Research in motion: the enigma of Parkinson's disease pathology spread

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Abstract | Neuropathological changes in Parkinson's disease progress slowly and spread according to a characteristic pattern. Recent papers have shed light on this progression of pathology by examining the fate of neurons grafted into the brains of patients with Parkinson's disease. Two of these studies demonstrate that grafted healthy neurons can gradually develop the same pathology as host neurons in the diseased brains. According to these studies, implanted neurons developed α-synuclein- and ubiquitin-positive Lewy bodies more than a decade after transplantation. We discuss the possible underlying mechanisms and their implications for how pathology spreads in Parkinson's disease.

Parkinson's disease (PD) is the most common cause of movement disorder and has a median age-of-onset of ~55 years. It presents with a classical triad of motor symptoms: akinesia, rigidity and tremor. Many patients also suffer from non-motor symptoms, including disturbances of autonomic functions and deterioration of cognition[1]. The aetiology and pathogenetic mechanisms that cause PD remain enigmatic, although some clues can be derived from the disease's neuropathological features. Classical descriptions of PD mostly focus on the progressive degeneration of dopaminergic neurons located in the substantia nigra pars compacta. This pathology is thought to underlie several motor symptoms, although it is not believed to explain other signs and symptoms that are associated with PD. Pathology in other brainstem, subcortical and cortical structures is also prominent[2,3].

Tests of neural transplantation have been undertaken for over two decades, with the aim of replacing the neurons that are lost in PD and reinstating dopaminergic neurotransmission in the striatum. Three recent studies, encompassing a total of eight patients who received grafts between four and sixteen years previously, investigated whether PD pathology can propagate to intrastriatal grafts of young, healthy neurons[4–6]. In three cases, a fraction of implanted neurons developed α-synuclein- and ubiquitin-positive Lewy bodies more than a decade after transplantation. In this Progress article, we discuss the mechanisms that might underlie the spread of the disease to the grafts and their implications for how α-synuclein pathology might spread in PD. We discuss evidence that suggests that inflammation, oxidative stress, excitotoxicity or failure of neurotrophic support can promote protein misfolding in the PD brain. We also describe the hypothesis that α-synuclein pathology might spread through a prion-like mechanism. Finally, we stress that these findings might be important for our understanding of how pathology normally progresses in the PD brain.

Lewy bodies in PD

The brain regions that are affected in PD exhibit neuronal intracytoplasmic inclusions that are termed Lewy bodies (LBs) when they are present in cell bodies and Lewy neurites (LNs) when they are present in neuronal processes. These inclusion bodies are particularly rich in aggregated α-synuclein, but also contain numerous other proteins, including components of the ubiquitin–proteasome system and molecular chaperones, and lipids[7–9]. α-Synuclein is normally enriched in nerve terminals and is involved in synaptic function. During normal aging and in PD, levels of natively folded α-synuclein increase in the cytoplasm of substantia nigra neurons[10]. α-Synuclein in LBs, however, is misfolded, post-translationally modified and ubiquitylated. The post-translational modifications include nitrosylations and phosphorylation at serine residue 129 (REF. 8).

The roles of LBs and LNs in PD are unclear. Their formation might contribute to cell death, they might have no functional relevance, or their occurrence might indicate that the cell is evading death by successfully rendering misfolded proteins harmless[8]. The proportion of nigral neurons that contain LBs in PD is relatively constant, at ~3–4% regardless of disease stage. Therefore, it has been suggested that LBs are continuously formed during the course of the disease and disappear when the affected neuron dies. This process takes ~6 months to complete[11].

LBs and LNs can also be found in other brainstem and olfactory structures, as well as in the basal ganglia and the neocortex[2,12]. Cases in which early cognitive impairment is accompanied by prominent cortical LB pathology are diagnosed as 'dementia with Lewy bodies' (REFS 12,13). Nigral LBs are also seen in ~10% of asymptomatic individuals above the age of 60. This is known as 'incidental LB disease', and might represent presymptomatic PD[14]. The identification of three rare missense mutations in the α-synuclein gene that cause autosomal-dominant PD with LBs, and subsequently of families with duplications of the wild-type α-synuclein gene that present with syndromes that include parkinsonism, autonomic failure and dementia, has brought the role of aggregated α-synuclein in the aetiology of PD to centre stage[8,15–19].

