REVIEW

Cell Therapeutics in Parkinson's Disease

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Abstract The main pathology underlying motor symptoms in Parkinson's disease (PD) is a rather selective degeneration of nigrostriatal dopamine (DA) neurons. Intrastriatal transplantation of immature DA neurons, which replace those neurons that have died, leads to functional restoration in animal models of PD. Here we describe how far the clinical translation of the DA neuron replacement strategy has advanced. We briefly summarize the lessons learned from the early clinical trials with grafts of human fetal mesencephalic tissue, and discuss recent findings suggesting susceptibility of these grafts to the disease process long-term after implantation. Mechanisms underlying graft-induced dyskinesias, which constitute the only significant adverse event observed after neural transplantation, and how they should be prevented and treated are described. We summarize the attempts to generate DA neurons from stem cells of various sources and patient-specific DA neurons from fully differentiated somatic cells, with particular emphasis on the requirements of these cells to be useful in the clinical setting. The rationale for the new clinical trial with transplantation of fetal mesencephalic tissue is described. Finally, we discuss the scientific and clinical advancements that will be necessary to develop a competitive cell therapy for PD patients.

Keywords Neural transplantation · Dopamine neurons · Parkinson's disease · Stem cells · Dyskinesias

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Introduction

Parkinson's disease (PD) is a chronic disorder characterized by deficits in the control of movement secondary to degeneration of the dopaminergic nigrostriatal pathway. Other dopaminergic and nondopaminergic systems are also affected with the development of many nonmotor problems. Whereas motor symptoms can be treated rather well with dopaminergic medication and deep brain stimulation (DBS), effective therapies for nonmotor symptoms, such as dementia, are lacking, and disease progression cannot be counteracted. Transplantation of fetal mesencephalic tissue to the striatum reverses behavioral deficits in animal models of PD through restoration of striatal dopamine (DA) transmission [1]. Based on the animal experimental data, 300 to 400 patients with PD were grafted with human fetal mesencephalic tissue in the 1980s to 1990s. These trials provided proof of the principle that cell replacement can work in the human PD brain, but they also revealed troublesome adverse effects, so-called graft-induced dyskinesias (GIDs), in a subpopulation of patients [2].

The main objective now is to develop DA cell replacement by transplantation into a clinically competitive treatment. Besides the use of fetal DA-rich mesencephalic tissue, other sources of DA neurons (e.g., generated from stem cells or by direct conversion of somatic cells) have

been suggested. However, it should be emphasized that several new treatments for the PD patient have been added during the past couple of decades. For example, DBS in the subthalamic nucleus has been shown to substantially improve motor deficits in advanced PD [3]. Therefore, to become clinically useful, DA cell therapy has to provide a therapeutic advantage, such as long lasting, major improvement of mobility, suppression of dyskinesias, amelioration of symptoms resistant to other treatments, or counteraction of disease progression.

In this article, we give a brief summary of the outcome in the clinical trials with fetal mesencephalic grafts. We discuss, in some detail, recent studies of GIDs and long-term survival and function of transplanted DA neurons. Then we describe how far advanced the use of stem cells and reprogramming of somatic cells are to being used in the clinic for PD. We also discuss the major scientific and clinical problems that need to be solved for the successful application of DA cell therapy in patients.

Can DA Neurons be Replaced and Neural Grafts Have Functional Effects in PD Patients?

The outcome in the initial clinical trials with intrastriatal transplantation of human fetal mesencephalic tissue has been reviewed extensively (for more detail see Lindvall and Björklund [2]); this will not be described in detail here. Based on the findings in these trials, the following 6 general conclusions of importance can be made for further development of a cell therapy in PD:

1. Dopaminergic Grafts Can Survive and Become Morphologically Integrated in the PD Patient's Brain

    Following implantation of postmitotic DA neuroblasts from the ventral mesencephalon of 6- to 9-week-old human fetuses, positron emission tomography (PET) detected increases in 6-L-[^{18}F]-fluorodopa (^{18}F-dopa) uptake (Fig. 1) [4–19], and histopathological studies have shown long-term, extensive synaptic reinnervation in the striatum [13, 20–27].

2. Dopaminergic Grafts Can Restore DA Release in the PD Patient's Striatum

    One patient with major clinical improvement, in whom ^{18}F-dopa uptake was normalized in the grafted putamen 3 years postsurgery, showed normal basal and drug-induced DA release (as assessed by ^{11}C-raclopride binding) 10 years after transplantation [28]. In a group of PD patients, there was no evidence for abnormal DA release from the grafted neurons [29].

