Lewy body pathology in fetal grafts

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Although fetal nigral transplants have been shown to survive grafting into the striatum, increased \(^{18}\)F-6-fluoroo-

\(\text{L}-3,4\)-dihydroxyphenylalanine (\(^{18}\)F-DOPA) uptake and improved motor function in open-label assessments have failed to establish any clinical benefits in double-blind, sham-controlled studies. To understand morphological and neurochemical alterations of grafted neurons, we performed postmortem analyses on six Parkinson’s disease (PD) patients who had received fetal tissue transplantation 18–19 months, 4 years, and 14 years previously. These studies revealed robust neuronal survival with normal dopaminergic phenotypes in 18-month-old grafts and decreased dopamine transporter and increased cytoplasmic \(\alpha\)-synuclein in 4-year-old grafts. We also found a decline of both dopamine transporter and tyrosine hydroxylase and the formation of Lewy body–like inclusions in 14-year-old grafts, which stained positive for \(\alpha\)-synuclein and ubiquitin proteins. These pathological changes suggest that PD is an ongoing process that affects grafted cells in the striatum in a manner similar to how resident dopamine neurons are affected in the substantia nigra.

Keywords: fetal tissue transplantation; Parkinson’s disease; dopaminergic phenotype; \(\alpha\)-synuclein; ubiquitin; thioflavin-s

Introduction and background

Fetal nigral transplantation has been considered a viable therapeutic strategy for Parkinson’s disease (PD) for over two decades. This strategy is based on the concept that new dopaminergic neurons can replace those that are lost in PD and, just as critically, replace dopaminergic innervation and dopaminergic synaptic connectivity to the denervated striatum. Several open-label reports indicate that patients experienced clinically meaningful benefits for a couple of years to a decade after transplantation.\(^1\)\(^-\)\(^7\) Patients who exhibited significant improvements showed lowering of scores for the total Unified Parkinson Disease Rating Scale (UPDRS) during “off” state (off medication) and/or required substantially lower doses of antiparkinsonian medications.\(^3\)\(^,\)\(^6\) These clinical changes have been associated with increased striatal \(^{18}\)F-6-fluoro\(\text{L}-3,4\)-dihydroxyphenylalanine (\(^{18}\)F-DOPA) uptake.\(^7\)\(^,\)\(^8\) Several years after transplantation, patients experienced progressive worsening of PD features and experienced difficulty in gait, balance, and falling that could not be controlled with medication.\(^9\)\(^-\)\(^11\) To date, none of the positive outcomes has been replicated in a double-blinded trial of fetal nigral transplantation in PD.\(^12\)\(^,\)\(^13\) In the course of both open-label and double-blinded trials, numerous patients have received fetal nigral transplants. These trials have employed a variety of parameters for tissue preparation, storage techniques, locus of implants, and others, and each case that ultimately comes to autopsy provides a rich reservoir of information that deserves careful examination. In the series of patients that have been part of a collaboration between Mt. Sinai, The University of South Florida at Tampa, and Rush University Medical Center, we have examined, using histochemical and immunohistochemical methods, six PD patients who received fetal tissue transplantation for 18–19 months \((n = 2)\), 4 years \((n = 2)\), and 14 years \((n = 2)\). It has been 14 years since our original study demonstrating that fetal grafts can survive, innervate, and form synaptic contacts in PD patients that survived 18 months posttransplantation.\(^5\) Since initial reporting, we\(^5\) and others\(^14\) have confirmed the robust
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Pathological alteration of graft neurons

A subset of grafted neurons displayed a progressive dysfunction although grafted fetal nigral neurons were aged only 14 years postnatally; which is far younger than the typical age at which PD affects nigral neurons. This dysfunction of grafts was not related to the shortage of blood supply, since most grafted neurons survived and functioned for several years. Whether the dysfunctional grafted neurons duplicate pathological alterations that occur in PD needs to be investigated. First, immunohistochemistry was employed to visualize α-synuclein (LB509) distribution and morphology in grafts. We found that α-synuclein was not detected at all in grafted neurons that survived for 18 months (Fig. 1A). Cytoplasmic, but not aggregated, α-synuclein immunoreactive (α-synuclein-ir) profiles were seen in 4-year-old grafts (Fig. 1B, C), and spherical α-synuclein-ir aggregated masses were deposited within neuromelanin laden neurons in 14-year-old grafts (Fig. 1D, E). Some of the spherical α-synuclein-ir masses displayed a lighter staining core surrounded by dark staining (Fig. 1E), survival and innervation provided by grafted nigral neurons over longer periods of time. Recently, long-term transplants have come to autopsy in which the patients survived for more than 10 years after initial transplantation. Observations from both our lab and from others (Isacson, personal communication; Bill Langston, personal communication; and Kordower, unpublished observations) have shown that transplanted fetal nigral cells undergo pathological changes analogous to those seen in PD. Two types of findings have been observed; those involving graft phenotype and those involving structural changes and the formation of Lewy bodies. These changes provide insight into the understanding of disease pathogenesis and cell replacement therapy.

