Cell Reports
Report

Long-Term Health of Dopaminergic Neuron Transplants in Parkinson's Disease Patients

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http://dx.doi.org/10.1016/j.celrep.2014.05.027

SUMMARY

To determine the long-term health and function of transplanted dopamine neurons in Parkinson's disease (PD) patients, the expression of dopamine transporters (DATs) and mitochondrial morphology were examined in human fetal midbrain cellular transplants. DAT was robustly expressed in transplanted dopamine neuron terminals in the reinnervated host putamen and caudate for at least 14 years after transplantation. The transplanted dopamine neurons showed a healthy and nonatrophied morphology at all time points. Labeling of the mitochondrial outer membrane protein Tom20 and α-synuclein showed a typical cellular pathology in the patients' own substantia nigra, which was not observed in transplanted dopamine neurons. These results show that the vast majority of transplanted neurons remain healthy for the long term in PD patients, consistent with clinical findings that fetal dopamine neuron transplants maintain function for up to 15–18 years in patients. These findings are critically important for the rational development of stem-cell-based dopamine neuronal replacement therapies for PD.

INTRODUCTION

There is a need to understand how transplanted neurons can survive despite ongoing disease processes in the brains of patients with Parkinson's disease (PD). Currently, there is some controversy surrounding the neural transplantation field and neuroscience research regarding interactions between potentially pathological toxic proteins as a cause of neurodegeneration, and the concept of "disease spread" from cell to cell (Desplats et al., 2009; Isacson and Mendez, 2010). The accumulation of Lewy-body-like inclusions in some transplanted fetal dopamine neurons after long-term survival (over a decade) in the PD brain has been described (Cooper et al., 2009; Kordower et al., 2008; Kurowska et al., 2011; Li et al., 2008). Such pathology is a rare occurrence, with only a very low frequency (~1%) of grafted neuromelanin-containing neurons in cell suspension grafts exhibiting signs of α-synuclein pathology even 22 years after grafting (Kurowska et al., 2011). These isolated cell inclusions are not observed in all patients (Mendez et al., 2008) and are usually found in less than 1%–5% of transplanted neurons depending on the transplantation method used, and clinical and postmortem data indicate that this rare pathology does not affect overall graft function (Cooper et al., 2009; Isacson and Mendez, 2010). It has been suggested that such Lewy-body-like pathology is a product of protein transfer from the parkinsonian host brain to the transplanted fetal cells (Kurowska et al., 2011). However, α-synuclein pathology is not definitive for PD, and incidental α-synuclein pathology has also been reported in the normal aging brain, with frequencies of 8%–22.5% in normal aging and up to 34.8% in centenarians (Ding et al., 2006; Klos et al., 2006; Mikolaenko et al., 2005; Saito et al., 2004; Wakisaka et al., 2003). Experimental paradigms of oxidative stress (e.g., rotenone exposure) or neuroinflammation can also induce α-synuclein accumulation in dopamine neurons (Gao et al., 2008; Sherer et al., 2003).

Recent results from postmortem examinations of fetal ventral mesencephalic grafts in PD patients suggested that dopamine transporters (DATs) are downregulated in the transplanted dopamine neurons (Kordower et al., 2008; Kurowska et al., 2011), and that such changes (which also include reduction of the dopamine neuron phenotypic marker tyrosine hydroxylase [TH]) are indicative of neuronal dysfunction and PD pathophysiological changes in the transplanted neurons. Since a cell therapy approach holds considerable promise as a therapeutic strategy for PD (C.R. Freed et al., 2013, Soc. Neurosci., conference; Kefalopoulou et al., 2014; Ma et al., 2010; Mendez et al., 2005; Politis et al., 2010, 2012), it is important to address the status of transplanted fetal dopamine cells in more detail. In previous studies, we reported surgical, clinical, and histopathological data obtained in five patients with advanced idiopathic PD who had received intracerebral transplantation of fetal dopaminergic cell suspension grafts 4–14 years earlier (Cooper et al., 2009; Mendez et al., 2005, 2008). In those studies, therapeutic improvements were seen without clinical side effects, such as off-period dyskinesias. Postmortem examinations demonstrated that grafted dopaminergic neurons survived for up to 14 years posttransplantation. In the current study, we examined DAT expression as a measure of neuronal function, and the mitochondrial marker Tom20 (translocase of outer mitochondrial membrane 20 kDa)

to assess mitochondrial morphology, to further understand the long-term phenotypical characteristics of the transplanted dopamine neurons and potential effects of the aging of transplants.

RESULTS

Dopamine Transporter Localization and Expression in Transplanted Fetal Dopamine Neurons

In the present study, we assessed DAT immunostaining in 4- to 14-year-old grafts in five patients from our previously published series (Mendez et al., 2005, 2008) in order to further understand the long-term phenotypical characteristics of the transplanted dopamine neurons and potential effects of the aging of transplants.