3. Dopaminergic Grafts Can Become Functionally Integrated into Neural Circuitries in the PD Patient's Brain

With a time course paralleling that of clinical improvement, bilateral intrastriatal dopaminergic grafts reversed deficits in the movement-related activation of supplementary motor area and dorsolateral prefrontal cortex, which are believed to underlie akinesia in PD [30].

4. Dopaminergic Grafts Can Give Rise to Functional Improvement in Some Patients but the Outcome Is Highly Variable

    Clinical benefits in patients with surviving grafts have been observed in open-label trials [4, 7–11, 13, 15–19, 31, 32], and the most successful cases have had L-dopa treatment withdrawn, exhibiting major clinical improvement for several years [4, 8, 18, 28]. In contrast, the improvements in 2 sham surgery-controlled clinical trials were only modest. Freed et al. [6] demonstrated 18% reduction in Unified Parkinson's Disease Rating Scale motor score compared to the sham group score at 12 months after bilateral intraputaminal grafts. Two patients who died only had between 7,000 and 40,000 grafted dopaminergic neurons in each putamen [6], which were much fewer than were found in 2 patients in an open-label trial (i.e., between 80,000 and 135,000 [22–24]). This may be due to having less tissue implants, or storage in cell culture for as much as 4 weeks, or lack of immunosuppression. In an open-label follow-up of the patients in this study, Ma et al. [19] observed sustained clinical benefit and graft viability for as much as 4 years after transplantation. In the other sham surgery-controlled clinical trial [14], tissue was implanted in the putamen and cyclosporine was given for 6 months thereafter. No difference between the grafted and sham groups was found in the change of Unified Parkinson's Disease Rating Scale motor score at 24 months as compared to baseline. Similar to the time course of improvement in the open-label trials, grafted patients showed progressive symptomatic relief during the first 6 to 9 months after surgery, but then reverted back to baseline. The lack of efficacy could be explained by the patients of Olanow et al. [14] being more severely disabled as compared to those, for example, in the Swedish series [4, 8, 18]. In support of this idea, Olanow et al. [14] found significant improvement in their less severely disabled patients compared to the sham group at 2 years. The function of the grafts in the study of Olanow et al. [14] may have been impaired due to an immune reaction. Deterioration after the initial improvement is consistent with such a reaction after withdrawal of immunosuppression at 6 months. In 2 patients who came to autopsy, the grafts were infiltrated by activated microglia, suggesting a delayed immune response [14].

In contrast, there were 2 patients who had been subjected to 6 months of immunosuppressive treatment in the study of Mendez et al. [13] who also showed clinical improvement and only a few macrophages and activated microglia were found in grafted regions 3 to 4 years after surgery.

5. Magnitude and Duration of Functional Improvement Induced By Dopaminergic Grafts Is Dependent on Patient Selection

The preoperative response to L-dopa has been found to be an important predictor of the degree of improvement after DA neuron transplantation [33]. By using ^{18}F-dopa PET, Piccini et al. [29] have shown that the occurrence of dopaminergic denervation in areas not reached by intraputaminal grafts, such as the ventral striatum, exerts a marked influence on the overall outcome following transplantation of human fetal mesencephalic tissue. In accordance, Ma et al. [19] observed that the symptomatic relief in patients grafted bilaterally in the posterior putamen was dependent on the extent of the loss of dopaminergic innervation in the ventrorostral putamen. Taken together, these results provide evidence that patients with denervations who remain restricted to caudate-putamen are likely to have major long-term benefits from dopaminergic grafts placed in these areas. In contrast, long-lasting successful outcomes in PD patients with more widespread denervations are unlikely, unless grafts are also placed in areas outside the caudate-putamen.

6. Adverse Effects Are Few with Dopaminergic Grafts, Except Dyskinesias in a Subgroup of PD Patients

The most serious complication after transplantation of fetal mesencephalic tissue has been GIDs in the off phase, which are encountered in approximately 15% of grafted patients. These dyskinesias can be effectively treated with DBS in the globus pallidus [34, 35]. Until now, the occurrence of GIDs and their unclear underlying mechanisms have stopped the clinical activities with cell therapy in PD.

Can Graft-Induced Dyskinesias be Avoided or Treated?

