

## REVIEW

## Modeling human neurological disorders with induced pluripotent stem cells

Yoichi Imaizumi\*<sup>†</sup> and Hideyuki Okano\*<sup>\*</sup>Department of Physiology, Keio University School of Medicine, Tokyo, Japan<sup>†</sup>Next Generation Systems CFU, Eisai Co. Ltd., Ibaraki, Japan**Abstract**

Human induced pluripotent stem (iPS) cells obtained by reprogramming technology are a source of great hope, not only in terms of applications in regenerative medicine, such as cell transplantation therapy, but also for modeling human diseases and new drug development. In particular, the production of iPS cells from the somatic cells of patients with intractable diseases and their subsequent differentiation into cells at affected sites (e.g., neurons, cardiomyocytes, hepatocytes, and myocytes) has permitted the *in vitro* construction of disease models that contain patient-specific genetic information. For example, disease-specific iPS cells have been established from patients with neuropsychiatric disorders, including schizophrenia and autism, as well as from those with neurodegenerative diseases, including Parkinson's dis-

ease and Alzheimer's disease. A multi-omics analysis of neural cells originating from patient-derived iPS cells may thus enable investigators to elucidate the pathogenic mechanisms of neurological diseases that have heretofore been unknown. In addition, large-scale screening of chemical libraries with disease-specific iPS cells is currently underway and is expected to lead to new drug discovery. Accordingly, this review outlines the progress made via the use of patient-derived iPS cells toward the modeling of neurological disorders, the testing of existing drugs, and the discovery of new drugs.

**Keywords:** human disease model, induced pluripotent stem cells, neurological disorders, Parkinson's disease.

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The advent of an aging society is accompanied not only by increases in cancer and heart disease but also by increases in chronic and age-related diseases. Analyses of the pathological mechanisms of various chronic diseases and the development of new therapies for their management are currently underway, based in large part on the impressive research advances of recent years. However, numerous disorders remain with no established means of treating the underlying cause. The fact that complete human disease models are unavailable for these conditions is cited as a major problem in terms of developing new drugs for their control. While animal disease models and human disease-mimetic cell lines have been developed, construction of models that can accurately and thoroughly reproduce human pathology remains difficult. Furthermore, there is ample room for debate as to whether animal and cell line disease models can correctly reflect the phenomena that actually occur in human patients, because of species-specific differences and differences in cell line specificity. Moreover, no disease models exist for many of the rarer conditions.

In recent years, however, Professor Yamanaka of Kyoto University (Kyoto, Japan) launched a method for the

preparation of induced pluripotent stem (iPS) cells that have almost the same pluripotency as embryonic stem (ES) cells. This was done by introducing four reprogramming genes, *Oct4*, *Sox2*, *Klf4*, and *c-Myc*, into differentiated somatic cells (Takahashi and Yamanaka 2006; Takahashi *et al.* 2007). Yamanaka's method made it possible (with some exceptions) to establish iPS cells from the somatic cells of any individual, regardless of race, genetic background, or state of health (i.e., whether afflicted with a disease). Moreover, the development of *in vitro* differentiation protocols for ES cells toward each

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Address correspondence and reprint requests to Hideyuki Okano, Department of Physiology, Keio University, School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: hidokano@a2.keio.jp

**Abbreviations used:** AD, Alzheimer's disease; APP, amyloid precursor protein; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazine; ERK1/2, extracellular signal-regulated kinase 1/2; ES cell, embryonic stem cell; FD, familial dysautonomia; GSH, reduced glutathione; iPS cell, induced pluripotent stem cell; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease.

embryonic germ layer paved the way for researchers to apply these techniques to iPS cells, allowing the production of a variety of iPS-derived cells, including hepatocytes, neurons, and cardiomyocytes (Takahashi *et al.* 2007). For example, the use of iPS cells derived from patients with certain neurological diseases permits the preparation of brain cells that contain the actual genetic information of the patients themselves. This is a notable feat, given that such cells have been technologically and ethically difficult to obtain in the past. Moreover, as long as the *in vitro* differentiation system is in place, it may be feasible to produce human disease models for diseases whose causative gene is unknown.

The pathological investigation of disease progression, including disease onset and the time course of disease advance, requires human materials. These materials are difficult to obtain in practice and until recently, researchers had to utilize the tissues of patients in both the early phase and the asymptomatic stage of a particular disease. However, thanks to the present revolution in iPS cell technology, cells differentiated *in vitro* from iPS cells can be used instead of human tissues for these purposes. Furthermore, iPS cell technology can be applied to chemical library screening for drug discovery, as well as to subsequent testing for drug toxicity and efficacy (Fig. 1). As a result, it is expected that the enormous cost and time involved in drug discovery research will be streamlined, and that the ability to discover new drugs will be improved.

In this review article, we outline the current status of neurological disease-specific iPS cell research. In particular, we describe recently obtained knowledge in the form of actual examples from the literature.

