Regenerative Medicine for Parkinson’s Disease

Takao YASUHARA,1 Masahiro KAMEDA,1 Takashi AGARI,1 and Isao DATE1

1Department of Neurological Surgery, Okayama University Graduate School of Medicine, Okayama, Okayama

Abstract

Regenerative medicine for Parkinson’s disease (PD) is expected to develop dramatically with the advancement of biotechnology as represented by induced pluripotent stem cells. Existing therapeutic strategy for PD consists of medication using L-DOPA, surgery such as deep brain stimulation and rehabilitation. Current treatment cannot stop the progression of the disease, although there is definite therapeutic effect. True neurorestoration is strongly desired by regenerative medicine. This review article describes the historical development of regenerative medicine for PD, with a focus on fetal nigral cell transplantation and glial cell line-derived neurotrophic factor infusion. Subsequently, the current status of regenerative medicine for PD in terms of cell therapy and gene therapy are reviewed. In the end, the future direction to realize regenerative medicine for PD is discussed.

Key words: cell therapy, dopaminergic neuron, gene therapy, induced pluripotent stem cells

Introduction

In the early 1900s, Cajal reported that neural tissue never regenerates in the central nervous system (CNS) once it undergoes maturation. For a long time after that people believed this hypothesis. In 1992, Reynolds and Weiss demonstrated that neural stem cells (NSCs) that are capable of self-renewal and differentiation into neurons or astrocytes exist in the adult mammalian brain.1 The era of stem cell research dawned and many studies followed.2–5 Various kinds of stem cells were explored and biological technology developed. In 2006, Takahashi and Yamanaka established a method to induce pluripotent stem cells from mouse embryonic and adult fibroblast cultures by four factors (i.e., Oct3/4, Sox2, c-Myc, and Klf4).6 This innovation might accelerate the clinical trial of stem cell transplantation for CNS disease patients and open up the possibility to clarify the mechanisms of several diseases and to discover new drugs.

Parkinson’s disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic neurons in the nigrostriatal system. The tetralogy of PD is resting tremor, rigidity, akinesia, and disturbance of postural reflex. As a definite therapy, dopamine replacement therapy was established and is still a gold standard for the treatment of PD.7 Stereotoxic surgery, including deep brain stimulation (DBS) and electrical coagulation, were also established as a definite therapy for PD.8,9 Rehabilitation is also a key treatment for functional maintenance and recovery of PD patients.10,11 Many PD patients reap benefits from the existing therapies. However, as time passes, the PD condition becomes exacerbated. Table 1 shows the advantages and disadvantages of the existing therapies.

Because the major pathology of PD is the degeneration of dopaminergic neurons in the nigrostriatal system, PD is a good target of regenerative medicine. As regenerative medicine, fetal nigral cell transplantation was initially considered to be the magic bullet for PD patients.12,13 In this review article, fetal nigral cell transplantation and infusion of glial cell line-derived neurotrophic factors are described as part of the historical development of regenerative medicine for PD in the following section. Subsequently, the current status of cell therapy and gene therapy are discussed. In conclusion, the future direction of regenerative medicine for PD is also described.

Historical Development of Regenerative Medicine for PD

I. Fetal nigral cell transplantation

In 1988, the clinical trial of fetal nigral cell transplantation for PD patients was reported by two groups.12,13 Fetal nigral cell transplantation was performed to achieve synapse formation between preserved host...
neurons and transplanted donor cells, as well as to supply dopamine. This method was considered as a newly developed, effective, and safe therapy at that time, despite the immunological problems and the ethical issues of using aborted fetuses. At the outset of the twenty-first century, randomized, double-blind studies revealed the insufficient functional recovery of older patients and delayed dyskinesia in some patients, although the ameliorated dopamine uptake was confirmed by positron emission tomography (PET) using 18F-fluorodopa.14–16) After the results of these studies became common knowledge, the momentum for fetal cell transplantation declined. Mendez et al. reported the more optimistic aspects of fetal nigral cell transplantation using a cell suspension rather than solid tissue, as used in the previous randomized, double-blind studies.17) Two patients showed amelioration in motor function, reduced L-DOPA-induced dyskinesia, and no dyskinesia in the off state. Four years after transplantation, many transplanted cells had survived with subtle immune reaction.17) Some patients receiving fetal nigral cell transplantation showed a continuous improvement of motor symptoms for over a decade.18) Recently, long-term symptomatic relief at 18 years and 15 years after transplantation was reported.19) Thus, fetal nigral cell transplantation might exert strong therapeutic effects for a long time with appropriate patient selection, transplant protocol, and optimal trial design. Recently, the current status of the TRANSEURO trial (http://www.transeuro.org.uk), an European Union-funded multicenter clinical trial of fetal nigral cell transplantation, was shown in detail.20) In order to increase the therapeutic effects and to minimize the risk for graft-induced dyskinesia, the overall protocol of TRANSEURO was strictly determined (Table 2). The results of this hopeful trial strongly affect the following cell therapies using dopaminergic neurons.