Recently it has been proposed that PD progresses in six stages that can be distinguished by the anatomical spread of LB pathology, on the basis of the correlation of neuropathological findings with preclinical and clinical disease[2]. The stereotypic disease has been proposed to begin in the gastrointestinal and olfactory systems. It spreads along axonal pathways, advances to the brainstem through the vagal nerve and subsequently ascends to the substantia nigra and mediotemporal and other limbic structures, eventually reaching the neocortex. Although it is now

known that not all cases follow this pattern of progression, this hypothesis for disease spread remains attractive and seems to be valid in most PD cases[20–22]. An alternative hypothesis is that neurons in different brain regions vary with respect to their proneness to protein misfolding, and that the pattern of LB formation reflects the susceptibility to α-synuclein misfolding. Regardless of the different pathogenic pathways that might underlie the development of LBs, the α-synuclein proteinaceous inclusions constitute an excellent marker for the advancement of disease.

Insights from transplants

Recent reports on patients with PD who received transplants of embryonic mesencephalic neurons provide new insight into how LB pathology might propagate in the CNS[4–6]. In 3 out of the 4 patients who died 11 to 16 years after the surgery, grafted neurons containing neuromelanin — a marker of nigral dopaminergic neurons — exhibited LBs and LNs[4,5] (TABLE 1). By contrast, 4 patients who survived only 4 or 9 years following transplantation did not exhibit α-synuclein pathology in the grafts[4,6], suggesting that at least one decade is required for the development of LBs in young, previously healthy neurons (TABLE 1). One study[6] reported no LBs in a patient 14 years after transplantation. The difference between this observation and those in the three other patients with transplants older than a decade, who developed LBs, could be related to histology protocols or might suggest that the graft environment and/or the susceptibility of the graft to the pathological process differ between patients.

The LBs that were found in the grafted neurons had features that were characteristic of those found in the substantia nigra of these and other PD patients: they contained ubiquitylated proteins and proteins that were phosphorylated at serine residue 129 (FIG. 1), and they were thioflavin S-positive, indicating the presence of β-sheet-rich protein fibrils[4,5,23]. The pathology seems to be progressive: preliminary findings suggested that, in one of the patients previously described in REF. 5, LBs were more common in the grafts that were implanted 16 years before death than in those that were transplanted in the contralateral hemisphere 4 years later[23]. Similarly, cytoplasmic α-synuclein accumulation, which is thought to be an age-related phenomenon[10], was time-dependent: elevated levels of α-synuclein were found in 80% of the

Table 1 | **Characteristics of eight grafted Parkinson's disease post-mortem cases***