The appearance of GIDs was first observed in the Denver/Columbia trial [6], and subsequently they have also been reported in some of the patients in the Swedish program [36] and in the Tampa/Mount Sinai trial [14]. This side effect came as a surprise, because none of the pre-clinical studies in rodent and primate models of PD have observed any adverse responses of this type. Most notably, however, all pre-clinical studies that preceded the clinical trials had

been performed in animals that were not exposed to chronic L-dopa. Only 1 transplantation study had been performed in rats made dyskinetic prior to transplantation, and in this experiment the established L-DOPA-induced dyskinesias were reduced by approximately 70% after transplantation, without any signs of off-state dyskinesias [37]. Studies performed after the report by Freed et al. [6] have reported similar findings, confirming that fetal mesencephalic grafts typically reduce established L-DOPA-induced dyskinesias, and do not induce any off-state dyskinesias, in 6-hydroxydopamine-lesioned rats (for more detail see Lane et al. [38]). A distinct type of GID, however, has been identified in these rodent studies (i.e., dyskinesia induced by the DA-releasing agent amphetamine) [39–41]. This phenomenon, which is never seen in nongrafted controls, appear gradually after transplantation, and is highly correlated with the number of DA neurons in the graft. Interestingly, this type of GID develops only in animals that have developed L-DOPA-induced dyskinesia prior to transplantation, suggesting that animals have become dyskinetic by chronic L-DOPA treatment are particularly susceptible to develop GID [40, 42].

The interpretation of the experimental studies is complicated by the fact that off-state dyskinesias of the type seen in patients have not been observed so far in transplanted rats or monkeys. Nevertheless, the studies on amphetamine-induced GID may be informative and give us some clues. In particular, the data obtained with this model suggest that some form of L-DOPA-induced postsynaptic supersensitivity, established prior to transplantation, may play a role in the induction of GID. There are also data to suggest that small, regionally restricted transplants may be more prone to cause this side-effect, forming "hot-spots" of DA release, whereas the surrounding striatum remains supersensitive [39, 43]. In addition, we have been interested in the possibility that serotonin neurons could take part. We know that the fetal mesencephalic tissue used for transplantation also contains serotonin neurons, in addition to DA neurons. With the landmarks commonly used for dissection of the fetal tissue, the number of serotonin neurons is likely to be quite large, probably in the order of half of the DA neurons present in these preparations. Experiments in the rat 6-hydroxydopamine model suggest that serotonin neurons can exacerbate dyskinesia induced by L-DOPA, and we have proposed that the presence of large numbers of serotonin neurons in the grafts, in combination with a suboptimal number of DA neurons could make grafted patients more prone to develop GID [44].

Two recent studies [45] of transplanted PD patients have provided support for this idea [46]. These studies included 3 patients who had received fetal mesencephalic grafts 13 to 16 years earlier. All 3 showed excellent clinical benefit that persisted in time, and were in the absence of
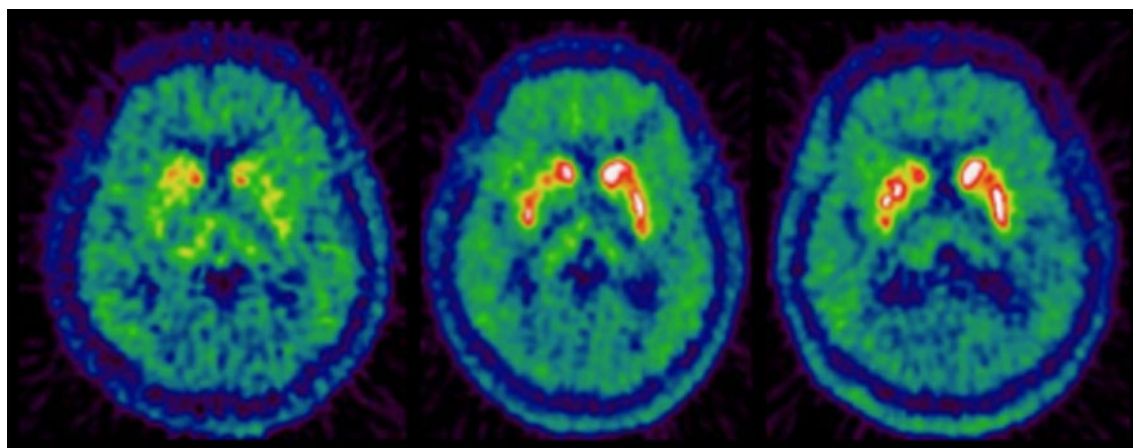
Fig. 1 Human fetal mesencephalic dopaminergic neurons survive and grow after bilateral transplantation into putamen and the head of caudate nucleus in a Parkinson's disease patient. The 6-L-[^{18}F]-fluorodopa (^{18}F-dopa) positron emission tomographic images show striatal uptake before (left), at 8 months (middle), and at 21 months (right) postsurgery. (Courtesy Dr. Marios Politis, Hammersmith Hospital, London, UK)