In the usage of autologous cells, there are no

Table 1 Advantages and disadvantages of the existing therapies for Parkinson’s disease

<table>
<thead>
<tr>
<th>Therapeutic option</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Medication (L-DOPA)</td>
<td>oral intake</td>
<td>drug-induced dyskinesia</td>
</tr>
<tr>
<td></td>
<td>less invasiveness</td>
<td>systemic side effects</td>
</tr>
<tr>
<td></td>
<td>prompt effectiveness</td>
<td>long-term side effects</td>
</tr>
<tr>
<td>Surgery</td>
<td>dramatic effect</td>
<td>invasiveness</td>
</tr>
<tr>
<td></td>
<td>selective target</td>
<td>undissolved mechanism</td>
</tr>
<tr>
<td></td>
<td>prompt effectiveness</td>
<td>expensive medical care (DBS)</td>
</tr>
<tr>
<td>Rehabilitation</td>
<td>safety</td>
<td>weak effect</td>
</tr>
<tr>
<td></td>
<td>low medical care</td>
<td>gradual effect</td>
</tr>
<tr>
<td></td>
<td>improved mental state</td>
<td>required continuity</td>
</tr>
</tbody>
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DBS: deep brain stimulation. The general advantages and disadvantages of medication using L-DOPA, surgery including thalamotomy, pallidotomy and subthalamic nucleus (STN)-deep brain stimulation (DBS), and rehabilitation are described.

Table 2 Detailed information on TRANSEURO

<table>
<thead>
<tr>
<th>Issues of criteria</th>
<th>Conditions</th>
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<tbody>
<tr>
<td>Patient selection</td>
<td>no cognitive impairment</td>
</tr>
<tr>
<td></td>
<td>younger than 65 years old</td>
</tr>
<tr>
<td></td>
<td>less than 10 years of disease duration</td>
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<tr>
<td></td>
<td>no significant L-DOPA-induced dyskinesia</td>
</tr>
<tr>
<td>Tissue composition</td>
<td>tissue collection in several centers</td>
</tr>
<tr>
<td></td>
<td>transfer of tissue between centers (across national borders)</td>
</tr>
<tr>
<td></td>
<td>stores up to maximum of 4 days in hibernation medium</td>
</tr>
<tr>
<td>Tissue placement</td>
<td>standardized instrument for grafting</td>
</tr>
<tr>
<td></td>
<td>delivery of tissue through 5–7 tracts to the posterior putamen</td>
</tr>
<tr>
<td>Trial design</td>
<td>observation study on 150 patients</td>
</tr>
<tr>
<td></td>
<td>randomized 40 patients in 150</td>
</tr>
<tr>
<td></td>
<td>assigned either to transplant/control group</td>
</tr>
<tr>
<td></td>
<td>1 year immunosuppression</td>
</tr>
<tr>
<td>Primary end-point</td>
<td>3 years post-transplantation</td>
</tr>
</tbody>
</table>

Issues of criteria and the conditions are described, respectively. The patients are selected not to cause graft-induced dyskinesia. Tissue is collected, stored, and transplanted in a stable fashion as a guarantee for the quality of cell transplantation.
ethic issues and little immune reaction. Autologous cell transplantation using various tissues was also reported as a potential treatment for PD.21,22) A phase I/II study was performed using intrastriatal transplantation of the autologous carotid body in patients with advanced stage PD.22) Bilateral intrastriatal transplantation was performed in 13 PD patients. No patients demonstrated grafts-induced dyskinesia. Clinical amelioration was found in 10 patients. The carotid body is a paraneuron derived from the neural crest that is similar to chromaffin cells of the adrenal medulla. Gial cell line-derived neurotrophic factor (GDNF) secreted from the carotid body might exert the neuroprotective effects of these transplanted cells.23) A well-designed, randomized, double-blind study with more patients is required to confirm the efficacy.

