacological rescue of mitochondrial deficits in iPSCs reveals that mutant SOD1 misregulates neurofilament balance in motor neurons. Cell Stem Cell. 2012;14(1):141ra90.

16. Devine MJ, Ryten M, Vodicka P, Thomson AJ, Burdon T, Houlden H, Cavaleri F, Nagano M, Drummond NJ, Taanman JW, Schapira AH, Gwinn K, Hardy J, Lewis PA, Kunath T. Parkinson’s disease induced pluripotent stem cells with tripllication of the alpha-synuclein locus. Nat Commun. 2011;2:440.

17. Eber AD, Yu J, Rose FF Jr., Mattis VB, Lorson CL, Thomson JA, Svendsen CN. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature. 2009;457(7227):277–280.

18. Egawa N, Kitaoka S, Tsukita K, Naitoh M, Takahashi K, Yamamoto T, Adachi F, Kondo T, Okita K, Asaka I, et al. Drug screening for ALS using patient-induced pluripotent stem cells. Sci Transl Med. 2012;4(145):145ra104.

19. Ferrarulo L, Meyer K, Sherwood TW, Vick J, Likhite S, Frakes A, Miranda CJ, Braun L, Heath PR, Pineda R, Beattie CE, Shaw PJ, Askwith CC, McGtigue D, Kaspar BK. Oligodenrocyes contribute to motor neuron death in ALS via SOD1-dependent mechanism. Proc Natl Acad Sci USA. 2016;113(42):E6496–E6505.

20. Fuller HR, Mandefro B, Shirron SL, Gross AR, Kaus AS, Botting CH, Morris GE, Sareen D. Spinal muscular atrophy patient iPSC-derived motor neurons have reduced expression of proteins important in neuronal development. Front Cell Neurosci. 2015;9:506.

21. Guo W, Naujock M, Fumagalli L, Vandoorne T, Baetsen P, Boon R, Ordovaln L, Patel A, Welters M, Vanwelden T, et al. HDAC6 inhibition reverses axonal transport defects in motor neurons derived from FUS-ALS patients. Nat Commun. 2017;8(1):861.

22. Ichiyangi N, Fujimori K, Yano M, Ishihara-Fujisaki C, Sone T, Akiya Y, Okada Y, Akamatsu M, Matsumoto T, Ishikawa M, Nishimoto Y, Ishihara Y, Sakuma T, Yamamoto T, Tsuji H, Suzuki N, Warita H, Aoki M, Okano H. Establishment of in vitro FUS-associated familial amyotrophic lateral sclerosis model using human induced pluripotent stem cells. Stem Cell Reports. 2016;6(4):496–510.

23. Imamura K, Izumi Y, Watanabe A, Tsukita K, Woltjen K, Yamamoto T, Hotta A, Kondo T, Kitaoa S, Ohta A, et al. The Src/c-Abl pathway is a potential therapeutic target in amyotrophic lateral sclerosis. Sci Transl Med. 2017;9(391): pii: eaaf3962.

24. Ishida Y, Kawakami H, Kitajima H, Nishiyama A, Sasai Y, Inoue H, Muguruma K. Vulnerability of Purkinje Cells generated from spinocerebellar ataxia type 6 patient-derived iPSCs. Cell Rep. 2016;17(6):1482–1490.

25. Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, Carson CT, Laurent LC, Marsala M, Gage FH, Remes AM, Koo EH, Goldstein LSB. Probing sporadic and familial Alzheimer’s disease using induced pluripotent stem cells. Nature. 2012;482(7384):216–220.

26. Jeon I, Lee N, Li JY, Park IH, Park KS, Moon J, Shim SH, Choi C, Chang DJ, Kwon J, Oh SH, Shin DA, Kim HS, Do JT, Lee DR, Kim M, Kang KS, Daeley GQ, Brundin P, Song J. Neuronal properties, in vivo effects, and pathology of a Huntington’s disease patient-derived induced pluripotent stem cells. Stem Cells. 2012;30(9):2054–2062.