any L-DOPA or agonist medication. With time, however, they developed moderately severe GID. A PET scan using a serotonin uptake ligand ^{11}C-3-amino-4-(2-dimethylamino-methylphenylthio)benzonitrile (^{11}C-DASB) showed excessive serotonergic innervation in the grafted striatum of these patients. Moreover, in a subsequent double-blind test, it was found that GID was markedly attenuated by systemic administration of a serotonin receptor 5-HT1A agonist, Buspirone (Fig. 2). Activation of 5-HT1A receptors is known to inhibit serotonin neuron activity and dampen transmitter release from serotonergic neurons.

Taken together, the experimental studies in rodents, and the recent observations in patients displaying GID, point to 3 major factors that may have contributed to the development of GID in grafted patients: 1) the presence of L-DOPA induced dyskinesias prior to transplantation; 2) the uneven distribution of the grafted tissue, creating "hot spots" of graft-derived innervation in an otherwise supersensitive striatum; and 3) the development of a serotonergic hyperinnervation in the grafted putamen. In future clinical trials, therefore, one should avoid the inclusion of severely dyskinetic patients, use a grafting procedure that ensures widespread distribution and high survival of the grafted DA neurons, and minimize the inclusion of serotonin neurons in the tissue used for transplantation.
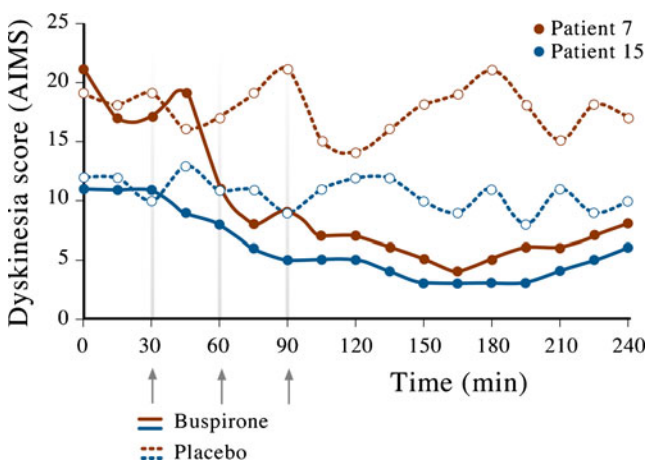
Can Neural Grafts Survive and Provide Long-Term Function?

The patients who received their transplants in the late 1980s and early 1990s have offered possibilities to evaluate the long-term efficacy of fetal dopaminergic grafts. Moreover, several patients whose brains have become available for analysis at autopsy have provided a unique opportunity to study the survival and function of the grafted dopaminergic neurons at survival times for as much as 22 years. Some of these patients, such as the 3 reported in the recent studies [45] and [46] have shown sustained clinical benefit of their transplants for more than 13 years, although in these cases it was associated with the emergence of dyskinesias (GIDs) unrelated to their ongoing medication. The recovery in striatal ^{18}F-DOPA uptake seen in these patients has been sustained, suggesting that the grafted dopaminergic neurons have survived well over time.

The microscopic analysis of long-term surviving grafts has been particularly interesting. To date, immunohistochemical analysis of transplants surviving longer than 10 years has been reported for 5 cases [20, 21, 25–27].