II. Infusion of gial cell line-derived neurotrophic factor
Alternatively, intraparenchymal injection of GDNF was demonstrated as another hopeful therapeutic option for PD patients. After the pilot study,24) strong therapeutic effects were shown in several open label clinical trials,25–27) although intraventricular GDNF administration demonstrated no improvement in a randomized, double-blind study.28) Previously in the basic research, we revealed that intrastriatal injection of GDNF was more effective than intraventricular administration.29) The discrepancy between the intraparenchymal and intraventricular administration might be due to the concentration of GDNF-acting striatal fibers. In a clinical study, Patel et al. demonstrated the safety and effectiveness of GDNF therapy for 2 years.26) Similarly, unilateral GDNF administration ameliorated bilateral function with no severe side effects.27) Moreover, neuronal sprouting was confirmed in the nigrostriatal area of a patient with unilateral GDNF infusion for 43 months who died of a myocardial infarction 3 months after discontinuing treatment.25) However, the positive results of open label studies were reversed by the randomized controlled trial of GDNF infusion.30) Moreover, anti-GDNF antibody was found in 3 of 34 patients. Amgen (Thousand, Oaks, California, USA), a company holding the patent, halted the clinical study using GDNF. After that clinical trials of GDNF infusion disappeared.

Current Status of Regenerative Medicine for PD

I. Cell therapy
In the previous section, the clinical trials of cell transplantation with fetal nigral dopaminergic neurons and autologous dopamine-producing cells were described. In this section, first we briefly review our strategy of regenerative medicine for PD. Subsequently, the current status of cell therapy using bone marrow-derived stem cells, embryonic stem cells (ESCs), and NSCs are described. Finally, the progress of the fast-evolving technology, that is, induced pluripotent stem (iPS) cells are shown. Our strategy for PD: Twenty-four and eighteen respective years have passed since encapsulated cell transplantation for a PD model of animals was initially reported in the world,31) and in Japan.32) Encapsulated cell transplantation enables us to safely use various kinds of cells, including genetically modified cells, to secrete a designed neurotrophic factor, growth factor, or neurotransmitter.33,34) Cells inside the capsule survived for at least 6 months in vivo with sufficient nutrients and oxygen available through a semi-permeable membrane. The capsule protects cells from immunological rejection and prevents problems by tumor formation (Fig. 1). Detailed information regarding our method is shown in the previous review article.35) In association with the development of NSCs, GDNF-secreting NSCs were used for PD model of rats.36) In the study, behavioral improvement and immunohistochemical preservation of dopaminergic neurons were demonstrated with many surviving transplanted cells. Mesenchymal stem cells (MSCs) were also shown to be good candidates for degenerated dopaminergic neurons.37) The intravenous administration of MSCs exerted therapeutic potentials at least partly through the
neuroprotective effects of stromal cell-derived factor 1α. In an experiment that explored the appropriate conditions of cell transplantation, the number of NSCs that survived in vivo was increased by GDNF pretreatment. In addition to the development of the cell source itself, the transplantation procedure and timing of transplantation should be thoroughly considered to ensure an appropriate evaluation of transplantation. In addition to cell therapy, therapeutic mechanisms of exercise, of carbamylated erythropoietin Fc fusion protein, and of spinal cord stimulation were explored for animal models of PD.11

Cell therapy using MSCs, ESCs, and NSCs: In the stem cell era, various kinds of stem cells were explored in terms of their potentials for PD treatment. MSCs are easily harvested and amplified with differentiation capacity. Dezawa et al. showed the way to induce the differentiation of MSCs into the neuronal lineages by gene transfection with Notch intracellular domain and subsequent administration of basic fibroblast growth factor, forskolin, and ciliary neurotrophic factor. Additional GDNF treatment increased the proportion of dopaminergic neurons. Recently, they found multi-lineage-differentiating stress-enduring (Muse) cells with stage-specific embryonic antigen. The protocol of isolation and culture takes less time and labor than that of other stem cells. The use of Muse cells for the treatment of CNS disorders is hopeful.

ESCs have also been studied vigorously. In 2000, two essential methods of neuronal differentiation from ESCs were reported. Kawasaki et al. found that the co-culture of ESCs and stromal cells [stromal cell-derived inducing activity (SDIA) method] make ESCs induce neurons with a high proportion of dopaminergic neurons. On the other hand, Lee et al. showed the method going through the embryoid body. In both methods, various types of refinement were performed to realize clinical application. The method to induce dopaminergic neurons has been now developed. Most recent one is a combination of dual SMAD inhibition and floor plate induction. With the SDIA method, Takahashi conducted the preclinical trial. The ethical issues and tumor formation are critical problems. The continuous efforts to overcome tumorigenesis are admirable.