	Patient 3; Sweden	Patient 8; Sweden	Patient A; United States	Patient B; United States	Patient C; United States	Patient 4; Canada	Patient 5; Canada	Patient 6; Canada
Age at surgery; gender	48 (L); 53 (R); M	43 (L); 45 (R); M	61; F	53; F	69; M	61; M	61; F	55; M
Age at disease onset	37	38	39	49	59	46	42	41
Years post-grafting at time of death	16 (L); 12 (R)	13 (L); 11 (R)	14	4	4	9	14	9
Lewy bodies in graft?	Yes (more in L)	Yes	Yes	No	No	No	No	No
Total number of surviving dopamine neurons in grafts	12,100–29,500 per tract (L); 14,400–27,600 per tract (R)	Numerous; not quantified	Numerous; not quantified	~100,000 per side	~30,000 per side	11,100 (R); 11,687 (L)	9,861 (R); NT (L)	10,917 (R); 21,552 (L)
Injection tracts per striatum	3 (L); 5 (R)	6 (L); 7 (R)	8	8	8	4	3 (R); NT (L)	4
Number of donor embryos	4 (L); 5 (R)	5 (L); 8 (R)	4 (L); 4 (R)	4 (L); 4 (R)	1 (L); 1 (R)	3 (L); 4 (R)	2–3 (L); NT (R)	3 (L); 4 (R)
Transplantation preparation	Fresh tissue, cell suspension with small aggregates	Fresh tissue, cell suspension with small aggregates	Small tissue pieces	Small tissue pieces	Small tissue pieces	Tissue hibernated 6 days, cell suspension	Tissue hibernated 6 days, cell suspension	Tissue hibernated 6 days with GDNF, cell suspension
Immunosuppression	Cyclosporin, Azathioprine, Prednisolone (up to 65 MoT)	Cyclosporin, Azathioprine, Prednisolone (up to 40 MoT)	Cyclosporin (6 MoT)	Cyclosporin (6 MoT)	Cyclosporin (6 MoT)	Cyclosporin (6 MoT)	Cyclosporin (6 MoT)	Cyclosporin (6 MoT)
Microglia number and activation state	Slight increase, resting microglia	NA	Increased, activated microglia	Increased, activated microglia	Increased, activated microglia	Slight increase, mostly resting microglia	Slight increase, mostly resting microglia	Slight increase, mostly resting microglia
Cause of death	Aspiration pneumonia	Aspiration pneumonia	Cardiac arrest	Myocardial infarct	Respiratory failure	Myocardial infarct	Myocardial infarct	Renal failure (owing to hypertension)

*The table header indicates the country in which each patient was operated on. Data for patients 3 and 8 from REFS 5,56; data for patients A, B and C from REF. 4 and from J.H. Kordower, T.B. Freeman and C.G. Goetz (personal communications); data for patients 4, 5 and 6 from REF. 6. F, female; GDNF, glial-cell-line-derived neurotrophic factor; L, left side of the brain; M, male; MoT, months of treatment; NA, data not available in literature; NT, not transplanted; R, right side of the brain.

16-year-old neurons, as opposed to 40% of the 12-year-old neurons[5]. The effect of the LBs on the function of the grafted neurons is uncertain. In one of the patients with LBs in the transplants, immunostaining for the dopamine transporter was reduced in the grafted neurons, which might indicate that the cells were beginning to fail[4]. Currently there are no systematic clinical and positron-emission tomography (PET) follow-up studies of patients beyond one decade after grafting; such studies might shed light on any functional decline of grafts.

These observations raise important questions regarding mechanisms of disease propagation in PD. The LBs formed in the 11-to-16-year-old neurons even though LB disorders mostly affect the aged. The observations made in these studies suggest that certain pathogenetic events in PD are not cell-autonomous, and that affected neurons or glia, or the extracellular environment, might transfer aspects of the disease process to healthy neurons. This concept has recently gained attention in the study of other neurodegenerative diseases, especially amyotrophic lateral sclerosis[24]. In the following sections we discuss what the events that underlie the spread of α-synuclein pathology to dopamine neurons that have been transplanted to the PD brain might be (FIG. 2), and their relevance to how neuropathology spreads in the PD brain.

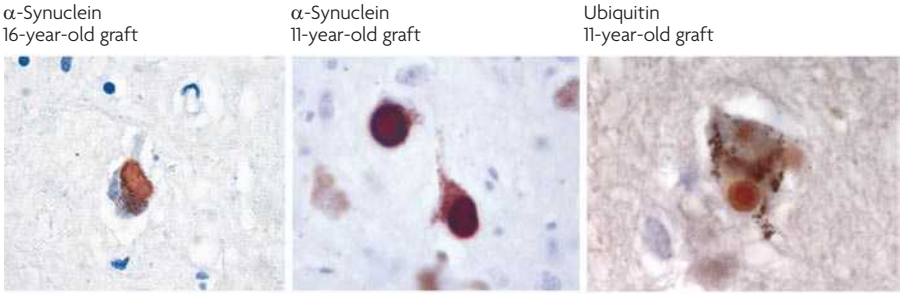

Figure 1 | **Lewy bodies develop in grafted neurons in Parkinson's disease.** Photographic images of Lewy bodies in grafted cells that had been stained with antibodies against α-synuclein (left-hand and middle panels) and ubiquitin (right-hand panel). The grafted neurons were implanted into the brains of two patients with Parkinson's disease 16 (left-hand panel) and 11 (middle and right-hand panels) years before death. The images shown are derived from the post-mortem material described in REF. 5.