Fig. 2 Studies in 2 grafted patients who had received their fetal dopamine neuron transplants 13 and 16 years earlier, show that the graft-induced dyskinesias can be markedly attenuated by administration of the 5-HT1A agonist Buspirone, given as 3 doses of 5 mg, 30 minutes apart. These patients show excellent long-term clinical improvement in the absence of any dopaminergic medication. (Data from Politis et al. [45])

Consistent with the imaging data, these transplants that had survived for as many as 16 years in the brain contained large numbers of dopaminergic neurons (Fig. 3). However, a fraction of the grafted DA neurons showed a pathological accumulation of α-synuclein, and some of them contained Lewy Body structures, which is a characteristic feature of affected neurons in PD. These α-synuclein-positive inclusions seem to be present only in transplants that have survived for 10 years, or longer, in the diseased brain, suggesting that the pathological accumulation of α-synuclein is a slow process that takes place during a timespan of many years. In 1 patient who received bilateral transplants with a 4-year interval, Li et al. [27] found α-synuclein-positive Lewy Body-like inclusion in 2% of pigmented, presumed dopaminergic neurons, contained in the 12-year-old graft compared to 5% in the 16-year-old graft in the same patient, suggesting that the α-synuclein-related pathology spreads slowly with time at a rate of approximately 1% per year.

To what extent the accumulation of α-synuclein in the grafted neurons is detrimental to the survival and/or function of the grafted cells is not known. Kordower et al.
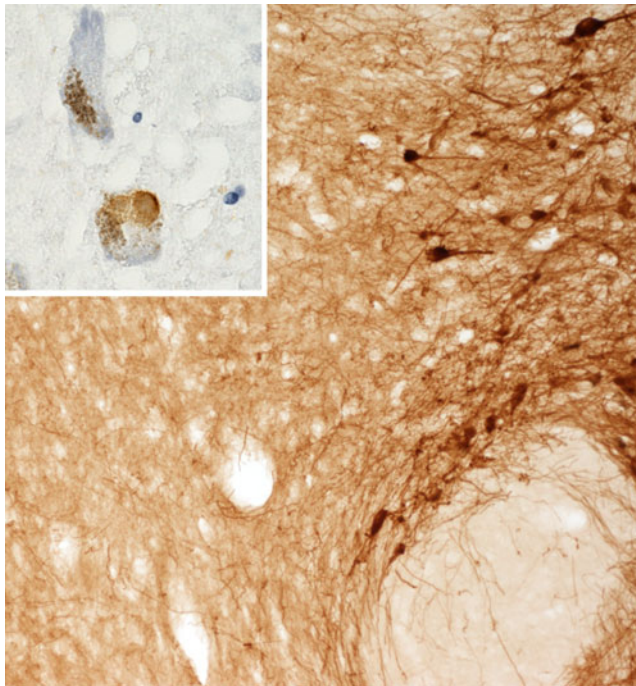
[20, 21] have reported that α-synuclein-expressing neurons in long-term surviving grafts have a reduced expression of the DA transporter (DAT), and the same group has previously reported that α-synuclein-expressing neurons in the substantia nigra in PD patients have a reduced expression of tyrosine hydroxylase [47]. These observations may be taken to suggest that the α-synuclein pathology seen in DA neurons in long-term grafts is associated with dysfunction at the synaptic level. Changes in DAT expression in grafted patients are possible to monitor noninvasively by single photon emission computed tomography or PET imaging. However, so far this has been done to a very limited extent. To our knowledge this method has been applied long-term only in 1 transplanted patient. In this case, DAT binding was increased after grafting and remained unchanged still at 14-years posttransplantation [46]. This suggests that reductions in DAT expression, as described by Kordower et al. [20, 21] may vary between patients.

Can Stem Cell-Derived Neurons Replace Fetal Grafts in PD Patients?

Stem cells could become a source of virtually unlimited numbers of DA neuroblasts for transplantation and subsequent neuronal replacement, and this strategy is the focus here. However, it should be pointed out that experimental studies have indicated that stem cells and precursor cells can also contribute to recovery through other mechanisms in animal models of PD. For example, transplantation of human neural progenitor cells genetically modified to secrete glial cell line-derived neurotrophic factor into the striatum and substantia nigra protected injured DA neurons [48]. Moreover, nonmodified, nondifferentiated neural stem cells (NSCs) injected into substantia nigra and striatum of nonhuman primates mediated recovery, possibly by giving rise to a small number of cells with DA neuron phenotype, but mainly through various homeostatic adjustments induced, for example, by glial cell line-derived neurotrophic factor-expressing progeny [49]. Finally, stimulation of endogenous neural precursors in adult rats by intraventricular injection of angiopoietin, alone or in combination with the Notch ligand Dll4, rescued injured DA neurons and ameliorated amphetamine-induced rotational asymmetry [50].