Figure 1. Low- (A, B, D, F) and high-power (C, E, G) photomicrographs from 18-month- (A), 4-year- (B, C), and 14-year-old (D, E) grafts and host nigra (SN; F, G) showing α-synuclein immunoreactivity (α-syn-ir; LB509). α-syn-ir was hardly detectable within 18-month-old grafted neurons (A). Conversely, cytoplasmic but not aggregative α-synuclein was distributed in perikarya and main processes in 4-year-old grafted neurons (B, C). Many α-syn-ir inclusions were observed within neuromelanin-laden neurons in 14-year-old graft (D, arrows). The inclusion with a lighter core surrounded dark staining was similar to the inclusion in host substantia nigra (G, SN). Scale bar = 80 μm in F (applies to A, B, D), 8 μm in C, E, G.
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**Figure 2.** Low- (A, B, C, E) and high-power (D, F) photomicrographs from 18-month- (A), 4-year- (B), and 14-year-old (C, D) grafts and host putamen (Pt; E, F) illustrating S-129 α-synuclein immunoreactivity (α-syn-ir). S-129 α-syn-ir was hardly detectable within 18-month- and 4-year-old grafted neurons (A, B). Conversely, S-129 α-syn-ir perikarya, processes, and inclusions were observed in 14-year-old grafted neurons (C, D). Darkly stained swollen fibers were distributed in the host putamen (E, F; Pt). Scale bar = 35 μm in E (applies to A, B, C), 8 μm in D, F.

similar to Lewy bodies in the PD substantia nigra (Fig. 1F, G). In order to confirm that α-synuclein inclusions are a pathological feature, a second α-synuclein antibody that only detects α-synuclein phosphorylated on Ser-129 was employed to stain grafted tissue. This immunostaining demonstrated that α-synuclein inclusions in 14-year-old but not 18-month-old or 4-year-old grafts were phosphorylated at Ser-129 (Fig. 2). Phosphorylation at Ser-129 is a specific marker of α-synucleinopathies including PD. The Ser-129 phosphorylation enhances α-synuclein toxicity, a fact verified in the Drosophila model of PD. Ubiquitination of α-synuclein is another pathological event in PD, and Lewy bodies are ubiquitin immunopositive. In this regard, we observed aggregated ubiquitin in 14-year-old but not in 18-month-old or 4-year-old grafts (Fig. 3). Some of the ubiquitin immunoreactive (ubiquitin-ir) inclusions in the grafts had the appearance of Lewy bodies similar to the PD substantia nigra (Fig. 3D, F). Finally, we confirmed that aggregates with α-synuclein and ubiquitin staining in grafts were Lewy bodies by classical neuropathological stains such as thioflavin-S, the definitive light microscopic marker of Lewy bodies (Fig. 4). Several of the intracytoplasmic aggregates in the grafted neurons displayed a dense core surrounded by a lighter halo. This is the classic morphology of Lewy bodies found in catecholaminergic neurons in the substantia nigra and locus ceruleus, two regions that degenerate in PD, but distinct from the homogeneously stained Lewy bodies seen in the cerebral cortex in cortical Lewy body disease.