### Driving iPS cell neural differentiation

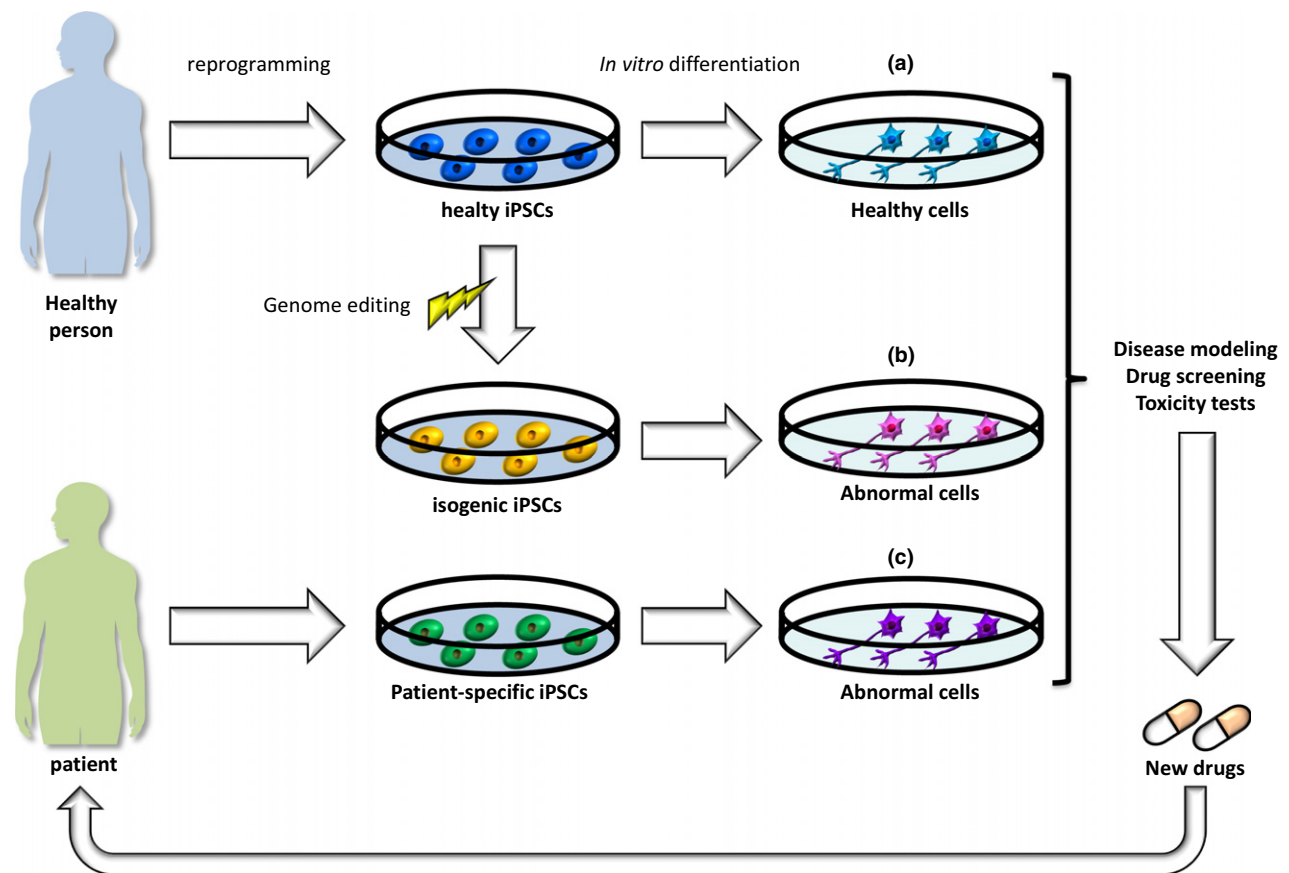
Modeling a neurological disease requires developing methods to mimic development to make defined cultures of neurons and/or glia. So far, many studies involving the induction of various types of neurons from ES cells have allowed following the developmental process *in vitro*. Although co-culture with stromal cells, such as PA6, and/or spontaneous aggregation called embryoid bodies were directed to form neural cells in early studies (Kawasaki *et al.* 2000; Okada *et al.* 2004), recent protocols provided us more efficient and specific neural differentiation with a combination of small-molecules in a feeder-free culture system. Dual SMAD signal inhibition by supplementing Noggin and SB431542, inhibiting bone morphogenetic protein (BMP) and transforming growth factor beta, respectively, contributed to rapid and high efficacy of neuroepithelial cells (Chambers *et al.* 2009). These neuroepithelial cells have potential to differentiate into different region-specific central nervous system neurons using appropriate cues, such as Sonic Hedgehog (Shh) and Wnt8 (for midbrain dopaminergic neurons) (Fasano *et al.* 2010; Kriks *et al.* 2011), retinoic

acid (RA) and Shh (for spinal cord motor neurons) (Li *et al.* 2005), Shh (for forebrain  $\gamma$ -aminobutyric acid (GABA) interneurons) (Liu *et al.* 2013a). These recent studies provide a promising strategy for controlled production of specific neurons for neurological disorders.

### Modeling neurological diseases in vitro with disease-specific iPS cells

The iPS cell technology has rapidly expanded worldwide in less than 5 years. Disease-specific iPS cells are now available from patients with a variety of conditions, including nervous system, hematopoietic system, and metabolic system diseases, and investigations of their pathology are progressing at a brisk pace (Dimos *et al.* 2008; Park *et al.* 2008; Bellin *et al.* 2012; Robinton and Daley 2012).

