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Preclinical evaluation of patient-derived cells shows promise for Parkinson's disease

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Commentary

Parkinson's disease (PD) is a neurodegenerative disease caused by the progressive loss of dopaminergic (DA) neurons in the midbrain projecting to the striatum, which leads to motor dysfunctions, such as bradykinesia (slowed movement), rigidity, and tremors. To replace the lost cells, the transplantation of DA neurons derived from embryonic stem cells or induced pluripotent stem cells (iPSCs) has been considered. In this issue of the *JCI*, Song et al. report on their development of an iPSC induction and differentiation protocol that can promote the realization of autologous transplantation to treat PD patients with their own cells.

Preclinical evaluation of patient-derived cells shows promise for Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disease caused by the progressive loss of dopaminergic (DA) neurons in the midbrain projecting to the striatum, which leads to motor dysfunctions, such as bradykinesia (slowed movement), rigidity, and tremors. To replace the lost cells, the transplantation of DA neurons derived from embryonic stem cells or induced pluripotent stem cells (iPSCs) has been considered. In this issue of the *JCI*, Song et al. report on their development of an iPSC induction and differentiation protocol that can promote the realization of autologous transplantation to treat PD patients with their own cells.

Cell transplantation for Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease in which the number of dopaminergic (DA) neurons that project from the midbrain substantia nigra to the striatum progressively decrease. As a result, patients mainly exhibit motor dysfunctions, such as bradykinesia (slowed movement), rigidity, and tremors. To replace the lost DA neurons, the transplantation of fetal midbrain, which contains DA neurons, has been attempted since 1987. A number of clinical cases have proved that this treatment can improve patient symptoms (1). Moreover, positive effects for over 10 years have been reported (2). Now, a new clinical trial is ongoing in Europe that seeks to optimize the treatment protocols of this strategy (3). However, there are several issues regarding fetal-based treatment, including ethical concerns using fetal tissue, difficulty in obtaining a sufficient amount of fetal brain tissue, and contamination of serotonergic neurons, which may cause dyskinesia (involuntary movement). Although the efficacy of fetal cell transplantation has been demonstrated, it is not yet admitted as a standard treatment.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are considered alternative cell sources for DA neurons because both can differentiate into somatic cells of every organ. ESCs are derived from the inner cell mass of a blastocyst, which defines the early stage preimplantation embryo (4). On the other hand, iPSCs are induced from somatic cells, such as skin fibroblasts or blood cells, by introducing reprogramming factors (5). As a result, iPSCs result in fewer ethical problems because they avoid embryo destruction and can potentially allow autologous transplantation by using the patient's own cells, in which case there is no need for immunosuppression. To take advantage of this, the team led by Kim developed an iPSC induction and differentiation protocol to treat PD patients by autologous transplantation (6).

Improving methods to generate DA neurons from iPSCs

The process of autologous transplantation can be divided into two parts. One part establishes the iPSC line and the corresponding quality checks. The other induces DA neurons from the iPSCs and again, checks the final product for quality, safety, and efficacy. In the current study, the Kim group made several improvements to both parts of this process (6).

In the original iPSC-reprogramming method, four transcriptional factors (c-Myc, Oct4, Sox2, Klf4) were introduced into dermal fibroblasts by retroviral vectors (7). Notably, the protooncogene c-Myc risks tumorigenesis, so it has since been replaced by L-Myc in the reprogramming method (8). Song et al. found that the addition of two metabolism-modulating microRNAs (miR-302s and miR-200c) facilitated the generation of human iPSCs in terms of efficient colony formation and the expression of pluripotent markers in each colony (6). Another problem with the original design was retroviral integration, which sometimes caused genomic mutations. This risk was avoided by using integration-free methods via plasmid vectors (9). Song et al. also used plasmid vectors and confirmed that the established iPSCs were free of integrated plasmid DNAs. By using this new protocol, they succeeded in establishing human iPSCs that contained no somatic mutations causally implicated in cancer (6).

The next improvement was related to the culture conditions. Song et al. found that DA induction by neurosphere culture is highly variable between experiments (10–12) and thus investigated a monolayer culture (6). After comparing several cell densities, they reached the conclusion that the spotting method was best, in which iPSCs are initially attached only to designated areas by precoating circular areas (or "spots") of 5 mm diameter using Matrigel on the cross points of a 2×2 cm^2 grid. By this technique, they could significantly reduce cell death and obtain a higher yield of DA neurons (6).