Possible underlying mechanisms

Several interesting hypotheses have been put forward to explain the propagation of PD pathology from diseased to healthy transplanted neurons. Here we discuss each of the main hypotheses in turn. The molecular events that we mention are not mutually exclusive, and it is possible that two or more of the suggested mechanisms co-operate to generate LBs in grafted neurons.

Inflammation. It is well established that inflammation is ongoing in the PD brain. Microglia are activated in PD[25], and increased levels of pro-inflammatory mediators such as tumour necrosis factor-α, interleukin-1β (IL1β), IL6, inducible nitric oxide synthase and cyclooxygenase 2 are found in the striatum and in the substantia nigra[26]. The neuronal grafting procedure itself is also known to induce a mild, transient inflammation in experimental animals[27]. Two of the grafts in which LBs were found were examined for the presence of activated microglia, an indicator of inflammation (TABLE 1): in one of these grafts, levels of activated microglia were elevated[4], whereas in the other there was little evidence of inflammation[5]. Inflammatory mediators and cells might promote upregulation of α-synuclein and α-synuclein aggregation. For example, IL1β treatment raises neuronal levels of α-synuclein[28], and activated macrophages increase α-synuclein nitrosylation in neural cells[29] (nitrosylation is known to promote protein aggregation[8]).

However, although the hypothesis that inflammation could cause the pathological changes that are observed in the grafts is valid, it is worth noting that LBs are not a defining feature of many neurodegenerative conditions or cerebral amyloid diseases in which chronic inflammation is also prominent. Moreover, in one of the patients in which LBs were found, there was only a low level of microglial activation in the vicinity of the grafts[5]. Therefore, even with a presumed mild transient inflammatory response to transplantation surgery, it seems unlikely that inflammatory mechanisms alone could explain the progressive α-synuclein accumulation and aggregation, as well as LB formation, in grafted neurons.

Oxidative stress and excitotoxity. Oxidative stress is another potentially important pathway that could be instrumental in the acquisition of PD-like pathology in grafted neurons. A large body of evidence from post-mortem studies implicates oxidative stress in the pathology of the PD brain[30]. Experimental data indicate that dopaminergic neurons might be particularly sensitive to oxidative damage[31], which can promote protein modifications, such as nitrosylation, with consequent enhanced protein misfolding and aggregation[8]. Neurons that have been exposed to such insults can also develop increased α-synuclein levels[32,33]. Because the grafted neurons are initially both young and healthy, their antioxidant defences could be expected to withstand the oxidative stress for some years. However, accelerated senescence of grafted neurons has been described[34], and this could hypothetically promote age-related atrophy and molecular crowding (when high-molecular-mass molecules take up a greater fraction of the cell's available space), which have been proposed to favour protein aggregation[8,35].

Another possible trigger of oxidative stress in the grafted cells is excitotoxicity, which has been suggested to contribute to pathogenesis in dopamine neurons in PD[36,37]. The grafts were placed in the striatum, which receives a dense excitatory input from the neocortex that could provide excessive extracellular glutamate. In fact, rodent studies have provided both electrophysiological and morphological evidence for an input from the host cortex to nigral grafts placed in the striatum[38–40]. Thus, grafted neurons might receive glutamatergic input, supporting an excitotoxicity hypothesis. Chronic exposure to low-grade excitotoxicity might trigger oxidative stress inside the ectopically placed graft and initiate α-synuclein changes.