Previous explorations of human neurological and psychiatric disorders were hampered by the difficulty in obtaining patient-derived neural cells or tissues because of the limited accessibility to the brain, except for autopsy samples. On the other hand, researchers have long used patient-derived fibroblasts or immortalized lymphoblasts for study, but these cells do not always recapitulate the pathogenic events of neurological and psychiatric disorders. To overcome these limitations, researchers now take advantage of olfactory tissue, with its enormous capacity for neurogenesis (Makay-Sim, 2013; Sawa and Cascella, 2009; Kano *et al.*, 2013), as well as neural cells induced from disease-specific iPS cells to examine the pathophysiology of these conditions. In fact, a variety of iPS cells derived from patients with the following neurological and psychiatric conditions are currently in wide use: Alzheimer's disease (AD) (Yagi *et al.* 2011; Israel *et al.* 2012; Kondo *et al.* 2013), Parkinson's disease (PD) (Devine *et al.* 2011; Nguyen *et al.* 2011; Seibler *et al.* 2011; Cooper *et al.* 2012; Imaizumi *et al.* 2012; Jiang *et al.* 2012; Liu *et al.* 2012a; Rakovic *et al.* 2012; Sanchez-Danes *et al.* 2012; Reinhardt *et al.* 2013), amyotrophic lateral sclerosis (Dimos *et al.* 2008; Mitne-Neto *et al.* 2011; Bilican *et al.* 2012; Egawa *et al.* 2012), Huntington's disease (Park *et al.* 2008; Zhang *et al.* 2010; An *et al.* 2012; Camnasio *et al.* 2012; HD iPSC Consortium 2012; Jeon *et al.* 2012; Juopperi *et al.* 2012), spinal muscular atrophy (Ebert *et al.* 2009; Chang *et al.* 2011), spinal and bulbar muscular atrophy (Nihei *et al.* 2013), Rett's syndrome (Marchetto *et al.* 2010; Muotri *et al.* 2010; Ananiev *et al.* 2011; Cheung *et al.* 2011; Ricciardi *et al.* 2012), schizophrenia (Brennand *et al.* 2011; Chiang *et al.* 2011; Pedrosa *et al.* 2011; Paulsen Bda *et al.* 2012), Down syndrome (Park *et al.* 2008; Li *et al.* 2012; Weick *et al.* 2013), Dravet syndrome (Higurashi *et al.* 2013; Jiao *et al.* 2013; Liu *et al.* 2013b), familial dysautonomia (FD) (Lee *et al.* 2009, 2012), adrenoleukodystrophy (Jang *et al.* 2011), Cockayne's syndrome (Andrade *et al.* 2012), fragile X-associated tremor/ataxia syndrome (Liu *et al.* 2012b; Crompton *et al.* 2013),



**Fig. 1** Application of induced pluripotent stem (iPS) cell technology in disease research. (a, c) iPS cells are established by introducing Yamanaka's four factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) into healthy human and patient-derived somatic cells. Later, the iPS cells are induced to differentiate into target cells. The differentiated target cells can then be applied to the analysis of disease pathology, the screening of chemical libraries to identify drug candidates, and toxicity and efficacy testing of the newly identified compounds. Thus, iPS cells are

crucially linked to new drug development. (b) Monogenic mutations are induced in iPS cells derived from healthy subjects via genome editing technologies by using helper-dependent adenoviral vectors, the zinc-finger nucleases (ZFNs), the TALENs, and the crisper-Cas9 system, which have all been developed in recent years. The use of isogenic iPS cells makes it possible to precisely analyze pathogenetic mechanisms that are attributable to the effects of a single gene.

and Machado-Joseph disease (Koch *et al.* 2011) (Table 1). We will introduce some examples of the use of these disease-specific iPS cells for the characterization of human neurological disorders in the following sections.

### Modeling Familial PDs with disease-specific iPS cells

A number of disease-specific iPS cells were originally obtained from patients with genetic diseases, in which the causative gene was identified mainly because of recent advances in sequencing technology. The incidence rate of these diseases is low, and accordingly, the development of treatment modalities has in general been delayed. These diseases may also be described as conditions in which the cause-effect relationship can readily be identified between the abnormality exhibited by neurons differentiated from the

disease-specific iPS cells and the mutation or deletion of the causative gene. The advantages of disease-specific iPS cells have been greatly exploited for rare genetic diseases.

Disease-specific iPS cell research directed toward familial PD is especially active. Parkinson's disease is the second most common neurodegenerative disease after AD. More than 4 million patients are afflicted with PD worldwide, and the prevalence in Japan is about 100–150 cases per population of 0.5 million individuals. There is currently no method of treatment for the underlying cause, and because many patients become symptomatic from the latter half of the 6th decade until the 7th decade of life, the management of PD is a major issue in countries facing an aging population. Although the exact cause-effect relationship of the disorder remains undetermined, PD is thought to stem from a loss of dopaminergic neurons in the substantia nigra of the midbrain. As a result, dopamine content falls below 20% of its normal