Many laboratories are now involved in producing DA neuroblasts for transplantation by predifferentiation from stem cells. In the clinical setting, the stem cell-derived DA neuroblasts most likely have to be of human origin and after maturation, work at least as well as those in fetal mesencephalic grafts to be therapeutically useful. It has been estimated that approximately 100,000 or more grafted

Fig. 3 Lewy bodies can develop in a fraction of dopaminergic neurons long term after transplantation, but extensive graft-derived reinnervation of the Parkinson's disease patient's striatum can still be observed. α-Synuclein immunostaining (*inset to the left*) showing dopaminergic neurons containing clustered pigmented granules in a 16-year-old graft. One of these neurons contains a compacted Lewy body. Tyrosine hydroxylase immunostaining (right) from the same patient showing surviving grafted DA neurons giving rise to a rich terminal network. (Courtesy Dr. Jiayi Li, Wallenberg Neuroscience Center, Lund, Sweden)

DA neurons need to survive long-term in each human putamen to induce substantial benefit [51]. Available animal and clinical data also indicate that the grafted DA neurons have to exhibit the phenotype of those neurons that have died (i.e., substantia nigra neurons). The definition of a dopaminergic nigral neuron includes expression of phenotypic markers typical for those cells using immunohistochemistry, as well as expression profiles of genes/transcription factors that define that area of the brain during normal nigral development. The cells need to exhibit neuronal properties using appropriate neurophysiological measures and DA release in response to standard *in vitro* stimuli.

For the clinical translation, the stem cell-derived cells should be tested in appropriate animal models, primarily in rats with unilateral 6-hydroxydopamine lesions. The cells must be able to survive long term in large numbers and differentiate into mesencephalic DA neurons. Ongoing cell division must not be present beyond 1 to 2 months following transplantation. A major portion of the striatum (>50%) should be evenly innervated by graft-derived dopaminergic fibers. The ability of the grafted cells to mediate sustained behavioral improvements must be shown not only by alleviating amphetamine-induced rotational asymmetry, but also by inducing significant recovery in either stepping, placing, or cylinder tests in rats, as well as in skilled reaching similar to fetal mesencephalic grafts.

Cells with at least some of the characteristics of mesencephalic DA neurons have been produced from stem cells of different sources. So far, the most promising results have been obtained using embryonic stem (ES) cells [52–62] or therapeutically cloned ES cells [63]. The examples shown (Fig. 4) are derived from mouse ES cells, using forced expression of the transcription factor Lmx1a, according to the protocol published by Friling et al. [62] in 2009. Other types of stem cells, such as NSCs and progenitors from the embryonic ventral mesencephalon [64–67], adult NSCs from the subventricular zone [68], bone marrow stromal cells [69], and fibroblast-derived induced pluripotent stem (iPS) cells [61, 70–72] have also been used for this purpose. It has also been shown that human stem cell-derived DA neuroblasts, which will be required for patient application, can survive in animal models of PD and after maturation exert functional effects [54, 58, 66, 69, 71, 72]. However, some properties that will be fundamental for successful clinical translation have not been demonstrated yet for human stem cell-derived DA neurons, including those that they can substantially reinnervate striatum, restore DA release *in vivo*, and markedly improve deficits resembling the symptoms experienced by patients with PD. Experimental work establishing these properties remains to be performed before a human stem cell-derived DA neuroblast can be selected as a candidate cell for patient application.

A major concern is the risk of tumor growth. Because life expectancy is virtually normal in PD patients, even a minor risk of tumor formation associated with stem cell therapy is unacceptable in this disorder. Human ES cells can give rise to unlimited numbers of NSCs, but they are associated with a risk of tumor formation [58]. A problem for the clinical translation is that the tumor risk with human cells derived from ES cells and most likely also from iPS cells (as follows) is difficult to assess in the preclinical xenograft situation [73].

Can Neurons for Transplantation be Generated from the PD Patient's Own Skin Cells?

Somatic cells, such as fibroblasts, can be reprogrammed to pluripotent stem cells by the introduction of transcription factors [74]. These iPS cells are then differentiated to specific neuron types for transplantation. With this technology, patient-specific cells can be produced without the need for immunosuppressive treatment after transplantation, and the ethical issue with the use of human ES cells is avoided. Wernig et al. [70] and Hargus et al. [71] reported that it has now been shown that DA neurons can be generated from iPS cells, derived from mouse [70] or human [71, 72] fibroblasts, and ameliorate behavioral deficit after transplantation in a rodent PD model.