Another pathological feature within long-term fetal grafts is an increase in inflammatory cells within the grafted region. CD45 immunostaining revealed that every graft, from those with short survival times (18 months) to those with long survival times (14 years), was surrounded by activated microglia. CD45 (called the common leukocyte antigen) is a protein tyrosine phosphotase that has been shown to be an essential regulator of T and B cell antigen receptor signaling. It plays an important role in signal transduction and inhibition or
Figure 3. Low- (A, B, C, E) and high-power (D, F) photomicrographs from 18-month- (A), 4-year- (B), and 14-year-old (C, D) grafts and host substantia nigra (SN; E, F) illustrating ubiquitin (UB) immunoreactivity. The UB was hardly detectable within 18-month- and 4-year-old grafted neurons (A, B). In contrast, several UB immunoreactive inclusions (C, arrows) were observed in neuromelanin-laden neurons and fibers in 14-year-old grafts. The inclusion had a lighter core surrounded by dark staining (D), which was similar to the inclusion in host substantia nigra (F, SN). Scale bar = 35 μm in E (applies to A, B, C), 8 μm in D, F.

up-regulation of various immunological functions. The commercially available CD45 antibody recognizes a common epitope on all of the CD45 isoforms. CD45 immunoreactive cells were much denser around grafts than the host striatum in PD (Fig. 5). Activated microglial cells were also observed within the substantia nigra of patients with PD at postmortem and were identified by their immunoreactivity to human leucocyte antigen DR (HLA-DR), a cell-surface receptor belonging to the MHC class II. This finding was confirmed by other investigators using additional markers, such as HLA-DP, HLA-DQ, HLADR, CD68, and ferritin. However, microglial activation was not found in the putamen. An increased expression of CD4+ and CD45RO+ T cells (indicative of activated T cells) have been reported in the serum of patients with PD, suggesting peripheral activation of lymphocytes. Although cellular and molecular studies indicate that there are neuro-inflammatory processes in the affected brain regions of patients with PD and in fetal tissue grafts, these studies do not help determine whether such changes are involved in Lewy body formation or are merely a consequence of neuronal degeneration. In regard to this, a long-term Huntington’s disease transplantation case (11 years posttransplant) had been examined. We found that there were clouded CD45 immunoreactive, activated microglia around the grafts but neither cytoplasmic α-synuclein-ir neurons nor Lewy bodies in the grafted cells (Fig. 6), indicating that Lewy body formation in grafts is not due to a generalized inflammatory response.

Alterations of dopaminergic phenotype in grafted neurons

To understand whether PD-like changes in dopaminergic phenotype were seen in grafted neurons, we studies neuromelanin, tyrosine hydroxylase (TH), dopamine transporter (DAT), and Vesicular

Figure 4. Photomicrographs from 14-year-old graft (A, B) and host substantia nigra (SN; C, D) illustrating thioflavin-S staining inclusions. Note that thioflavin-S-stained inclusions displayed a dense core surrounded by a lighter halo that is similar to host nigral thioflavin-S-stained inclusions. Scale bar = 4 μm in D (applies to B), 40 μm in A, C.

Figure 5. Low- (A, C) and high-power (B, D) photomicrographs from 14-year-old graft (A, B) and host putamen (Pt; C, D) illustrating CD45 immunoreactivity. Note that there were increased CD45 stained microglial cells in the graft (A, B) as compared with the host putamen (Pt; C, D) Scale bar = 35 μm in D (applies to B), 180 μm in A, C.
Figure 6. Laser confocal microscopic images from an 11-year posttransplant Huntington’s disease case illustrating the absence of cytoplasmic α-synuclein cells and inclusions (A), but increased CD45 (B, C) (arrowheads point to graft). Scale bar = 60 μm in C (applies to all).

Monoamine Transporter 2 (VMAT2) expression in all cases.

Neuromelanin is an easily discernible endogenous marker for nigral dopaminergic neurons in the human brain, which appears after the first 2 to 3 years of life, accumulates during aging, and typically becomes extracellular following neural degeneration in PD. We tested whether neuromelanin accumulated in grafted cells at a rate similar to normal human nigral neurons. Neuromelanin was observed in 4-year-old grafts and 14-year-old grafts but not in 18-month-old grafts (Fig. 7). The density of neuromelanin was greater in 14-year-old grafts than in 4-year-old grafts. The majority of neuromelanin-laden neurons in grafts were immunopositive for Girk2, a marker of dopaminergic neurons in the substantia nigra pars compacta. To verify alteration of neuromelanin with age, the density of neuromelanin in grafted neurons was compared with normal aged nigral neurons. We found that the density of neuromelanin in 14-year-old grafted neurons appeared similar to 18-year-old nigral neurons (Fig. 7C, D) and interpret this expression of neuromelanin in grafted neurons as normal.