**Table 1** Neurological diseases modeled with iPSCs

Disease	Gene	Cell type differentiated from iPSCs	Drug tests	References
Adrenoleukodystrophy	ABCD1	Oligodendrocytes and neurons	Lovastatin, 4-phenylbutyrate	(Jang <i>et al.</i> 2011)
Alzheimer's disease	PS1, PS2, APP, sporadic	Cortical neurons	$\gamma$ -, $\beta$ -secretase inhibitor, docosahexaenoic acid	(Yagi <i>et al.</i> 2011; Israel <i>et al.</i> 2012; Kondo <i>et al.</i> 2013)
Amyotrophic lateral sclerosis	SOD1, VAPB, TDP43	Motor neurons and glial cells	Anacardic acid, trichostatin A, spliceostatin A, garcinol	(Dimos <i>et al.</i> 2008; Mitne-Neto <i>et al.</i> 2011; Bilican <i>et al.</i> 2012; Egawa <i>et al.</i> 2012)
Cockayne's syndrome	ERCC6	iPSCs and neurons	N/A	(Andrade <i>et al.</i> 2012)
Down syndrome	Trisomy 21	Neurons and neural progenitors	N/A	(Park <i>et al.</i> 2008; Li <i>et al.</i> 2012; Weick <i>et al.</i> 2013)
Dravet syndrome	SCNA1A	GABAergic neurons	N/A	(Higurashi <i>et al.</i> 2013; Jiao <i>et al.</i> 2013; Liu <i>et al.</i> 2013b)
Familial dysautonomia	IKBKAP	Neural crest progenitor cells	Kinetin, SKF-86466	(Lee <i>et al.</i> 2009, 2012)
Fragile X-associated tremor/ataxia syndrome	FMR1	Neurons	N/A	(Urbach <i>et al.</i> 2010; Liu <i>et al.</i> 2012b)
Huntington's disease	HTT	Glutamatergic neurons and GABAergic neurons	N/A	(Park <i>et al.</i> 2008; Zhang <i>et al.</i> 2010; An <i>et al.</i> 2012; Camnasio <i>et al.</i> 2012; HD iPSC Consortium 2012; Jeon <i>et al.</i> 2012; Juopperi <i>et al.</i> 2012)
Machado-Joseph disease	ATXN3	Glutamatergic neurons	Calpain	(Koch <i>et al.</i> 2011)
Parkinson's disease	LRRK2, PINK1, SNCA, PARKIN, sporadic	Dopaminergic neurons	Coenzyme Q10, rapamycin, GW5074, LRRK2-IN1, PD0325901	(Devine <i>et al.</i> 2011; Nguyen <i>et al.</i> 2011; Seibler <i>et al.</i> 2011; Cooper <i>et al.</i> 2012; Imaizumi <i>et al.</i> 2012; Jiang <i>et al.</i> 2012; Liu <i>et al.</i> 2012a; Rakovic <i>et al.</i> 2012; Sanchez-Danes <i>et al.</i> 2012; Reinhardt <i>et al.</i> 2013)
Rett's syndrome	MECP2, CDKL5	Neurons and neural progenitor cells	IGF1, gentamicin	(Marchetto <i>et al.</i> 2010; Muotri <i>et al.</i> 2010; Ananiev <i>et al.</i> 2011; Cheung <i>et al.</i> 2011; Ricciardi <i>et al.</i> 2012)
Schizophrenia	DISC1, sporadic	Neurons	Loxapine, valproic acid	(Brennand <i>et al.</i> 2011; Chiang <i>et al.</i> 2011; Pedrosa <i>et al.</i> 2011; Paulsen Bda <i>et al.</i> 2012)
Spinal muscular atrophy	SMN1	Motor neurons	Valproic acid, tobramycin	(Ebert <i>et al.</i> 2009; Chang <i>et al.</i> 2011)
Spinal and bulbar muscular atrophy	CAG repeat in the androgen receptor gene	Motor neurons	17-allylaminogeldanamycin	(Nihei <i>et al.</i> 2013)

level, defects develop in the circuits linking the cerebrum and the basal ganglia to which dopaminergic neurons project, and patients exhibit stereotypical motor symptoms, including bradykinesia, rigidity, tremors, and postural instability.

Previous research indicates that approximately 10% of PD patients have a form of disease that is caused by a mutation in a specific gene, and whose occurrence is termed 'familial'. For example, familial PD develops because of mutations in

the *LRRK2*, *PINK1*, *SNCA*, *PARKIN*, *DJ-1*, *ATP1A3*, *UCHL1*, and *GBA* genes. These genes are also thought to play an important role in the pathogenetic mechanism of sporadic Parkinson's disease. Because the motor symptoms do not develop until close to 70% of the dopaminergic neurons in the substantia nigra are lost, the molecular mechanism that predominates during the initial stage of PD remains unknown. However, iPS cells derived from PD patients can theoretically serve as a useful disease model by providing a tool to investigate the time course of changes from the onset of PD until the pathology has become apparent. Especially, the iPS-derived dopaminergic neuron is reasonable as PD patients have substantial loss of these neurons during the disease. We therefore focused our recent research efforts toward establishing iPS cells from early-onset familial PD (PARK2) patients with a mutation in the *PARKIN* gene to investigate the efficacy of disease-specific iPS cells for modeling purposes (Imaizumi *et al.* 2012).

We prepared PARK2 iPS cells by using recombinant retrovirus vectors to introduce the four aforementioned reprogramming genes, that is, *Oct4*, *Klf4*, *Sox2*, and *c-Myc*, into the skin cells of two PARK2 patients, patient PA and patient PB (Imaizumi *et al.* 2012). First, based on previous research on animal models of PD, we assumed that these patients would have higher levels of oxidative stress than normal individuals. Hence, we analyzed the amounts of reactive oxygen species (ROS) and reduced glutathione (GSH) in the neurons induced from PARK2 iPS cells. The results showed an inverse relationship between low GSH content and high ROS levels. We also observed activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Zhang *et al.* 2011), which serves as a defense mechanism against intensified oxidative stress. These observations are consistent with previous data showing a predominant decrease in GSH and a concomitant increase in Nrf2 signaling in the substantia nigra of the postmortem brains of PD patients (Venkateshappa *et al.* 2012; Bassik *et al.* 2013). Thus, oxidative stress is apparently already high in the brain before the onset of Parkinson's disease and during the initial stages after its onset.

