Stem Cell Research for Regenerative Medicine/Personalized Medicine

Therapeutic Application of Stem Cell Technology toward the Treatment of Parkinson’s Disease

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Parkinson’s disease (PD) is one of the candidate diseases for cell transplantation therapy, since successful clinical experiments have accumulated using human fetal tissue grafting for PD patients. Although some grafted PD patients have shown drastic improvements, several issues still remain with regard to using human fetal tissue. This review highlights the recent advances in stem cell technology towards clinical applications using human pluripotent stem cells. In particular, pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells (iPSCs), are the focus as a source of cell transplantation therapy that can be used instead of human fetal tissues. Additionally, efficient methods for stem cell maintenance and differentiation have been developed and improved towards the clinical transition. These advances in the basic technologies have helped accelerate the realization of regenerative medicine. We also review the current topics regarding disease modeling and drug screening using iPSC technology. Finally, we also describe the future prospects of these stem cell research fields toward clinical application.

Key words Parkinson’s disease; dopaminergic neuron; stem cell therapy; disease modeling; drug discovery

1. INTRODUCTION

Parkinson’s disease (PD) is one of the most common neurodegenerative disorders. It is characterized by the progressive death of dopaminergic (DA) neurons in the substantia nigra pars compacta, resulting in muscle rigidity, resting tremors, bradykinesia, and akinesia. Currently, the main clinical treatment for PD is dopamine replacement therapy using \(\text{\textit{L}}\)-dihydroxyphenylalanine (\(\text{\textit{L}}\)-DOPA) and/or dopamine receptor agonists.\textsuperscript{11} Although pharmacotherapy can improve parkinsonian symptoms during the initial stage of PD, the efficacy of pharmacotherapy is gradually lost during long-term treatment, and the on–off phenomenon, wearing-off phenomenon and drug-induced dyskinesia occur in later stages. In addition, pharmacotherapy cannot delay the progression of the loss of DA neurons, and also cannot recover the lost DA neurons. If human beings had regenerative potential similar to planarians\textsuperscript{9} and newts,\textsuperscript{5} some neural disorders could be spontaneously resolved by autonomous cells. Since this is not possible, the clinical strategy of “regenerative medicine,” including cell transplantation therapy, has been developed to recover the lost neural function and treat neural diseases. In particular, the establishment of human pluripotent stem cells, such as embryonic stem cells (ESCs)\textsuperscript{5} and induced pluripotent stem cells (iPSCs),\textsuperscript{5,6} has accelerated the progress of regenerative medicine using these cells (Fig. 1A), and has also improved the ability to better model human diseases.

2. IN VITRO DIFFERENTIATION OF MIDBRAIN DA NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

PD is one of candidate diseases for the application of cell transplantation therapy, because it could provide a long-term therapeutic option for PD. The first clinical trial of cell transplantation therapy for PD patients was reported in 1987 using aborted human fetal ventral midbrain tissue\textsuperscript{7} (Fig. 2A). So far, more than 400 PD patients have been treated as part of this clinical trial. Some grafted PD patients have exhibited drastic improvements in their symptoms. However, strict ethical issues still remain regarding the use of human fetal tissue for the treatment of human disease. Therefore, it is necessary to develop efficient methods to generate midbrain DA neurons from pluripotent stem cells, such as ESCs and/or iPSCs, instead of human ventral midbrain tissue (Figs. 2B–D).

Recently, the molecular and cellular mechanisms of mammalian brain development have become better understood,\textsuperscript{8} and one proposed strategy for DA neuronal induction from ESCs/iPSCs \textit{in vitro} is to mimic the midbrain developmental process \textit{in vivo}. Some secreted factors facilitate neural induction and define the anterior–posterior and dorso–ventral axis of the developing mammalian brain. For instance, important factors for mammalian midbrain development are Sonic hedgehog (Shh) and fibroblast growth factor 8 (FGF8), which are locally expressed in the ventral neural tube and the midbrain–hindbrain boundary, respectively.\textsuperscript{9} In addition, midbrain DA neuronal specification is regulated by several transcription factors, such as Lmx1a, FoxA2, Nurr1 and Pitx3.\textsuperscript{10–13} Therefore, the cellular aspects and transcriptional regulation of
midbrain development may provide a means for improving the protocol to differentiate authentic DA neuronal subtype cells from ESCs/iPSCs in vitro (Fig. 1B).