We conducted immunofluorescence staining for DAT using a monoclonal antibody that recognizes the N terminus of DAT (Miller et al., 1997), and performed colabeling with a TH antibody to label dopaminergic neurons and fibers. A general assessment of the integrity of the grafted TH-immunoreactive neurons in all patients revealed cells with a healthy appearance, including a robust cell soma and absence of signs of atrophy (Figures 1A–1C, 1F–1H, 1K–1M, and 1P–1R). In two independent patients at 4 years posttransplantation (Figures 1A–1J), an examination of DAT/TH immunostaining at low magnification (Figures 1A and 1F) showed dense DAT and TH expression in the reinnervated putamen and caudate in areas both near to and farther away from the graft. Although DAT was also expressed in the grafted cell soma, the intense punctate staining pattern in the reinnervated areas was most striking (Figures 1B and 1G). This expression, consistent with that of synaptic proteins, was easily observed at high magnification (Figures 1C, 1D, 1H, and 1I) where DAT was localized along TH-immunoreactive fibers.

To determine whether DAT expression was maintained in the long term, we examined DAT immunolabeling in transplanted neurons at 9 years and 14 years posttransplantation (Figures 1K–1T). As also seen at the younger time points, a robust punctate expression in the reinnervated striatum was observed (Figures 1K–1M and 1P–1R) and higher-magnification imaging verified the coexpression of DAT puncta along TH-immunoreactive dopaminergic fibers (Figures 1N and 1S). The intensity of DAT immunofluorescence was quantified in the reinnervated putamen at 4–14 years after transplantation (Figure S1) and compared with DAT labeling intensity in the contralateral (nontransplanted) putamen from subject 2. As expected, very low levels of DAT labeling in the nontransplanted parkinsonian putamen were observed, consistent with the severe loss of DAT expression in the putamen in PD (Miller et al., 1997). In contrast, at 4, 9, and 14 years after transplantation, DAT expression was significantly increased within the grafted putamen.

Parallel control immunostainings in which the primary antibodies were omitted showed no immunoreactivity of DAT or TH (data not shown). To further confirm the specificity of the DAT labeling observed in the reinnervated putamen and caudate, we also examined DAT immunolabeling in adjacent anatomical regions on the same tissue sections (see Figure 1). As expected, in the lateral and medial globi pallidi, which are regions that receive comparatively little dopaminergic innervation and normally exhibit little DAT expression in the human brain (Ciliax et al., 1999), we observed weak DAT immunolabeling and a sharp boundary from high to low DAT and TH expression (Figures 1E, 1J, 1O, and 1T).

Mitochondrial Localization and Expression in Transplanted Fetal Dopamine Neurons

Tom20 was used to label mitochondria in grafted neurons and also in the host substantia nigra and globus pallidus. In the remaining substantia nigra TH-immunoreactive neurons from PD patients (subjects 2, 5, and 6; Figures 2A, 2A', 2D, 2D', 2G, and 2G'), Tom20 labeling often appeared intensely labeled in the cell soma, with accumulation in the perinuclear area and little immunostaining in the axon and processes. In neurons costained with Tom20 and α-synuclein, the host patient's substantia nigra showed Lewy bodies and variable or reduced distribution of Tom20-stained mitochondria (Figure 3A). In grafted TH-immunoreactive neurons at 4 years posttransplantation (Figures 2B and 2B'), Tom 20 immunostaining was robust in the perikarya and neuronal processes, similar to what was observed in the normal brain. At 9 and 14 years posttransplantation, Tom20 labeling was generally less intense in the grafted TH-immunoreactive neurons (Figures 2E, 2E', 2H, and 2H') compared with the Tom20 staining pattern observed in subject 2 at 4 years posttransplantation; however, there was no abnormal accumulation of mitochondria in the cell soma as was observed in the host substantia nigra. The localization of Tom20 in neurons within the host medial globus pallidus (Figures 2C, 2F, and 2I) exhibited a homogeneous localization throughout the cell soma and processes, and showed no evidence of abnormal perinuclear accumulation as was observed in the patients' own substantia nigra. In neurons within the transplants costained with Tom20 and α-synuclein, a normal distribution of Tom20 staining was observed in the absence of Lewy bodies (Figures 3B–3D).

DISCUSSION

Efficacious fetal ventral mesencephalic grafts can reduce both PD motor symptoms and levodopa-induced dyskinesia for many years, and can reduce or negate the requirement for dopamine replacement therapy. Months to years are required for the newly replaced dopaminergic neurons to mature, integrate into the host brain, and function (Barker et al., 2013), and most fetal ventral mesencephalic cell transplants provide improvement in PD motor symptoms starting at ~1 year after transplantation (Evans et al., 2012). However, successful transplants can survive and function for many years. Recent studies by Kefalopoulou et al. (2014) and Politis et al. (2010, 2012) described two patients who were still improving (as shown by PET neuroimaging of dopamine uptake and reduction of the Unified Parkinson's Disease Rating Scale score) more than 18 years after they had undergone transplantation of fetal ventral mesencephalic cells.

The study presented here shows long-term graft survival in PD patients with maintained DAT localization along TH-immunoreactive axons in the reinnervated striatum, indicating functional dopaminergic neurons. Abnormalities in mitochondrial localization, as indicated by accumulation in the cell soma in dopaminergic neurons in the host substantia nigra, were not observed