TH, the rate-limiting enzyme involved in the biosynthesis of the catecholamines dopamine and norepinephrine from tyrosine, is a useful marker for dopaminergic and noradrenergic neurons. To verify function of grafted neurons we first examined TH expression. Immunohistochemistry revealed that robust survival of TH immunoreactive (TH-ir) neurons was observed in 18–19-month- and 4-year-old grafts placed in the postcommissural putamen (Fig. 8). TH-ir neurons in grafts in both groups appeared relatively normal with respect to morphological appearance. These neurons survived in an organotypic pattern and extended neurites, which extensively innervated the postcomissural putamen in a patch-matrix fashion. An electron microscopic analysis revealed that the graft and host made bidirectional synaptic contacts. In the 4-year-old transplantation group, TH-ir neurons were present around the periphery but absent in center of grafts (Fig. 8C, E). In the 18-month and 4-year grafts, virtually all neuromelanin-positive cells expressed TH. In contrast, many neuromelanin-laden neurons displayed loss of TH immunoreactivity (Fig. 8F) in one of our two 14-year-old cases. That melanin-laden grafted neurons failed to express TH was similar to the alteration in PD nigral neurons. For the most part, however, the grafted fetal mesencephalic tissue survived for a long period in the brain and restored dopaminergic innervation to the host putamen. This observation was associated with enhanced 18F-DOPA uptake and clinical benefit. Although there were robust TH-ir...
neurons in some grafts even after a decade, grafted fetal mesencephalic tissue became dysfunctional. Some neuromelanin-laden neurons lost TH expression in 14-year-old grafts, indicating that there was a down-regulation of dopaminergic phenotype in implanted neurons. Therefore, it persuaded us to examine different dopaminergic phenotypes in grafted neurons.

Dopamine transporter is another dopaminergic neuronal marker, which mediates uptake of dopamine into neurons from the synapse and is a major target for various pharmacologically active drugs and environmental toxins. Dopamine nerve terminals and consequently DAT are reduced 30 to 50% in early PD and to a greater extent in more severe PD. To verify that alterations in DAT levels in grafted neurons resembles that seen in PD, DAT was examined using immunohistochemistry. DAT immunoreactive (DAT-ir) neurons were found in most of the grafts, but intensity of DAT-ir neurons declined with graft age (Fig. 9). In the 18-month-old grafts, dark DAT-stained neurons were distributed around grafts and DAT-ir neurites extensively innervated the host putamen. The pattern of distribution of DAT-ir neurons was similar to that of TH-ir neurons. In the 4-year-old graft cases, some grafts contained dark DAT stained neurons while others enclosed few light DAT-ir neurons. In contrast, DAT staining revealed very light to no staining in neuromelanin-laden neurons in both of our 14-year-old graft cases (Fig. 9F), which is similar to what was observed in the PD nigra. One concern was that DAT severely declined, although there were robust TH-ir neurons in 14-year-old grafts. A decrease in DAT, although potentially serving as a compensatory mechanism in early disease, may ultimately result in increased dopamine turnover and higher oscillations in synaptic dopamine concentrations, thereby predisposing patients to motor complications during disease progression.

This morphological alteration of dopaminergic neurons in grafts from 18-month- to 14-year-old cases correlated with changes in motor function in the patients, although nondopaminergic and levodopa-unresponsive features emerge over this time. Although the grafted human ventral

Figure 7. Photomicrographs of grafts (A–C) and nigra (D) illustrating alteration of neuromelanin (NM). NM was hardly detected in 18-month-old graft (A). As aging advanced, granular NM appeared in 4-year-old grafted neurons (B) and accumulated in 14-year-old grafted neurons (C). The density of NM in 14-year-old graft is similar to 18-year-old nigra (D). Scale bar = 18 μm in D (applies to all).
mesencephalons were derived from different embryos, all the grafts underwent a process of being functional to becoming dysfunctional and showed decreases in dopaminergic phenotypes over time. The decline in DAT occurred much earlier and faster than the decrease in TH in grafted neurons. These features suggest that reduced dopaminergic transmission may occur over time, a compensatory response may occur, and an earlier manifestation of dopaminergic injury may be represented in the graft. As the level of dopaminergic transmission was much lower in the synapse, decrease of DAT in grafted neurons may be a response intended to enhance functional dopamine at the synapse. Molecular imaging analysis revealed that higher DAT decrease in PD is in fact related to an advanced stage of neuronal loss. Loss of DAT observed in SPECT and PET can be seen even before the onset of symptoms, since clinical manifestations take place after more severe dopamine neuron deterioration.31 Grafted cells also displayed diminished dopamine transporter, a PD-related pathogenesis. On the other hand, staining for VMAT2 was preserved in our cases, suggesting that it is a less sensitive index of cell injury than