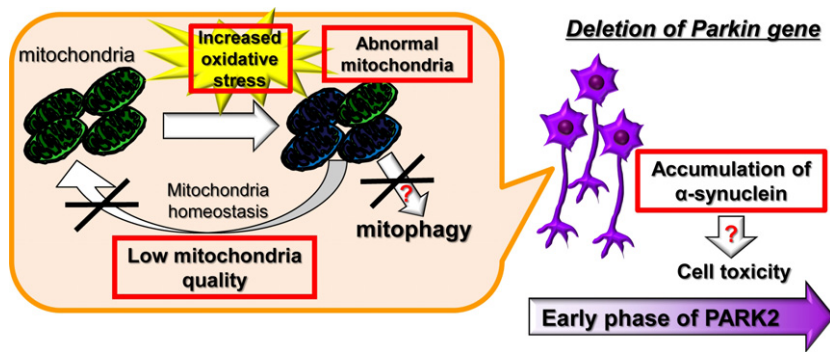
In support of the above-described work, the dopamine D1/D2 receptor antagonist apomorphine (Jang *et al.* 2013), the selective monoamine oxidase B inhibitor deprenyl (Benraiss *et al.* 2013), and the dopamine D2 receptor antagonist bromocriptine (Merkle and Eggan 2013), all of which are used clinically as drugs for the treatment of PD, up-regulate the Nrf2 pathway together with their main pharmacological effects. Moreover, the anti-convulsant drug zonisamide has a protective effect on dopaminergic neurons that is mediated by its dopamine synthesis-stimulating and mild monoamine oxidase-inhibiting actions; however, the drug also activates the synthesis of GSH, which is governed by the Nrf2 pathway (Liu *et al.* 2012b). Therefore, the therapeutic indications for zonisamide have been expanded to include

its use as an anti-parkinsonian agent. Thus, if zonisamide can directly activate the central Nrf2 pathway in a more efficient manner, it is expected to find utility as a highly effective drug for the prevention of the onset and/or progression of PD.

Mitochondria are essential intracellular organelles that function in energy production. Mitochondria release significant amounts of ROS as byproducts of energy metabolism. For this reason, mitochondrial energy production is considered to be the main cause of oxidative stress. Up until now, it has been a technical challenge to make detailed structural observations of the mitochondria obtained from the post-mortem brain tissue of PD patients, because of the need to minimize chemical changes in the tissues caused by cell death. As an alternative approach, we conducted detailed electron microscopic observations of the mitochondria in neurons induced from the iPS cells of PARK2 patients. Interestingly, abnormal mitochondria were observed in the iPS-derived neurons originating from both of the PARK2 patients investigated in this study, patient PA and patient PB (Fig. 2). Because no abnormal mitochondria were observed in PARK2-derived fibroblasts or in the PARK 2 iPS cells themselves, these abnormal organelles would appear to be a neuron-specific phenomenon.

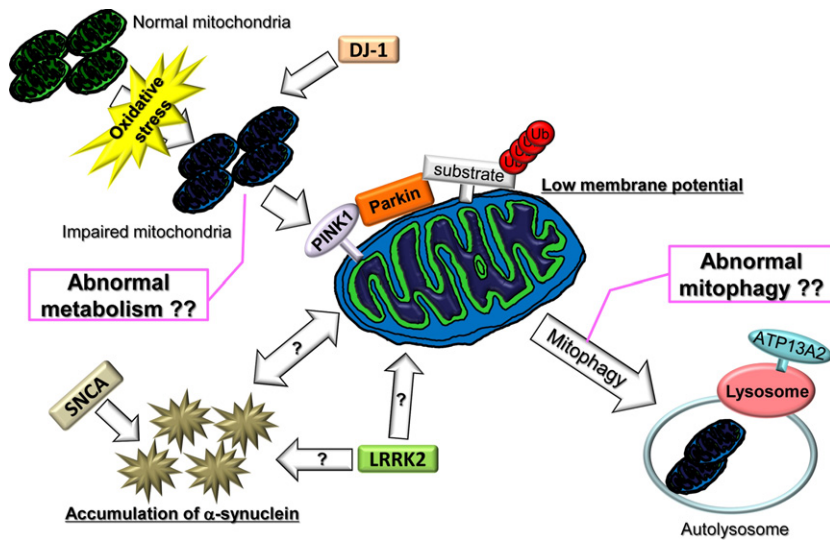
In addition, the PARKIN protein is reportedly involved in mitochondrial autophagy (mitophagy) (Narendra *et al.* 2008; Matsuda *et al.* 2010). Therefore, we employed the mitochondria uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone to reduce mitochondrial membrane potential. carbonyl cyanide *m*-chlorophenyl hydrazone caused the inner membrane of the mitochondria of neurons derived from healthy subjects to disappear, whereas no disappearance of the inner membrane was observed in the neurons derived from the iPS cells of PARK2 patients. These findings suggest that abnormal mitochondria may have accumulated in the cells, because the mechanism that controls mitochondrial quality does not function properly in PARK2 iPS cell-derived neurons (Fig. 3).