27. Kiskinis E, Sandoe J, Williams LA, Bouling GL, Moccia R, Waininger BJ, Han S, Peng T, Thams S, Mikkilineni S, et al. Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1. Cell Stem Cell. 2014;14(6):781–795.

28. Koch P, Breuer P, Peitz M, Jungverdorben J, Kesavan J, Poppe D, Doerr J, Ladewig J, Mertens J, Tütting T, Hoffmann P, Klockgether T, Evert BO, Wüllner U, Bürstle O. Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. Nature. 2011;480(7378):543–546.

29. Kondo T, Asai M, Tsukita K, Kutoy Y, Ohsawa Y, Sunada Y, Imamura K, Egawa N, Yahata N, Okita K, et al. Modeling Alzheimer’s disease with iPSCs reveals stress phenotypes associated with intracellular Abeta and differential drug responsiveness. Cell Stem Cell. 2013;12(4):487–496.

30. Kondo T, Imamura K, Funayama M, Tsukita K, Miyake M, Ohta A, Woltjen K, Nakagawa M, Asada T, Arai T, Kawakatsu
S, Izumi Y, Kaji R, Iwata N, Inoue H. iPSC-based compound screening and in vitro trials identify a synergistic anti-amyloid beta combination for Alzheimer’s disease. Cell Rep. 2017; 21(8):2304–2312.

31. Lin X, Li JJ, Qian WJ, Zhang QJ, Wang ZF, Lu YQ, Dong EL, He J, Wang N, Ma LX, Chen WJ. Modeling the differential phenotypes of spinal muscular atrophy with high-yield generation of motor neurons from human induced pluripotent stem cells. Oncotarget. 2017;8(26):42030–42042.

32. Miller JD, Ganat YM, Raja WK, Mungenast AE, Lin YT, Ko T, Abdurrob F, Seo J, Ou Z, Luo M, Niu X, Chen Y, Xie Y, He W, Song B, Xian Y, Nekrasov ED, Vigont VA, Klyushnikov SA, Lebedeva OS, Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Mitne-Neto M, Machado-Costa M, Marchetto MC, Bengtson YA, Paschon DE, Vangipuram M, Sundararajan R, Urnov FD, Klahr DJ, Dranovsky A, Arancio O, Crary JF, Gandy S, Nagae SA. Characterization and molecular profiling of PSEN1 familial Alzheimer’s disease iPSC-derived neural progenitors. PLoS One. 2014;9(1):e84547.

33. Weick JP, Held DL, Bonadurer GF III, Doers ME, Liu Y, Maguire C, Clark A, Knackert JA, Molinarolo K, Musser M, Yao L, Yin Y, Lu J, Zhang X, Zhang SC, Bhattacharyya A. Deficits in human trisomy 21 iPSCs and neurons. Proc Natl Acad Sci USA. 2013;110(24):9962–9967.

34. Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, Yamanaka S, Okano H, Suzuki N. Modeling familial Alzheimer’s disease with induced pluripotent stem cells. Hum Mol Genet. 2011;20(18):3642–3652.

35. Nekrasov ED, Vigont VA, Klyushnikov SA, Lebedeva OS, Vassina EM, Bogomazova AN, Chestkov IV, Semashko TA, Kiseleva E, Sarkhel I, Labkovsky PA, Zimin OA, Ryazantseva MA, Skopin AY, Illarionshik SN, Kazmacheyeva EV, Lagarkova MA, Kiselev SL. Manifestation of Huntington’s disease pathology in human induced pluripotent stem cell-derived neurons. Mol Neurodegener. 2014;23(13):3523–3536.

36. Ou Z, Luo M, Niu X, Chen Y, Xie Y, He W, Song B, Xian Y, Fan D, Ou Yang S, Sun X. Autophagy promoted the degradation of mutant ATXN3 in neurally differentiated spinocerebellar Ataxia-3 human induced pluripotent stem cells. Biomed Res Int. 2016;2016:6701793.