Figure 8. Low- (A, C, E) and high-power (A, D, F) photomicrographs from 18-month- (A, B), 4-year- (C, D), and 14-year-old (E, F) grafts illustrating alterations of tyrosine hydroxylase (TH) immunoreactivity. Note that TH immunostaining intensity was diminished (E) and some neuromelanin-laden neurons appeared TH immunonegative (F; arrows) in 14-year-old graft. Scale bar = 35 μm in F (applies to B, D), 180 μm in A, C, E.
Figure 9. Low- (A, C, E) and high-power (B, D, F) photomicrographs from 18-month- (A, B), 4-year- (C, D), and 14-year-old (E, F) grafts illustrating alterations of dopamine transporter (DAT) immunoreactivity. Note that DAT immunostaining intensity was diminished in 4-year-old grafted neurons (C, D) and severely decreased in 14-year-old grafts (E, F). Scale bar = 35 μm in F (applies to B, D), 180 μm in A, C, E.

either TH or DAT. DAT and VMAT2 are important implications for brain imaging of patients with PD and should be pursued.

Possible pathogenesis of Lewy body formation in grafts

Inflammation, oxidative stress and excitotoxicity, loss of neurotrophic support, and prion disease–like mechanisms have all been proposed to explain the propagation of PD pathology from the host diseased brain to healthy transplanted neurons. Our findings, accumulating cytoplasmic α-synuclein in 4-year-old and aggregating α-synuclein in 14-year-old grafted neurons, support the concept that Lewy body formation is a chronic process and grafted nigral neurons can be attacked by PD even though they are placed in an ectopic location (putamen). Increase in cytoplasmic α-synuclein in grafted neurons is the basic condition required to form Lewy bodies. Phosphorylation and nitration of cytoplasmic α-synuclein are the processes involved in Lewy body formation. Apparently these processes occur in the host and in the graft in a similar fashion.

It is known that the clinical manifestations of tremor, rigidity, and bradykinesia generally occur...
when there is a loss of 80% of striatal dopamine; this implies the existence of a relatively long preclinical period. This latency between disease onset and appearance of clinical symptoms suggests that some of these changes may be of a compensatory nature, aimed at maintaining high enough synaptic dopamine levels to allow for relatively normal motor function. A critical site in the regulation of synaptic dopamine levels is DAT. Physiologically, DAT is a membrane-spanning protein that binds the neurotransmitter dopamine. DAT transports dopamine against a concentration gradient from the synapse into the intracellular space as the primary mechanism through which dopamine is cleared from synapses. Under specific circumstances (e.g., exposure to amphetamines), DAT can reverse its normal functional role and mediate the transport of dopamine from the intracellular space into the synapse. In PD pathophysiology, a decrease in DAT occurs early in the disease process, possibly providing compensation that ultimately results in enhanced dopamine function at the level of the synapse. This change may also predispose individuals to motor complications as the disease progresses. This compensatory change was also exhibited in grafted neurons that consistently had decreases in DAT expression.