Finally, Song et al. modified the differentiation protocol to induce DA neurons. The differentiation of pluripotent stem cells into various somatic cells is mainly

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controlled by bone morphogenic protein (BMP), TGF/activin/nodal and Wnt signals (6, 13). In order to induce neural cells, it is important to inhibit both BMP and TGF/activin/nodal signals. Because this method inhibits the SMAD1/5/8 and SMAD2/3 intracellular pathways, it is called dual SMAD inhibition (10). In addition to the above, to generate DA neurons, the ventral midbrain is induced by moderately activating Wnt signals and ventralizing with Sonic hedgehog (Shh) (11, 12, 14). The combination of these conditions results in a highly efficient method to induce DA neurons, but the possibility of residual undifferentiated cells with neoplastic potential remains (15, 16). One possible solution to avoid this risk is the removal of unwanted cells by sorting DA neurons with antibodies against CORIN (17), a marker for floor plate, or ALCAM (18), a marker for vascular endothelial cells in the central nervous system. Song et al. took another path. Based on the previous findings that *BIRC5* (encoding survivin) is highly expressed in human ESCs/iPSCs compared with somatic cells (19), they hypothesized that the chemically inhibiting survivin would selectively eliminate any remaining undifferentiated iPSCs. Among survivin inhibitors, they chose the flavonoid quercetin and found that it eliminated undifferentiated iPSCs with greater than 99.99% efficiency (6).

Future directions

One of the merits of autologous transplantation relates to the immune response by the host brain. Previous studies have confirmed that there is only a minimum immune response to the autologous transplantation of iPSC-derived neural cells in nonhuman primate brains (20). Therefore, there is no need for immunosuppressant drugs upon transplantation, which avoids adverse effects such as liver or kidney dysfunction and a compromised host. In addition, there is minimal risk of transmitting pathogens from donor tissue. However, autologous transplantation requires establishing iPSCs, inducing DA neurons, and evaluating the induced cells for each patient. At the moment, these processes are costly, laborious, and time-consuming. Moreover, it remains unclear whether patient-derived DA neurons are appropriate for treatment, especially for familial patients in which genetic mutations exist in the cells derived for transplantation.

Although details remain unknown, the new protocol presented by Song et al. will contribute to reducing the cost and time of generating DA neurons from somatic cells (6). Especially in autologous transplantations, the efficiency of obtaining cells of good quality is critical. According to the current study, the authors analyzed five independent iPSC lines derived from a sporadic PD patient by karyotyping, quantitative real-time PCR (qRT-PCR), and whole-exome sequencing and found that four clones out of five were free of integrated plasmid DNAs and contained no somatic mutations causally implicated in cancer. Considering that the establishment of iPSC lines and these genomic evaluations are time- and cost-consuming, this efficiency is remarkable. At the same time, however, the authors made no statement about the genomic stability during differentiation. It is possible that mutations may occur during differentiation, which could prohibit the transplantation. Therefore, quality checks will be needed for the final product, not only to evaluate genomic integrity, but also to check for the contamination of animal-derived proteins, sterility, and the presence of mycoplasma or endotoxins. There is still debate about the interpretation of the genomic analysis results, in part because iPSCs are a new cell type and not all genetic mutations cause malignant phenotypes. Future genomic analyses and clinical data will help to establish quality check criteria.

Finally, Song et al. performed in vivo studies to confirm the efficacy of their DA neurons induced from sporadic PD patient–derived iPSCs and found that the cells improved the behaviors of 6-OHDA–lesioned rats (6). Previously, Kikuchi et al. reported that DA neurons induced from sporadic PD patient–derived iPSCs improved the behaviors of MPTP-treated nonhuman primates (21). Although there are several reports that iPSCs derived from familial PD patients cannot give rise to healthy DA neurons, it seems that DA neurons derived from sporadic PD patients can function in the brain. Nevertheless, there is concern that PD patient–derived DA neurons might be more vulnerable to the pathology of PD than neurons from healthy individuals. In the cases of fetal cell transplantation to PD patients, an accumulation of α-synuclein in the grafted cells was reported (2). The same observations should be made for iPSC studies, especially in the cases of autologous transplantation. Therefore, before any firm conclusions can be made about autologous treatments of PD, clinical observation over a long time period is needed.

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