NSCs are another source of hope. There are several research papers on the human-derived neural stem cell line for a PD model of animals. The neuroprotective effects of NSCs were mediated by secreted trophic factor, as well as neuronal differentiation. Clonal human dopaminergic neuron precursors might exert stable therapeutic effects and be a good design of in vivo experiments.

Current status of iPSC: As described briefly in the Introduction section, biotechnology using iPSC cells opened new doors for regenerative medicine. After mouse- and human-derived iPSC cells were established, the technology has been ameliorated at a phenomenal speed. Tumorigenesis is a matter of grave concern in terms of the clinical application of iPSC cells. Various modifications were developed all over the world to reduce the risk of tumor formation. Methods have been identified to generate iPSC cells without c-Myc, with only Oct3/4 and Klf4, with Oct4 from mouse neural stem cells, with recombinant proteins, without viral vectors, or without exogenous reprogramming factors. In 2011, Glis1, enriched in unfertilized oocytes, was shown as another important factor to promote the direct reprogramming of somatic cells during iPSC cell generation. Thus, the efficient generation of iPSC cells has been explored with safe methods. In Japan, the clinical application of iPSC cell-derived tissue may commence for age-related maculopathy. After the clinical study reveals safety, PD might be a hopeful target with iPSC cell technology. There are several planned clinical trials of iPSC cell-based therapies around the world. iPSC cell technology is also expected to reveal pathological conditions using patient-derived iPSC cell research. Various possibilities of iPSC cells are shown in Fig. 2. Alternatively, the direct conversion or trans-differentiation from fibroblasts into neurons without going through iPSC cells is another hopeful technique.

II. Gene therapy

Gene therapy is classified into two groups, that is, ex vivo and in vivo gene therapy. We mainly performed basic research using ex vivo gene therapy, as briefly described in the Cell therapy section. In this section, in vivo gene therapy, the main focus on recent clinical trials, is shown. Direct gene delivery using vectors with subsequent potent transfection and therapeutic effects are the prominent characteristics of in vivo gene therapy. Herpes simplex virus (HSV), retrovirus, adenovirus, adeno-associated virus (AAV), lentivirus, and other nonviral vectors such as liposome are usable for gene therapy. The strategies for PD can be roughly classified into the following
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Fig. 2 The possibility of induced pluripotent stem (iPS) cell technology. Dopaminergic (DA) neurons from iPS cells of a Parkinson’s disease (PD) patient can be used for autologous cell transplantation, genetic/proteomic analyses for PD pathogenesis, and drug discovery/evaluation. DA neurons from iPS cells of a healthy volunteer can be used for allogeneic cell transplantation, analyses for PD pathogenesis after genetic manipulation, and drug discovery/evaluation.

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four groups. The purposes are to increase local dopamine concentration, to exert neuroprotective/neurorestorative effects for degenerated dopaminergic neurons, to ameliorate the microenvironment of dopaminergic/non-dopaminergic systems involved in PD, and to normalize genetically abnormal cells related to the pathology of PD. Several genes might be related to the pathology of PD, including α-synuclein, leucine-rich repeat kinase 2 (LRRK2), and ubiquitin C-terminal hydrolase L1 (UCH-L1).69–72) Recent developments in genetic manipulation, such as dominant negative or small interfering RNA (siRNA), suppress the target gene expression, as well as the conventional way of over-expression of the target genes.73) Furthermore, animal models of PD by genetic manipulation might be useful to understand the pathology of PD.74) The enhancement or suppression of target genes might exert therapeutic effects on some types of PD patients. Concepts of gene therapy for PD are briefly shown in Fig. 3.