In addition, a 3-dimensional (3 d) culture system (i.e., serum-free embryoid body-like aggregates; SFEb) has been established for this purpose. The SFEb method may lead to the acquisition of sub-regional identities because the cells respond to positional information signals, and strongly contributes to mimicking the complicated organogenic process. 14)

Recent advances in the neural induction from ESCs/iPSCs in vitro revealed by dual inhibition of SMAD signaling using a transforming growth factor-β (TGF-β)/activin/nodal inhibitor (SB431542) and bone morphogenic protein (BMP) inhibitor (noggin and dorsomorphine) drastically enhanced neural lineage commitment from undifferentiated human ESCs and iPSCs in both SFEb (3D culture) and stromal (PA6) feeder co-cultures (2D culture). 15,16) Moreover, it was reported that there were no apparent differences between the human ESCs and iPSCs, such as the propensity for differentiation, observed in these culture systems. 15) Additionally, Kriks et al. reported that a glycogen synthase kinase 3β (GSK3β) inhibitor (CHIR99021) activates wnt signaling to induce Lmx1a expression in FoxA2-positive floor plate precursors, and facilitates the neurogenic conversion of human ESC-derived midbrain floor plates towards DA neurons. 17) More recently, Kirkeby et al. reported that the dose-dependent activation of wnt signaling by CHIR99021 controls the brain positional specification in neural induction based on dual SMAD inhibition via embryoid body culture in human ESCs, and that the gene expression profile of human ESC-derived DA neurons can be recapitulated in the human fetal midbrain. 18) These findings suggested that 3D cultures are suitable for mimicking brain organogenic processes in authentic midbrain DA neurons.

3. PRECLINICAL EXPERIMENTS INVOLVING CELL TRANSPANTATION THERAPY FOR PARKINSON’S DISEASE

The benefits and safety of grafted ESCs/iPSCs-derived DA neurons should be evaluated in in vivo animal experiments before human trials. In general, DA neurotoxins, such as rotenone, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), are used to create parkinsonian animal models using rodents and non-human primates. 19) In particular, since the symptoms and brain anatomy of monkey models are similar to those of humans, and high-resolution neuroimaging techniques are available to evaluate the function of DA neurons using positron emission tomography and magnetic resonance imaging, the same as they are for human patients, 20) the knowledge from monkey trials strongly contributes to the transition to human clinical trials.

In 2005, Takagi et al. first revealed that primate ESC-derived DA neurons survive in the putamen in MPTP-lesioned primate parkinsonian models, and that the [18F]-DOPA uptake increased in grafted monkeys 14 weeks after transplantation. Additionally, the neurological scores of the grafted monkeys improved in comparison with those of sham-operated monkeys starting from 10 weeks after transplantation. This study was the first report to show the functional efficiency of primate ESC-derived DA neurons, and opened up the possibility for transplantation therapy using ESC-derived DA neurons. 21)

Human ESC-derived DA neurons that were differentiated using an optimized protocol had the potential to improve motor function in the 6-OHDA-induced hemi-parkinsonian rat model after intrastriatal grafting. 17,18) However, several issues, such as possibility of tumorigenicity, remain obstacles to clinical trials. Doi et al. recently revealed that long-term neural induction (over 28 d) of human ESCs reduces their tumorigenicity and/or overgrowth after grafting in primate parkinsonian models for twelve months. In addition, the motor symptoms are also improved by grafting human ESC-derived DA neurons that have maturated over a long term (42 d). These results suggest that human ESC-derived DA neurons that are differentiated for appropriate terms strongly contribute to both reducing the risk of tumorigenesis and improving parkinsonian motor dysfunction. 22) Recently, Kikuchi et al. first revealed that human iPSC-derived DA neurons that are differentiated under feeder-free and serum-free conditions survived in an
MPTP-lesioned parkinsonian monkey for six months. This report supported the therapeutic potential of human iPSCs for future clinical trials.