37. Raja WK, Munogenst AE, Lin YT, Ko T, Abdurrob F, Seo J, Tsai LH. Self-organizing 3D human neural tissue derived from human induced pluripotent stem cells of ALS8 patients. Mol Neurodegener. 2016;11:27.

38. Sanchez-Danes A, Richaud-Patin Y, Carballo-Carballo I, Jimenez-Delgado S, Caig C, Mora S, Di Guglielmo C, Ezquerra M, Patel B, Giral A, Canals JM, Memo M, Alberch J, Lopez-Barneo J, Vila M, Cuervo AM, Tolosa E, Consiglio A, Raya A. Disease-specific phenotypes in dopamine neurons from human iPSC-based models of genetic and sporadic Parkinson’s disease. EMBO Mol Med. 2012;4(5):380–395.

39. Sanders LH, Laganiere J, Cooper O, Mak SK, Vu BJ, Huang YA, Paschon DE, Vangipuram M, Sundararajan R, Uren FV, Langston JW, Gregory PD, Zhang HS, Greenamyre JT, Isacson O, Schulte B. LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson’s disease patients: reversal by gene correction. Neurobiol Dis. 2014;62:381–386.

40. Serio A, Bilican B, Barnada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phattanai HP, Shum C, Wilmut I, Maniatis T, Shaw CE, Finkbeiner S, Chandran S. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. Proc Natl Acad Sci USA. 2013;110(12):4697–4702.

41. Shi Y, Kirwan P, Smith J, MacLean G, Orkin SH, Livesey FJ. A human stem cell model of early Alzheimer’s disease pathology in Down syndrome. Sci Transl Med. 2012;4(124):124ra29.

42. Sproul AA, Jacob S, Pre D, Kim SH, Nestor MW, Navarro-Sobrino M, Santa-Maria I, Zimmer M, Aubry S, Steele JW, Kahler DJ, Dranovsky A, Arancio O, Crary JF, Gandy S, Nagae SA. Characterization and molecular profiling of PSEN1 familial Alzheimer’s disease iPSC-derived neural progenitors. PLoS One. 2014;9(1):e84547.
human cells with synthetic modified mRNA. Cell Stem Cell. 2010;7(5):618–630.

51. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell. 2009;4(6):472–476.

52. Huangfu D, Maehr R, Guo W, Ejikelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol. 2008;26(7):795–797.

53. Mali P, Chou BK, Ye Z, Zou J, Dowey S, Brodsky RA, Ohm JE, Yu W, Baylin SB, Yusa K, Bradley A, Meyers DJ, Mukherjee C, Cole PA, Cheng L. Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. Stem Cells. 2010;28(4):713–720.

54. Zhang Z, Wu WS. Sodium butyrate promotes generation of human induced pluripotent stem cells through induction of the miR302/367 cluster. Stem Cells Dev. 2013;22(16):2268–2277.

55. Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K, Ge J, Xu J, Zhang Q, Zhao Y, Deng H. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science. 2013;341(6146):651–654.

56. Liu P, Chen M, Liu Y, Qi LS, Ding S. CRISPR-based chromatin remodeling of the endogenous Oct4 or Sox2 locus enables reprogramming to pluripotency. Cell Stem Cell. 2018;22(2):252–261 e4.

57. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145–1147.

58. Nakagawa M, Taniguchi Y, Senda S, Takizawa N, Ichisaka T, Asano K, Morizane A, Doi D, Takahashi J, Nishizawa M, Yoshida Y, Toyoda T, Osafune K, Sekiguchi K, Yamanaka S. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. Sci Rep. 2014;4:3594.

59. Beers J, Gulbranson DR, George N, Siniscalchi LI, Jones J, Thomson JA, Chen G. Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. Nat Protoc. 2012;7(11):2029–2040.

60. Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, Smuga-Otto K, Howden SE, Diol NR, Propson NE, Wagner R, Lee GO, Antosiewicz-Bourget J, Teng JM, Thomson JA. Chemically defined conditions for human iPSC derivation and culture. Nat Methods. 2011;8(5):424–429.

61. Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conard KR, Piekarz MS, Llanas RA, Thomson JA. Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol. 2006;24(2):185–187.

62. Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Nishikawa S, Muguruma K, Sasai Y. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol. 2007;25(6):681–686.

63. Ogushii M, Matsumura M, Eiraku M, Murakami K, Aramaki T, Nishiyama A, Muguruma K, Nakano T, Suga H, Ueno M, Ishizaki T, Suemori H, Narumiya S, Niwa H, Sasai Y. Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. Cell Stem Cell. 2010;7(2):225–239.

64. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPSCs by dual inhibition of SMAD signaling. Nat Biotechnol. 2009;27(3):275–280.

65. Chen SM, Lee MS, Chang CY, Lin SZ, Cheng EH, Liu YH, Pan HC, Lee HC, Su HL. Prerequisite OCT4 maintenance potentiates the neural induction of differentiating human embryonic stem cells and induced pluripotent stem cells. Cell Transplant. 2015;24(5):829–844.

66. Petros TJ, Tyson JA, Anderson SA. Pluripotent stem cells for the study of CNS development. Front Mol Neurosci. 2011;4:30.

67. Perrier AL, Tabar V, Barberi T, Rubio ME, Bruses J, Topf N, Harrison NL, Studer L. Derivation of midbrain dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci USA. 2004;101(34):12543–12548.

68. Fritzsch B, Jahan I, Pan N, Elliott KL. Evolving gene regulatory networks into cellular networks guiding adaptive behavior: An outline how single cells could have evolved into a centralized neurosensory system. Cell Tissue Res. 2015;359(1):295–313.

69. Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, Yang L, Beal MF, Surmeier DJ, Kordower JH, Tabar V, Studer L. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson’s disease. Nature. 2011;480(7378):547–551.

70. Xi J, Liu Y, Liu H, Chen H, Emborg ME, Zhang SC. Specification of midbrain dopamine neurons from primate pluripotent stem cells. Stem Cells. 2012;30(8):1655–1663.

71. Bain J, Plater L, Elliott M, Shipiro N, Haste CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, Cohen P. The selectivity of protein kinase inhibitors: a further update. Biochem J. 2007;408(3):297–315.

72. Doi D, Samata B, Katsukawa M, Kikuchi T, Morizane A, Ono Y, Sekiguchi K, Nakagawa M, Parmar M, Takahashi J. Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Reports. 2014;2(3):337–350.

73. Samata B, Doi D, Nishimura K, Kikuchi T, Watanabe A, Sakamoto Y, Kakuta J, Ono Y, Takahashi J. Purification of functional human ES and iPSC-derived midbrain dopaminergic progenitors using LRTM1. Nat Commun. 2016;7:13097.

74. Sanches S, Bruijn LI, Chandran S, Eggan K, Ho R, Klim JR, Livesey MR, Lowry E, Macklis JD, Rushton D, Sadegh C, Sareen D, Wichterle H, Zhang SC, Svendsen CN. Modeling ALS with motor neurons derived from human induced pluripotent stem cells. Nat Neurosci. 2016;19(4):542–553.
75. Maury Y, Come J, Piskorowski RA, Salah-Mohellibi N, Chevaleyre V, Peschanski M, Martinat C, Nedelee S. Combinatorial analysis of developmental cues efficiently converts human pluripotent stem cells into multiple neuronal subtypes. Nat Biotechnol. 2015;33(1):89–96.

76. Du ZW, Chen H, Liu H, Lu J, Qian K, Huang CL, Zhong X, Fan F, Zhang SC. Generation and expansion of highly pure motor neuron progenitors from human pluripotent stem cells. Nat Commun. 2015;6:6626.

77. Liu Y, Liu H, Sauvey C, Yao L, Zarnowska ED, Zhang SC. Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. Nat Protoc. 2013;8(9):1670–1679.

78. Maroof AM, Keros S, Tyson JA, Ying SW, Ganat YM, Merkle FT, Liu B, Goulburn A, Stanley EG, Elefanty AG, Widmer HR, Eggan K, Goldstein PA, Anderson SA, Studer L. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell. 2013;12(5):559–572.