There are several good reviews on gene therapy for PD.75,76) Since 2003, a total of seven phase I and three phase II trials of in vivo gene therapy have been performed.77) AAV was used in nine trials and lentivirus was used in one trial after obtaining good results in a preclinical study using non-human primates.78) During et al. has injected AAV-glutamic acid decarboxylase (GAD) omit into the STN and has achieved suppressive effects on the hyper-activated STN, which is similar to the underlying mechanism of DBS.79) After preclinical evaluations, they proceeded to the first clinical trials of in vivo gene transfer for PD patients.80) Recent data on the randomized controlled phase II study of AAV-GAD showed the reduction of off-medication Unified Parkinson’s disease rating scale (UPDRS) scores compared to a sham surgery control.81) Intrastriatal infusion of AAV with the human aromatic l-amino acid decarboxylase (hAADC) gene resulted in effective dopamine conversion from systemically administered l-dopa. The safety was demonstrated in the phase I study.82) AAV containing neurturin (CERE-120) were stereotaxically administered into the putamen for the neuroprotective effects of neurturin.83) For stronger therapeutic effects, AAV-neurturin was administered into the substantia nigra and putamen.84) However, strong efficacy was not shown, although the safety/feasibility was demonstrated. Thus far, AAV has been the central player of in vivo gene therapy for PD. Very recently, the first in vivo gene therapy for PD with lentiviral vector was successfully reported from Europe.85) They used ProSavin (Oxford BioMedica, Oxford, United Kingdom), a tricistronic lentiviral vector encoding tyrosine hydroxylase, AADC, and cyclohydrolase1. Previous studies showed that transfected non-dopaminergic neurons can produce dopamine.86) Fifteen PD patients were enrolled in the study and followed for 12 months. All patients had motor behavior improvements. The study also showed the safety and tolerability. Safety hurdles have been overcome in all of the clinical trials of in vivo gene therapy for a decade. However, improved clinical trial design, patient selection, and outcome measures are needed to achieve greater efficiency. Furthermore, the management of non-motor problems, mainly related to a non-dopamine factor, is also a challenge.87)

Future Direction of Regenerative Medicine for PD

In this article, regenerative medicine for PD is
Fig. 3 Targets of gene therapy for Parkinson’s disease (PD). Genetic interventions are shown on simplified scheme of PD pathogenesis. There are four targets of gene therapy for PD. (1) Pathogenic genes can be suppressed or normalized in dopaminergic (DA) neurons. α-synuclein (SNCA), leucine-rich repeat kinase 2 (LRRK2), Parkin, or PTEN-induced kinase 1 (PINK1) might be good candidates. (2) Local dopamine can be increased. Enzymes related to DA-synthesis, such as tyrosine hydroxylase (TH), GTP cyclohydrolase 1 (GTPCH1), aromatic L-amino acid decarboxylase (AADC), and tetrahydrobiopterin (BH4) are representative examples. (3) Trophic effects can be obtained through upregulation of glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), or other trophic/growth factors. Anti-apoptotic effects are also expected through enhanced anti-apoptotic factor like bcl-2. (4) Correction of hyper-excitability is a different approach. Transduction of glutamic acid decarboxylase (GAD) into the subthalamic nucleus (STN) is representative. GPe: external globus pallidus; GPi: internal globus pallidus.

Cell therapy and gene therapy are all hopeful. A trigger of a sort might bring innovation to the treatment for PD and cause a paradigm shift in PD therapy. In terms of cell therapy, iPS cell technology is promising. As a transplant source, autologous dopaminergic neurons from patient iPS cells are ideal. For drug discovery, iPS cells from patients as well as the clarification of mechanisms are useful, although the variable characteristics of the clones of respective iPS cells might be considered. Preclinical tests using iPS cells from healthy volunteers to evaluate safety, pharmacokinetics, or drug efficacy are also meaningful. With regard to gene therapy, the safety of AAV was secured. Additionally, the lentiviral vector might be usable. For further confirmation of efficiency, clinical trials should be continued. We should also consider the cost-benefit performance so as not to collapse medical economy. In applying regenerative medicine for PD, one paradoxical issue should be considered. The most hopeful candidates for regenerative medicine might be younger PD patients in the relatively early stage. These patients are usually treated by the therapeutic options in existence. It is easily understood that patients with better conditions might enjoy more powerful benefits from the treatment. In contrast, the conditions of candidates of regenerative medicine are not so good. In other words, regenerative medicine for PD is expected to exert therapeutic effects on PD patients in the advanced stage, who will receive fewer benefits from some treatments, including regenerative medicine. To overcome this, the fundamental restoration of dopaminergic neurons is desired, despite the difficult challenge.

Based on the historical achievements in this field, technological developments have often opened new epochs. However, randomized, controlled trials are often discordant with the favorable results of early open label trials. We repeatedly experienced difficulty in obtaining good results from randomized, controlled studies. In order to overcome the somewhat static current status, we should not only refine the protocol of clinical trials, but should also search for more predictive...
animal models to re-create clinical trials, perform carefully retrospective analyses, and continue our efforts to realize regenerative medicine for PD.

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Conflicts of Interest Disclosure

There is no conflict of interest to be declared.

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Address reprint requests to: Takao Yasuhara, MD, PhD, Department of Neurological Surgery, Okayama University Graduate School of Medicine, 2-5-1, Shikata-cho, Kita-ku, Okayama, Okayama 700-8558, Japan. e-mail: tyasu37@cc.okayama-u.ac.jp