Accumulation of  $\alpha$ -synuclein-rich Lewy bodies has been reported in the neurons of patients with sporadic Parkinson's disease, based on analyses of postmortem brain tissue (Shults 2006). However, it appears that Lewy bodies usually do not accumulate in the postmortem brain tissue of PARK2 patients (Farrer *et al.* 2001). We therefore analyzed the postmortem brain tissue of PARK2 patient PA. Surprisingly, we observed an abundance of  $\alpha$ -synuclein in the brain tissue of this patient. On the other hand, an analysis of the postmortem brain tissue of the father of patient PB, who had an identical *PARKIN* gene mutation and a similar genetic background as patient PB, revealed no  $\alpha$ -synuclein buildup. Next, we analyzed the expression of  $\alpha$ -synuclein in the iPS cell-induced neurons of patient PA and patient PB. A significant accumulation of  $\alpha$ -synuclein was found only in the iPS cell-induced neurons of patient PA, thus mimicking the situation in the patient's postmortem brain tissue. No such



**Fig. 2** Summary of the results of our study with PARK2 induced pluripotent stem (iPS) cell-derived neurons. An analysis of familial Parkinson's disease (PD) (PARK2) patient-derived iPS cells in which the *PARKIN* gene is defective revealed an increase in oxidative stress, abnormal mitochondrial morphology, and low mitochondrial quality. In addition,  $\alpha$ -synuclein accumulation was observed in the neurons originating from PARK2 patient (patient PA)-derived iPS cells.

The same patient showed Lewy body accumulation in the postmortem brain. Based on these results, further analyses using disease-specific human iPS cell-derived neurons should make it possible to reproduce the pathology and degree of disease progression in patients' brains, and to analyze pathological conditions that are close to those observed *in situ*.



**Fig. 3** Working model for familial Parkinson's disease (PD) risk factors: mediated mitochondrial quality control. Impaired mitochondria that have a low membrane potential develop as a result of increased oxidative stress and other factors. Mitochondrial quality is maintained by the familial PD factors PINK1 (PARK6) and PARKIN (PARK2). These factors pass into impaired mitochondria and guide the mitochondria toward degradation. DJ-1 (PARK7), another familial Parkinson's disease factor, is involved in a thioredoxin-mediated mechanism that protects mitochondria against oxidative stress, and a low mitochondrial membrane potential has been reported in LRRK2 mutants (PARK8). In addition, mutation of ATPase type 13A2

(ATP13A2, or PARK9), an  $H^+$ -ATPase involved in lysosomal acidification, causes abnormal lysosome function and abnormal protein degradation. On the other hand,  $\alpha$ -synuclein oligomers cause mitochondrial impairment in familial PD (PARK1, 4) and are associated with a missense abnormality of the gene (SNCA) that encodes  $\alpha$ -synuclein. In this case, a negative feedback loop is formed in which the mitochondrial impairment acts as a trigger for the formation of  $\alpha$ -synuclein oligomers. These results strongly suggest that a breakdown of quality control in the impaired mitochondria plays a critical role in the onset of PD.

accumulation was observed in the iPS cell-induced neurons of patient PB, which was consistent with the analysis of the patient's father's postmortem brain tissue.

This series of findings demonstrated that is possible to model many aspects of the cellular changes seem in patients. We still have a long way to go in modeling the

disease—especially the 3D organization and different cell types including glia, etc.

Many additional studies of iPS cells originating from familial PD patients (involving *LRRK2*, *PINK1*, *SNCA*, and *PARKIN* gene mutations) have been reported by other groups (see references in Table 2). Thus, by utilizing these disease-specific iPS cells, an investigator can actually reproduce the pathology of an individual patient's disease in a test tube. Furthermore, disease-specific iPS cells are proving extraordinarily useful in analyzing disease pathology in greater detail than that afforded by other methods (Table 2).

In addition, replication experiments with isogenic iPS cells, and rescue experiments via genetic repair, are likely to allow the precise analysis of pathogenetic mechanisms resulting from changes in single genes. A variety of innovations have made the performance of these experiments tenable, including the development of the helper-dependent adenoviral vector (Suzuki *et al.* 2008), zinc-finger nucleases (Zhou *et al.* 2009), transcription activator-like effector nucleases (TALENs) (Miller *et al.* 2011), and the CRISPR-Cas9 system (Cong *et al.* 2013; Mali *et al.* 2013) (Fig. 2). For example, Reinhardt *et al.* (2013) demonstrated neurite outgrowth abnormalities, induction of dopaminergic neuron death by the addition of 6-hydroxydopamine, tau and  $\alpha$ -synuclein deposition, and gene expression changes in dopaminergic neurons induced from iPS cells prepared from a patient with a *LRRK2* gene mutation (G2019S mutation). The investigators also established patient-specific iPS cells in which the G2019S mutation was repaired with zinc-finger nucleases, as well as isogenic leucine-rich repeat kinase 2 (*LRRK2*) iPS cells in which the G2019S mutation was introduced into normal cells. These iPS cells were used to show that the dopaminergic neuron abnormalities stemmed from a gain-of-toxic-function mutation of *LRRK2* and an enhanced extracellular signal-regulated kinase 1/2 phosphorylation. However, it is essential to conduct further isogenic control and rescue experiments to confirm the phenotype obtained using patient/disease-specific iPS cells for the analyses of disease-specific iPS cells derived from many monogenic mutation diseases, and not just PD.