79. Lu J, Zhong X, Liu H, Hao L, Huang CT, Sherafat MA, Jones J, Ayala M, Li L, Zhang SC. Generation of serotonin neurons from human pluripotent stem cells. Nat Biotechnol. 2016;34(1):89–94.

80. Li XJ, Zhang X, Johnson MA, Wang ZB, Lavaute T, Zhang SC. Coordination of sonic hedgehog and Wnt signaling determines ventral and dorsal telencephalic neuron types from human embryonic stem cells. Development. 2009;136(23):4055–4063.

81. Muguruma K, Nishiyama A, Ono Y, Miyawaki H, Mizuhara E, Hori S, Kakizuka A, Obata K, Yanagawa Y, Hirano T, Sasai Y. Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. Nat Neurosci. 2010;13(10):1171–1180.

82. Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. Cell Rep. 2015;10(4):537–550.

83. Chandrasekaran A, Avci HX, Leist M, Kobolak J, Dinnyes A. Astrocyte differentiation of human pluripotent stem cells: new tools for neurological disorder research. Front Cell Neurosci. 2016;10:215.

84. Dezonne RS, Sartore RC, Nascimento JM, Saia-Cereda VM, Romao LF, Alves-Leon SV, de Souza JM, Martins-de-Souza D, Rehen SK, Gomes FC. Derivation of functional human astrocytes from cerebral organoids. Sci Rep. 2017;7:45091.

85. Wang S, Bates J, Li X, Schanz S, Chandler-Militello D, Levine C, Maherali N, Studer L, Hochedlinger K, Windrem M, Goldman MA. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell. 2013;12(2):252–264.

86. Muffat J, Li Y, Yuan B, Mitalipova M, Omer A, Corcoran S, Bakiasi G, Tsai LH, Aubourg P, Ransohoff RM, Jaenisch R. Efficient derivation of microglia-like cells from human pluripotent stem cells. Nat Med. 2016;22(11):1358–1367.

87. Pandya H, Shen MJ, Ichikawa DM, Sedlock AB, Choi Y, Johnson KR, Kim G, Brown MA, Elkahlon AG, Marie D, Sweeney CL, Gossa S, Malech HL, McGavern DB, Park JK. Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. Nat Neurosci. 2017;20(5):753–759.

88. Elstner M, Morris CM, Heim K, Bender A, Mehta D, Jaros E, Klopstock T, Meitinger T, Turnbull DM, Prokisch H. Expression analysis of dopaminergic neurons in Parkinson’s disease and aging links transcriptional dysregulation of energy metabolism to cell death. Acta Neuropathol. 2011;122(1):75–86.

89. Naoi M, Maruyama W. Cell death of dopamine neurons in aging and Parkinson’s disease. Mech Ageing Dev. 1999;111(2-3):175–188.

90. Song J, Kim J. Degeneration of dopaminergic neurons due to metabolic alterations and Parkinson’s disease. Front Aging Neurosci. 2016;8:65.

91. Caires-Junior LC, Goulart E, Melo US, Araujo BSH, Alvizzi L, Soares-Schansoski A, De Oliveira DF, Kobayashi GS, Gries-Oliveira K, Musso CM, et al. Discordant congenital Zika syndrome twins show differential in vitro viral susceptibility of neural progenitor cells. Nat Commun. 2018;9(1):475.

92. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y. Self-organized generation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell. 2008;3(5):519–532.

93. Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, Sekiguchi K, Adachi T, Sasai Y. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature. 2011;472(7341):51–56.

94. Kadoshima T, Sakaguchi H, Nakano T, Soen M, Ando S, Eiraku M, Sasai Y. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. Proc Natl Acad Sci USA. 2013;110(50):20284–20289.

95. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA. Cerebral organoids model human brain development and microcephaly. Nature. 2013;510(7516):373-379.