Another characteristic in grafted neurons was an increase in α-synuclein. A decrease in DAT was accompanied by an increase in cytoplasmic α-synuclein in 4-year-old grafted neurons. We hypothesize that decreases in DAT in grafted neurons may be compensated for with increases in some other synaptic proteins, in particular an augmentation of cytoplasmic α-synuclein. α-synuclein is normally seen mainly in presynaptic terminals. Thus we observed intense α-synuclein immunoreactivity within the perikarya of 4-year-old grafts. The increase in cytoplasmic α-synuclein appears to be associated with a decrease in DAT. The function of α-synuclein is still unknown, although several studies suggest that it plays an important role in synapse maturation and maintenance. From co-immunoprecipitation studies, wild-type α-synuclein and its A30P mutant form were found to interact directly with the DAT, forming a protein:protein heteromeric complex in transfected cells, primary cultures of mesencephalic neurons and rat. In cotransfected Ltk2 cells, α-synuclein negatively modulated human dopamine transporter activity by attenuating the reuptake of dopamine and decreasing DAT levels. This negative modulation was further verified by an inverse correlation between DAT expression in the striatum and the burden of pathological α-synuclein aggregates in the substantia nigra. Increase in α-synuclein following a decrease in DAT in grafted neurons may initiate synucleinopathy. Whether an increase in α-synuclein is beneficial or toxic to a cell is debated. α-synuclein-knockout mice are grossly normal and display no neurodegenerative phenotype. However these mice display decreased numbers of synaptic vesicles and show subtle changes in dopamine homeostasis. Dopaminergic mouse neurons and human neuroblastoma cells lacking α-synuclein have been shown to be resistant to the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its active metabolite 1-methyl-4-phenylpyridine (MPP+). These data indicate that α-synuclein is a protein susceptible to neurotoxicity. Overexpression of wild-type or mutant α-synuclein in transgenic (tg) mice, Drosophilia, and neurons can cause alterations in nigrostriatal function and in some instances dopamine neuronal degeneration with inclusion bodies. Similarly targeted overexpression of α-synuclein in the nigrostriatal system of rats and nonhuman primates induces α-synuclein inclusions and neuronal degeneration and mimics several of the pathological and behavioral features of PD. Age-related increase of cytoplasmic α-synuclein without aggregation and decrease of DAT in nigral neurons indicate that there may be an imbalance of presynaptic proteins in PD that does not occur in controls; effects mediated impaired lysosomal and proteasomal systems.

It is interesting that α-synuclein was robustly increased in 4-year-old grafted neurons and aggregated in 14-year-old grafted neurons. This accumulating α-synuclein could not be cleared by lysosomal and proteasomal systems. We hypothesized that the continued accumulation of α-synuclein in grafted neurons may be caused by active protein-modifying processes such as phosphorylation and oxidative stress since pathological modification of α-synuclein is a characteristic in familial and sporadic Lewy body disease. Therefore we analyzed the specific forms of α-synuclein in grafted neurons using immunohistochemistry. We found heavy phosphorylated Ser-129 α-synuclein immunoreactive...
inclusions in 14-year-old grafts but not in 18-month-old grafts. Small amounts of phosphorylated Ser129 α-synuclein were observed in 4-year-old grafted neurons. This cytosolic form of phosphorylated α-synuclein may thus be the precursor to the predominant pathogenic form of α-synuclein in Lewy bodies. In order to understand whether accumulated α-synuclein in grafted neurons was ubiquitinated, ubiquitination was examined using immunohistochemistry. We found a pattern of labeling for ubiquitin inclusions and neurites that was similar to that of α-synuclein labeling in 14-year-old grafted neuron but not in 18-month- and 4-year-old grafts. The absence of ubiquitin labeling in 4-year-old grafted neurons containing heavy cytoplasmic α-synuclein immunostaining strongly suggests that ubiquitination occurs after α-synuclein aggregation. Immunobots of two-dimensional polyacrylamide gel electrophoresis (PAGE) gels stained with antibodies to synuclein and ubiquitin show that the major ubiquitinated species correspond in molecular mass to α-synuclein with ubiquitins attached in isolated Lewy bodies.62, 63 Ubiquitination was not detected in the soluble fraction of α-synuclein in dementia Lewy body brain.63 It is possible that ubiquitinated α-synuclein with phosphorylated modification may not go through the proteasome to be degraded and instead may accumulate to form Lewy bodies.

In summary, patients grafted with dopaminergic neurons experience improvements in their PD syndrome for a decade and then show a progressive worsening of PD features. The neurochemical and morphological alterations of grafted dopaminergic neurons appeared to cause a decrease in dopaminergic phenotypes, especially in DAT, which caused an unbalance of proteins in grafted neurons. The decrease in DAT is accompanied by an increase in cytoplasmic α-synuclein. The increased cytoplasmic α-synuclein was modified by oxidation or phosphorylation. In particular, the modified synuclein species Ser-129, which cannot be cleared through lysosomal and proteasomal systems, preferentially and consistently accumulates in the intracellular space and therefore may drive Lewy body formation.

Conflicts of interest

The authors declare no conflicts of interest.

References