Loss of neurotrophic support. Dopamine neurons, including those in the grafts, are likely to depend on trophic stimuli to maintain a normal internal milieu. Several reports have indicated that the basal ganglia of PD patients express reduced levels of neurotrophic factors, such as glial-cell-line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF)[41]. It is possible that, in the absence of appropriate levels of growth factors, the grafted neurons eventually fail to maintain their homeostasis, including appropriate antioxidant defences[42] or the molecular machineries that are required for the refolding or the targeted degradation of misfolded α-synuclein.

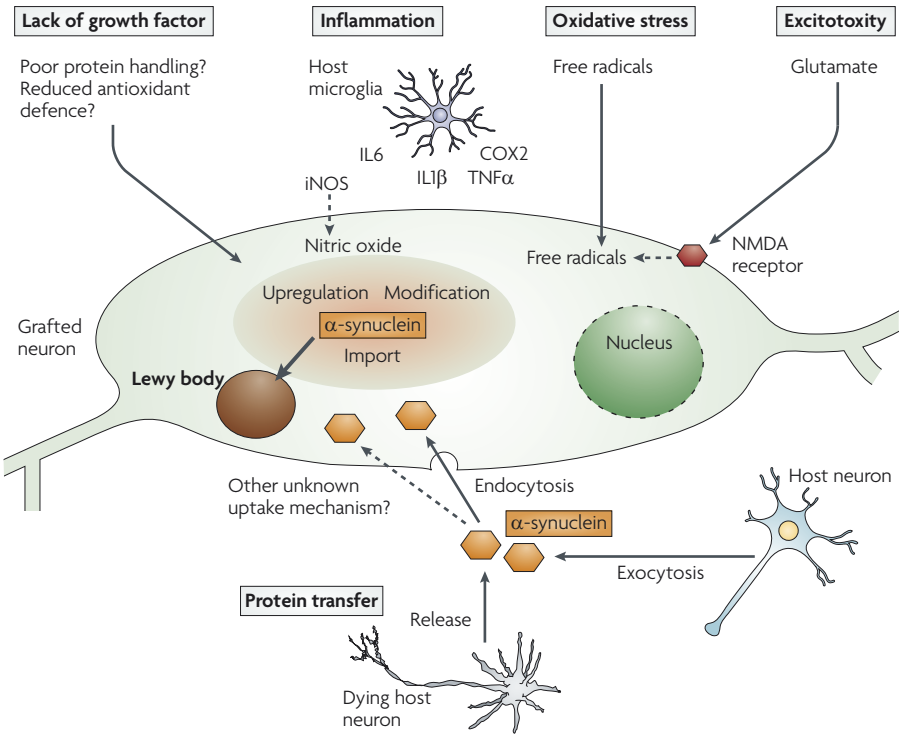

Figure 2 | **Mechanisms that might explain why Lewy bodies form in grafted neurons.** This diagram illustrates cellular mechanisms that might underlie the spread of Parkinson's disease (PD) pathology from the host brain to grafted neurons. Neurotrophic factors have been reported to be deficient in the PD striatum. Certain neurotrophic factors are known to counteract oxidative stress, and one could speculate that they normally also promote the molecular defence against misfolded proteins. A relative lack of these growth factors could therefore lead to the accumulation and aggregation of misfolded α-synuclein in the grafted cells. Inflammatory mediators (including tumour necrosis factor-α (TNFα), interleukin-1β (IL1β), IL6, inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) are upregulated in the parkinsonian brain. Some of them have been shown experimentally to promote upregulation of α-synuclein and α-synuclein nitrosylation, and could thereby promote the generation of Lewy bodies (LBs) in grafted neurons. There is ample evidence for oxidative stress in the PD brain. Free radicals from the host brain might attack grafted neurons, leading to modifications of α-synuclein and increasing its propensity to misfold. Excitotoxicity, potentially triggered by excessive glutamate in the PD striatum, could lead to increased free-radical stress inside grafted neurons. Host cells adjacent to the grafted neurons might also be a source of misfolded, LB-generating α-synuclein. α-Synuclein can be released from living cells into the surrounding extracellular milieu, and dying neurons in the PD brain could conceivably also release α-synuclein. Recent studies have shown that neurons take up different molecular forms of α-synuclein through different pathways, one of which is endocytosis. Once inside the grafted neuron, the exogenous α-synuclein could act as a template that promotes misfolding of endogenously produced α-synuclein, ultimately leading to the formation of LBs. NMDA, *N*-methyl-D-aspartate.