### Challenges in modeling sporadic PD with patient-derived iPS cells

Unlike diseases caused by deletions or mutations within a specific gene (i.e., the familial Parkinsonism-related genes *LRRK2*, *PINK1*, *SNCA*, or *PARKIN*), it is not easy to construct human disease models for sporadic diseases in a test tube, even by using disease-specific iPS cells. The difficulty is because of the fact that these diseases are impacted by environmental as well as genetic factors. Nonetheless, a recent study successfully modeled certain aspects of the sporadic PD and *LRRK2* PD by using neurons derived from a variety of patient-specific iPS cells

(Sanchez-Danes *et al.* 2012). The results showed that either sporadic PD- or *LRRK2*-PD-iPS-derived dopaminergic neurons showed morphological alterations as well as alterations in autophagic clearance. This study demonstrates that phenotypes in neurons representing *LRRK2* mutation were also observed in those from sporadic PD patients. These phenotypes associated with sporadic and *LRRK2* PD might be helpful to understand pathogenesis of sporadic PD.

### iPS cells based human disease models for genetic and sporadic AD

Recent work using neurons derived from the iPS cells of sporadic AD patients revealed an accumulation of amyloid  $\beta$ , as was observed previously in neurons derived from the iPS cells of familial AD patients with mutations in *PRESENILIN1*, *PRESENILIN2*, or amyloid precursor protein (APP) (Yagi *et al.* 2011; Israel *et al.* 2012). Presenilin 1 and 2 function as components of the  $\gamma$ -secretase intra-membrane protease complex. Although a  $\gamma$ -secretase inhibitor effectively inhibited the amyloid  $\beta$  buildup in neurons derived from the iPS cells of familial AD patients with *PRESENILIN1* and *PRESENILIN2* mutations, a  $\beta$ -secretase inhibitor, but not a  $\gamma$ -secretase inhibitor, engendered the same effect in neurons derived from the iPS cells of sporadic AD patients and APP-mutant familial AD patients (Yagi *et al.* 2011; Israel *et al.* 2012). Moreover, neurons derived from the iPS cells of APP-mutant patients and one of two sporadic AD patients (patient 1) showed an accumulation of A $\beta$  oligomers, as well as increased endoplasmic reticulum and oxidative stress. Interestingly, docosahexaenoic acid treatment ameliorated the stress response in the sporadic AD iPS cell-derived neurons of patient 1, but had no effect on the iPS cell-derived neurons originating from the other sporadic AD patient (Kondo *et al.* 2013).

Thus, while there continues to be room for further experimentation, these observations reveal the impressive capacity of iPS cell research to elucidate pathological phenomena and to judge the clinical effectiveness of drugs in sporadic as well as genetic diseases. Furthermore, the likelihood that iPS cell technology will lead to the development of new strategies and methods of disease treatment seems high.

### Potential applications of disease-specific iPS cells for drug screening

In recent years, several analyses have utilized disease-specific iPS cells to explore the pathogenetic mechanisms of disease. In addition, a number of attempts to utilize these cells for drug screening have also been reported. For instance, Lee and colleagues established iPS cells from patients with FD, a disorder caused by a point mutation in

**Table 2** Familial PD modeled with iPSC cells

Gene	Mutations	iPSC-derived cells	Phenotype in human iPSC-derived cells	Use of isogenic & gene corrected iPSC	Drug testing	References
SNCA Parkin	Triplication of SNCA	Dopaminegic neurons	Double amount of alpha-synuclein protein	N/A	N/A	Devine <i>et al.</i> 2011
	Heterozygous deletions of exon 3 and exon 5/Homozygous deletion of exon 3	Dopaminegic neurons	Increased spontaneous dopamine release; Decreased dopamine uptake and dopamine transporter-binding sites; Elevated ROS by increasing MAO transcripts	Lentiviral expression of WT-parkin	N/A	Jiang <i>et al.</i> 2012
PINK1	Homozygous deletion of exon 2-4/Homozygous deletion of exon 6,7	Neural cells including dopaminegic neurons	Increased oxidative stress; Abnormal mitochondrial morphology and impaired mitochondrial homeostasis; Accumulation of alpha-synuclein protein	N/A	N/A	Imaizumi <i>et al.</i> 2012
	Q456X nonsense/V170G missense	Dopaminegic neurons	Impaired recruitment of PARKIN to mitochondria; Increased mitochondrial copy number; Up-regulation of PGC-1alpha	Lentiviral expression of wild-type PINK1	N/A	Seibler <i>et al.</i> 2011
PINK1/LRRK2	V170G missense	Dopaminegic neurons	Lack of Valinomycin-induced mitophagy	N/A	N/A	Rakovic <i>et al.</i> 2012
	Q456X nonsense (PINK1)/Heterozygous R1441C and homozygous G2019S mutation (LRRK2)	Neural cells including dopaminegic neurons	Production of reactive oxygen species (PINK1); Mitochondrial respiration (PINK1 and LRRK2); Proton leakage (PINK1); Intraneuronal movement of mitochondria (LRRK2)	N/A	Coenzyme Q10, rapamycin, or GW5074	Cooper <i>et al.</i> 2012
LRRK2	Homozygous G2019S mutation	Dopaminegic neurons	Increased expression of oxidative stress-response genes (HSPB1, NOX1, and MAOB) and alpha-synuclein protein; Sensitive to caspase-3 activation and cell death caused by hydrogen peroxide, MG-132, and 6-hydroxydopamine	N/A	N/A	Nguyen <i>et al.</i> 2011
	Homozygous G2019S mutation	Neural stem cells	Susceptibility to proteasomal stress; Passage-dependent deficiencies in nuclear-envelope organization; Clonal expansion and neuronal differentiation	Isogenic corrected LRRK2 iPSC/miLRRK2 KI ESC	LRRK2-In-1	Liu <i>et al.</i> 2012a