Regarding the clinical application of cell therapy using ESCs/iPSCs, it is important to suppress the potential for graft versus host disease. The iPSC technology allows for autologous grafting that can theoretically avoid the immunological rejection of cell therapy, since personalized iPSC lines can be generated from individual patients (Fig. 2C). Alternatively, the iPSC technology can be used to construct a cell bank with human leukocyte antigen (HLA) haplotype-matching iPSCs to reduce the immunological rejection. In particular, HLA-homozygous iPSCs may be useful for allografting, which is likely to be needed soon after injury (Fig. 2D). Therefore, iPSC technology may provide benefits in that it can allow for the use of autologous and allogeneic cell therapy.

4. DISEASE MODELING USING iPSC TECHNOLOGY

The iPSC technology opened a new research field for disease modeling with patient-derived iPSCs. It is now possible to obtain somatic cells, such as neurons, which cannot be directly obtained from patients, by differentiation from patient-derived iPSCs. In the case of PD, although “sporadic” PD patients are more common, approximately 5–10% of PD patients have a familial etiology, which shows an autosomal recessive or dominant Mendelian inheritance. Recently, several groups generated iPSC lines from sporadic PD patients and familial PD patients with mutations in α-synuclein (SNCA), phosphatase and tensin homologue deleted on chromosome 10-induced putative kinase 1 (PINK1), leucine-rich repeat kinase 2 (LRRK2), and parkin. Interestingly, these patient-derived iPSCs have the potential to differentiate into DA neurons, the same as control iPSCs.

The most common pathological feature of PD is Lewy bodies, cytoplasmic inclusions in the remaining DA neurons in substantia nigra, and α-synuclein accumulates in Lewy bodies as the main component protein. Additionally, triplication of the α-synuclein gene causes a familial form of PD. Genome-wide association studies revealed that variations in the SNCA locus are significantly related to the risk of the onset of sporadic PD. The α-synuclein protein level in DA neurons derived from SNCA triplication patient iPSCs was elevated by long-term culture compared to control iPSCs. Additionally, these neurons showed high sensitivity to oxidative stress induced by hydrogen peroxide.

Likewise, Nguyen et al. demonstrated that the DA neurons derived from iPSCs obtained from PD patients with a LRRK2 (G2019S) mutant showed higher sensitivity to hydrogen peroxide, MG132 (a proteasome inhibitor) and 6-OHDA exposure compared to control iPSCs. In addition, the α-synuclein protein level in the LRRK2 (G2019S) mutant DA neurons was increased compared to the control neurons in long-term culture (60d). These phenotypic analyses using iPSCs derived from PD patients may be useful to recapitulate the PD phenotypes, which will be helpful to elucidate novel therapeutic targets.
On the other hand, in the case of cell transplantation therapy, especially autologous grafting, it is necessary to verify that DA neurons differentiated from PD patient-derived iPSCs would be applicable for cell transplantation therapy. In 2010, Hargus et al. demonstrated that DA neurons differentiated from three patients with sporadic PD-derived iPSCs survived in the striatum of 6-OHDA-induced hemi-parkinsonian rat models and contributed to improvement of amphetamine-induced rotation behavior.37 These results indicated that patient-derived iPSCs may have therapeutic potential, the same as control iPSCs, and can be used for cell transplantation therapy. Nevertheless, more accumulation of knowledge and discussion regarding the use of PD patient-derived iPSCs for cell transplantation therapy are needed in the future.

Patient iPSC-derived neurons can also be used to elucidate an individual’s response to specific drugs and determine whether they are candidates for that treatment.38 In the case of amyotrophic lateral sclerosis (ALS), Egawa et al. reported a drug screening protocol using motor neurons differentiated from ALS patient-derived iPSCs, and found that one candidate compound, anacardic acid, reduced the abnormal motor neuron phenotype.39 This report seems to be a good model for drug screening to isolate new drug candidate(s), which can be used in combination with iPSC technology and disease modeling.

5. CONCLUSION AND FUTURE STUDIES

Although stem cell biology, in vitro culture technology and in vivo evaluation systems have made dramatic progress in the past decade, there are still several issues regarding cell transplantation therapy that need to be resolved before clinical trial for PD. These are: (1) clinical-grade materials need to be prepared to avoid contamination with animal-derived factors (xeno-free) and unknown factors (chemically-defined materials) before clinical trials,40 (2) appropriate patient recruitment may contribute to more beneficial and reproducible trials,41 and (3) regulatory hurdles should be negotiated in the future to supply the safer cell transplantation therapies to PD patients. Such studies may lead to cell transplantation therapy becoming a beneficial clinical option for PD treatment.

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