Prion-disease-like mechanisms. PD is a protein-folding disease, and it shares fundamental biological properties with other neurodegenerative diseases, including Alzheimer's disease, polyglutamine diseases, motor neuron diseases and prion diseases. In some of these conditions, protein misfolding and aggregation is thought to follow a seeding–nucleation mechanism. Recently, cerebral amyloid disease was successfully induced in young amyloid-precursor-protein (APP)-transgenic mice by locally injecting either amyloid-β-containing human brain extract from patients with Alzheimer's disease or brain extracts from older APP-transgenic mice[43]. On the basis of anatomical considerations, a prion-disease-like propagation of α-synuclein pathology has been proposed for PD[21,44]. It is therefore tempting to speculate that a prion-disease-like mechanism, involving 'permissive templating' — that is, misfolded α-synuclein acting as a template for the conversion of the native α-helix form to a pathogenic β-sheet structure[45] — could explain the α-synuclein pathology in the grafted cells.

In transplanted patients with end-stage PD, α-synuclein deposits were present in cells in the striatum[5], which could indicate that the host milieu is enriched in the 'transmission friendly' β-sheet conformation of the protein (consistent with LN morphology). Furthermore, α-synuclein deposits have been seen in the striatum of patients with PD — in cellular processes that might belong to nigrostriatal dopaminergic neurons and striatal neurons, as well as glia. Their levels correlated with the overall extent of the pathology and with the clinical disease stage[46]. This might imply that LB pathology can only spread to the grafts when the disease process has advanced sufficiently to involve the part of the striatum that is in direct contact with the transplanted neurons.

The presence of LBs in neurons that were transplanted a long time previously, but not in relatively recently transplanted neurons[4,47], suggests that aging of transplanted cells promotes the propagation of α-synuclein aggregation from host to graft. It will be important to establish whether and how the α-synuclein leaves the host cells and subsequently enters grafted cells. Interestingly, *in vitro* studies have demonstrated that α-synuclein is secreted by living neurons and enters the surrounding medium[48], but the precise mechanism for the physiological release is debated[49]. It is also possible that dying cells in the brain release α-synuclein into the extracellular space. Consistent with these observations, α-synuclein is present in measurable quantities in cerebrospinal fluid and plasma[49–52].

Taken together, the evidence indicates that it is plausible that α-synuclein is released from diseased neurons and is therefore available for uptake by other cells. Although it should be emphasized that this has still not been demonstrated *in vivo*, different *in vitro* experimental paradigms have shown that fibril and oligomer forms of α-synuclein can be taken up from the extracellular space by neurons — through an endocytic pathway[53] and through another, as yet undetermined, route[53,54]. α-Synuclein that has been taken up through RAB5A-dependent endocytosis has even been reported to form intracytoplasmic inclusions in cultured neurons[55].

Conclusions

At present, the relative contributions of the different possible mechanisms of PD-pathology propagation are unknown. Further studies are needed, in animal and cell-culture models, to examine the factors that control cell-to-cell transfer of α-synuclein, as well as the impact of inflammation, oxidative stress, excitotoxicity and growth-factor deprivation on the accumulation of misfolded proteins. The neural-transplantation paradigm in

experimental animals can be used as a tool and might shed further light on how misfolded-protein pathology spreads to healthy cells. Hopefully, insight from future studies using *in vivo* and *in vitro* models will translate into an improved molecular understanding of the enigmatic spread of α-synuclein pathology. This will be invaluable when trying to devise novel therapeutic strategies that interfere with key steps in the pathogenetic mechanism, thereby altering the course of PD.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
α-synuclein
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
PD

FURTHER INFORMATION

Patrik Brundin's homepage:
http://www.med.lu.se/expmed/nesu

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