96. Dang J, Tiwari SK, Lichinchi G, Qin Y, Patil VS, Eroshkin AM, Rana TM. Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. Cell Stem Cell. 2016;19(2):258–265.

97. Pasca AM, Sloan SA, Clarke LE, Tian Y, Makinson CD, Huber JA, Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. Cell Rep. 2015;10(4):537–550.

98. Pasca AM, Sloan SA, Clarke LE, Tian Y, Makinson CD, Huber JA. Cerebral organoids model human brain development and microcephaly. Nature. 2013;510(7516):373-379.

99. Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van der Weerd T, Jarazo J, Walter J, Bruggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC. Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells in 3D culture. Nat Methods. 2015;12(7):671–678.

100. Sakaguchi H, Kadoshima T, Soen M, Narri N, Ishida Y, Ohgushi M, Takahashi J, Eiraku M, Sasai Y. Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. Nat Commun. 2015;6:8896.
Derivation of human midbrain-specific organoids from neuroepithelial stem cells. Stem Cell Reports. 2017;8(5):1144–1154.

100. Jo J, Xiao Y, Sun AX, Cukuroglu E, Tran HD, Goke J, Tan ZY, Saw TY, Tan CP, Lokman H, et al. Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. Cell Stem Cell. 2016;19(2):248–257.

101. Ranga A, Girgin M, Meinhardt A, Eberle D, Caiazzo M, Tanaka EM, Lutolf MP. Neural tube morphogenesis in synthetic 3D microenvironments. Proc Natl Acad Sci USA. 2016;113(44):E6831–E6839.

102. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci USA. 1996;93(3):1156–1160.

103. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2010;11(9):636–646.

104. Urnov FD, Miller JC, Lee YL, Beausejour CM, Porteus MH, Gregory PD, Holmes MC. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature. 2005;435(7042):646–651.

105. Bogdanove AJ, Voytas DF. TAL effectors: Customizable proteins for DNA targeting. Science. 2011;333(6051):1843–1846.

106. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. Breaking the code of DNA binding specificity of TAL-type III effectors. Science. 2009;326(5959):1509–1512.

107. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009;326(5959):1501.

108. Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013;31(3):230–232.

109. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819–823.

110. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823–826.

111. Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F, Baru V, Lou Y, Freyzon Y, Cho S, Mungenast AE, Muffat J, Mitalipova M, Pluth MD, Jui NT, Schüle B, Lippard SJ, Tsai LH, Kraince D, Buchwald SL, Jaenisch R, Lindquist S. Identification and rescue of alpha-synuclein toxicity in Parkinson patient-derived neurons. Science. 2013;342(6161):983–987.

112. Young CS, Hicks MR, Ermolova NV, Nakano H, Jan M, Younesi S, Karumbayaram S, Kumagai-Cresse C, Wang D, Zack JA, Kohn DB, Nakano A, Nelson SF, Miceli MC, Spencer MJ, Pyle AD. A single CRISPR-Cas9 deletion strategy that targets the majority of DMD patients restores dystrophin function in hiPSC-derived muscle cells. Cell Stem Cell. 2016;18(4):533–540.

113. Park CY, Kim DH, Son JS, Sung JJ, Lee J, Bae S, Kim JH, Kim DW, Kim JS. Functional correction of large factor VIII gene chromosomal inversions in hemophilia A patient-derived iPSCs using CRISPR-Cas9. Cell Stem Cell. 2015;17(2):213–220.

114. Jiang J, Jing Y, Cost GJ, Chiang JC, Kolpa HJ, Cotton AM, Carone DM, Carone BR, Shivak DA, Guschin DY, Pearl JR, Rebar EJ, Byron M, Gregory PD, Brown CI, Urnov FD, Hall LL, Lawrence JB. Translating dosage compensation to trisomy 21. Nature. 2013;500(7462):296–300.

115. Liu XS, Wu H, Ji X, Stelzer Y, Wu X, Czauderna S, Shu J, Dadon D, Young RA, Jaenisch R. Editing DNA methylation in the mammalian genome. Cell. 2016;167(1):233–247e17.