Table 2 (continued)

Gene	Mutations	iPSC-derived cells	Phenotype in human iPSC-derived cells	Use of isogenic & gene corrected iPSC	Drug testing	References
	Heterozygous G2019S mutation	Dopamine neurons	Dysregulation of CPNE6, MAP7, UHRF2, ANXA1, and CADPS2; Increased extracellular-signal-regulated kinase 1/2 (ERK) phosphorylation; Increased expression of MAPT mRNA and TAU protein as well as increased alpha-synuclein protein; Neurite outgrowth phenotype; Increased sensitivity to 6-Hydroxydopamine, rotenone and oxidative stress	Isogenic corrected LRRK2 iPSC/miLRRK2 KI iPSCs	LRRK2-IN1, PD0325901	Reinhardt <i>et al.</i> 2013
LRRK2/sporadic	G2019S mutation/Sporadic	Dopamine neurons	Increased accumulation of alpha-synuclein; Fewer and shorter neurites; Increased apoptotic cells; Abnormal autophagic clearance	N/A	N/A	Sanchez-Danes <i>et al.</i> 2012

the *IKBKAP* (inhibitor of nuclear factor- $\kappa$ B kinase complex-associated protein) gene (Lee *et al.* 2009). Neural crest progenitor cells induced from the iPS cells of FD patients exhibited decreases in *IKBKAP* expression levels, reduced differentiation efficiency into neurons, and attenuated migratory ability (Lee *et al.* 2009). The same investigators also screened 6912 compounds in an effort to uncover drugs capable of increasing the expression of the *IKBKAP* gene in the induced FD patient-derived neural crest precursor cells. One of the hit drugs was termed 'SKF-86466', which has since been confirmed to augment the expression of nervous system marker genes in the induced neural crest precursor cells (Lee *et al.* 2012). These results indicate that large-scale screening of chemical libraries with patient-derived cells, an impossible goal in the past, is rapidly becoming a reality through the utilization of disease-specific iPS cells.

### Future directions using disease-iPS cells

Recent advances in the iPS cell technology enable us to study not only monogenetic diseases but also sporadic disease. Furthermore, isogenic iPS cell controlled with the same genetic background would be more adequate to analyze the subtle changes in disease models. However, there are still some limitations in iPS cell technology. For example, current iPS cell-derived neurons showed poor maturational state. Patani *et al.*, showed that transcriptome profile of ES cell-derived neurons was more similar to fetal neurons rather than adult ones (Patani *et al.* 2012). Therefore, current iPS cell-derived neurons were not fully suitable for late-onset neurological disease, such as AD and PD, and disorders of neuronal circuits, such as autism and epilepsy. Moreover, the iPS cell-derived neurons following well-established neural differentiation protocols were still showed mixed population. For example, purity of ES cell-derived midbrain dopaminergic neurons was 60–80% and their properties *in vitro* were different from those in the A9 area and the striatum. However, recent direct reprogramming technology using neural factors on fibroblasts or iPS cells showed rapid production, nearly 100% purity, and functional maturation of neurons (induced neurons; iNs) (Vierbuchen *et al.* 2010; Caiazzo *et al.* 2011; Zhang *et al.* 2013). Therefore, inducing neural transcription factors on fibroblasts or iPS cells has a potential to achieve neuronal potential like adult neurons.

Furthermore, major challenges in modeling neurological disease focused on genetic disorders. However, most neurological disorders are mainly polygenic and dependent of environmental factors. To address those common diseases using iPS technology, it is because of make epigenetic modulation and/or common variant on iPS cell-derived neurons. Improving culture conditions to recapitulate the *in vivo* environment using tissue engineering techniques,

such as three dimensional culture system or microfluidics technology, is a need for future approaches.

## Conclusions

In this review, we turned our attention toward neurological disease-specific iPS cells and described the current status of research in the field. While there continue to be many issues that must be resolved from the standpoint of conducting iPS cell research, including generating homogenous populations of iPS cells and formulating methods of efficiently inducing and authenticating target cell types, research in this area has been expanding worldwide since the first report of the establishment of disease-specific iPS cells in 2008 (Dimos *et al.* 2008; Park *et al.* 2008; Bellin *et al.* 2012; Robinton and Daley 2012). Current research indicates that disease-specific iPS cell technology can accurately reflect conditions before the onset of clinical disease or, in many cases, during the initial stages of the disease. By combining disease-specific iPS cell technology with whole-genome analysis and non-invasive imaging technology, dramatic progress in the elucidation of pathogenetic mechanisms is expected. Moreover, applications in new drug development, as well as in sensitivity testing, safety testing, and toxicity testing of existing drugs, are likely to proceed at an accelerated pace through the use of disease-specific iPS cell technology. Our fervent hope is that the achievements in the field of disease-specific iPS cell research will be made available to all patients for whom no treatment of the underlying disease cause currently exists.

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