Several problems have to be solved before transplantation of DA neurons derived from iPS cells could be considered in a clinical setting. First, the risk of tumor formation, which resembles that of ES cells, has to be eliminated. Wernig et al. [70] and Hargus et al. [71] minimized this risk by separating contaminating pluripotent cells and committed neural cells using fluorescence activated cell sorting (FACS). Second, much work also remains to optimize the survival, growth capacity, and functionality of the DA neurons generated from iPS cells. Hargus et al. [71] reported only few graft-derived axons reinnervating the rat striatum, which is clearly less than that with human fetal mesencephalic tissue transplants [75]. Moreover, only amelioration of rotational asymmetry, but no effect on more complex behaviors, has been demonstrated in animal models following iPS-derived DA grafts. Third, the use of the patient's own cells might be associated with increased susceptibility to the degenerative process in PD. Consistent with this idea, increased α-synuclein expression has been reported in PD patient fibroblasts [76]. Finally, it can be questioned whether all of the efforts to produce patient-specific DA neurons for transplantation in PD are justified. Immune reactions to brain allografts are moderate and survival can be obtained even without immunosuppression
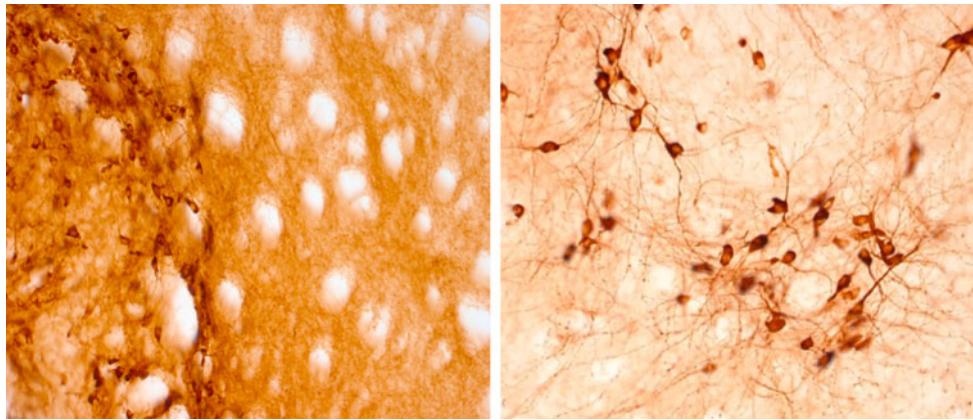
Fig. 4 Friling et al. [62] have shown that the production of midbrain dopamine (DA) neurons from mouse embryonic stem (ES) cells can be promoted by forced expression of the transcription factor Lmx1a. The DA neurons generated with this technique survive well after grafting to the rat striatum and express all the markers characteristic of authentic midbrain DA neurons. The 2 micrographs show examples of surviving ES cell-derived DA neurons, their characteristic cellular morphology, and their extensive axonal terminal network at 4 weeks after transplantation, as visualized in sections stained for tyrosine hydroxylase

[6], although most investigators favor immunosuppression for 6 to 12 months after transplantation.

Functional neurons can also be generated by directly reprogramming mouse somatic cells, such as fibroblasts, by expressing neural lineage-specific transcription factors [77]. This conversion does not occur through a pluripotent stem cell stage, and thereby the risk for tumor formation is eliminated. Very recently it was shown that human-derived fibroblasts can be directly converted to DA neurons with a nigral phenotype [78, 79]. Prior to their clinical use in PD is considered, it has to be shown that these DA neurons can survive transplantation and give rise to substantial improvements in animal models.

Can Neuronal Replacement Be Optimized with Fetal Grafts while We Are Waiting for Stem Cells?

It seems clear that fully validated, functional, and safe stem cell-derived DA neurons, suitable for use in clinical trials, are not going to be available any time soon. This raises the question on how to promote further development in cell therapy for PD while we are waiting for the new opportunities offered by stem cell technology. Roger Barker (Cambridge, UK) has gathered a group of investigators from the main European and North American sites involved in fetal cell transplantation during the last few years to review the experience gained from the clinical trails performed so far. In these discussion meetings, sponsored by Parkinson's UK (formerly UK Parkinson's Disease Society), the investigators have reviewed primary data and clinical observations and discussed every step in the grafting procedure and the key elements of the clinical protocols used at each site, with the goal to devise a new protocol in which every single step (including dissection, storage, and preparation of the fetal tissue; stereotaxic procedure; patient selection; immunosuppressive treatment; and clinical evaluation) is optimized and standardized. This formed the basis of a project proposal to the European FP7 program, called TransEuro, which was funded in 2009 and is now in progress.

The purpose of this project is to reactivate fetal cell grafting in less severe PD patients, in 2 steps: 1) first an open-label trial for a 2-year duration in a group of 20 patients, recruited at 4 different European centers, followed by a double-blind placebo-controlled trial in which the patients will be evaluated for a 5-year period. In these patients, ^{18}F-dopa PET scanning will be used as an element in the evaluation of patients before transplantation to make sure that the patients included in the trial have a well-preserved DA innervation in ventral striatal regions, inline with the observations made in the recent Piccini et al. [29] and Ma et al. [19] studies. Hopefully, this trial will pave the way for subsequent trials using DA neurons derived from stem cells and also serve as a model for how such trials should be designed.

Toward an Effective and Safe Cell Therapy for PD

To be clinically competitive in PD, a DA cell replacement therapy has to induce substantial (at least 50-70%) amelioration of motor symptoms without significant side effects. Neither in the open-label nor in the sham-surgery controlled clinical trials, improvements after intrastriatal transplantation of fetal mesencephalic DA-rich tissue in PD patients [4, 6, 8, 9, 13, 14] have exceeded those found with DBS in the subthalamic nucleus [80]. Moreover, there is no

convincing evidence that drug-resistant symptoms are reversed by these grafts [81]. The following 3 advancements will be necessary for the development of a clinically competitive cell therapy in PD.

1. Generation of Dopaminergic Neuroblasts in Large Numbers and in Standardized and Quality-Controlled Preparations

    Dopamine-releasing cells expressing markers and electrophysiological properties of substantia nigra neurons have to be produced under GMP conditions *in vitro*. It has to be demonstrated that these cells survive, effectively reinnervate the striatum, release DA *in vivo*, and give rise to substantial improvement of clinically relevant behavioral deficits after transplantation in a rodent PD model. Issues of scaling up, cell migration, and risks and toxicity should be assessed in rodents and large animals.

2. Improved Patient Selection and Tailor-Made Transplantation Procedure for Increased Efficacy

    Dopaminergic cell therapy will only be successful in patients who exhibit marked improvement of motor symptoms in response to L-DOPA, and in whom the main pathology is a loss of DA neurons. The dose and site of implantation of DA cells needs to be determined based on preoperative PET and single photon emission computed tomographic imaging so that the repair of the DA system becomes as complete as possible in each patient's brain. Based on the data of Piccini et al. [29] and Ma et al. [19], intrastriatal grafts should only be applied to patients with dopaminergic denervation largely restricted to striatal areas.

3. Strategies to Avoid Adverse Effects

    The risk of GIDs has to be minimized by excluding serotonergic neurons from the graft material, by carefully monitoring immunosuppressive treatment for at least 6 to 12 months, and by using a surgical procedure that gives rise to optimum distribution of cells over the putamen, and complete and even reinnervation without "hot-spots." The risk of tumor formation when the grafted DA neurons have been derived from pluripotent stem cells, and the consequences of the introduction of new genes in stem cell-derived neurons should be carefully evaluated after transplantation in animal models prior to clinical application.

Concluding Remarks

With the rapid development of stem cell and cell reprogramming technologies, it is conceivable that it will soon be possible to produce DA neurons of the correct phenotype for replacement in patients with PD. If these cells can be effectively manufactured, and proven to be safe, cell replacement therapies could become accessible to larger populations of PD patients. Importantly, the clinical trials with fetal mesencephalic grafts have provided proof of the principle that DA neuronal replacement can work in the PD patient's brain and give rise to long-lasting, dramatic improvements in some cases. We now have much better knowledge as to how to avoid GIDs and increase the efficacy of transplanted dopaminergic neurons. However, the nearly 25-year history of cell therapy in patients with PD illustrates that there is no fast track for the clinical translation. Only with long-term commitment to high-quality basic and clinical research addressing the crucial issues, some of which are discussed in this article, will it be possible to offer cell-based treatments to PD patients that lead to substantial improvements of their quality of life.

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