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The Prion Hypothesis of Parkinson's Disease

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Abstract The discovery of alpha-synuclein's prion-like behaviors in mammals, as well as a non-Mendelian type of inheritance, has led to a new concept in biology, the "prion hypothesis" of Parkinson's disease. The misfolding and aggregation of alpha-synuclein (α-syn) within the nervous system occur in many neurodegenerative diseases including Parkinson's disease (PD), Lewy body dementia (LBD), and multiple system atrophy (MSA). The molecular basis of synucleinopathies appears to be tightly coupled to α-syn's conformational conversion and fibril formation. The pathological form of α-syn consists of oligomers and fibrils with rich in β-sheets. The conversion of its α-helical structure to the β-sheet rich fibril is a defining pathologic feature of α-syn. These kinds of disorders have been classified as protein misfolding diseases or proteopathies which share key biophysical and biochemical characteristics with prion diseases. In this review, we highlight α-syn's prion-like activities in PD and PD models, describe the idea of a prion-like mechanism contributing to PD pathology, and discuss several key molecules that can modulate the α-syn accumulation and propagation.

Keywords Alpha-synucleinopathies · Proteopathy · Prion-like · Propagation · Transfer

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Introduction

A neuropathological hallmark in synucleinopathies is the formation of protein inclusions termed Lewy bodies and Lewy neurites that can be found both intracellularly and extracellularly. These aggregates are composed mainly of α-syn. α-syn is a terminal protein mainly located in the presynaptic element and axon [1] with little in the nucleus [2]. In the development of the central nervous system, perikaryal expression of α-syn is observed as early as 11-weeks post-conception in the cortical plate. Several neuronal groups in the hippocampus, basal ganglia, and brain stem express perikaryal α-syn which persist throughout the first few years of life. Perikaryal α-syn starts disappearing in early childhood, and only the neuropil is retained into adulthood [3]. In normal aging and certain neurodegenerative diseases, α-syn reappears in neuronal perikarya. Normally, α-syn is not seen in glial cells in adult human brain [4]. In transgenic mouse models overexpressing mutated or wild-type human α-syn, Lewy body-like inclusions are observed in neurons, but not in glial cells [5–7]. Several studies demonstrated that there is absence of α-syn mRNA expression in oligodendroglia in normal and multiple system atrophy (MSA) brains [8–10]. However, α-syn-positive inclusions are observed within neuronal perikarya, astrocytes, and oligodendrocytes in patients with PD, LBD, and MSA [11–13]. How α-syn accumulates in perikarya is unanswered. Interestingly, Lewy body pathology has been observed in fetal grafts [14–16] placed into PD brains more than a decade prior. These findings led to the current provocative hypothesis that the α-syn protein itself might transmit from a diseased neuron to its connective neuron like prion proteins, thereby spreading pathology in the brains of PD and other α-synucleinopathies [17–21]. Recent studies have demonstrated that seeding α-syn fibrils into the striatum or substantia nigra causes PD pathology to spread through the brain [22••, 23•], further supporting "prion hypothesis" in synucleinopathies. In this review, we described the α-syn's prion-like mechanism in disease progression that the abnormal α-syn forms β-sheets fibril by self-assembly

in vitro and in vivo transports α-syn fibril cell to cell and enhances α-syn fibril toxicity by inflammatory activity.

Alpha-synuclein is a Prion-like Molecule

β-pleated sheets have been implicated in the formation of the protein aggregates and fibrils in several neurodegenerative diseases including PD and prion diseases. In its aggregated form, α-syn is enriched in β-sheet structure, orderly organized into oligomers, and aggregated into amyloid fibrils. The conformational conversion of α-syn is speculated to be an important feature of self-propagation like prion proteins [18, 21]. Under natural conditions, α-syn is intrinsically unfolded at neutral pH and temperature [24]. Binding to membranes or vesicles containing acidic phospholipids, it assumes an α-helical structure [25]. Using immunohistochemistry, unfolded and α-helical α-syn is typically detected in axons and terminals but not in perikarya [26]. Unexpectedly, α-syn can be detected in some perikarya including nigral neurons during normal aging [26]. The pathological form of α-syn consists of oligomers and fibrils rich in β-pleated sheets. The conversion of α-helical structure to the β-sheet rich fibrils is a defining pathologic feature of α-syn, like prion proteins [27–29]. Though the precise molecular mechanisms underlying the propagation of the α-syn aggregation are unknown, it is generally accepted that α-syn aggregation is a process that follows a nucleated polymerization model. Unfolded α-syn molecules are initially distributed among several related conformations (unfold and α-helical). At the onset of the fibrillization process, soluble species undergo self-assembly into stable oligomeric intermediates and are able to grow through monomer accretion thereby evolving into final amyloid-like fibrils [28, 30]. Several studies indicated that α-syn dimerization is a key step to form inclusions [31, 32]. The nitrated tyrosine 125 can initiate dimerization [31]. The oxidative formation and accumulation of a dimeric, tyrosine cross-linked prenucleus is a critical rate-limiting step in the nucleation of α-syn fibrils. The α-syn dimer has a greater propensity to self-interact and strongly affect the aggregation properties of the molecules [32–34]. Consequently, the α-syn dimer acts as a template upon which native α-syn monomers is refolded into protofibrils. Extensive in vitro studies have shown that, when incubated at 37 °C, monomeric α-syn forms fibrils that are similar to those contained in Lewy bodies in PD [28, 35, 36]. Additional studies observed that α-syn fibrils can act as seeds, promoting fibrillization of surrounding monomeric α-syn [28, 37]. Interestingly, seeding A30P mutant fibril to wild-type monomeric α-syn leads to the generation of fibrils with the same character as A30P fibrils [29]. This assembly of wild-type fibrils induced by A30P seeds involves a conformational conversion from wild type to A30P mutant α-syn. This process is remarkably similar to that described for the template conversion of cellular prion protein (PrP^{C}) to prion in the scrapie form (PrP^{SC}). Several in vitro experiments have demonstrated that exogenous α-syn fibrils seed the formation of intracellular α-syn inclusions recapitulating several key features of Lewy bodies including size, subcellular localization, β-pleated sheet conformation, and hyperphosphorylation and polyubiquitination of constituent α-syn [38, 39•]. Neurons take up fibrillar α-syn via conventional endocytosis [40] and transport α-syn fibrils via axonal transport [41]. The monomeric and aggregated forms of α-syn are released from neurons by exocytotic mechanism [42], these studies above demonstrate that α-syn by self-assembly can complete conformational conversion from dimer, oligomer to fibril and α-helical structure to β-pleated sheet that is like the PrP^{SC}.

Intracellular Inclusion Replicated from Seeding α-Syn

Recent evidence indicates that α-syn seeding can induce endogenous α-syn inclusions in wild-type mice [22••]. In this study, injections of α-syn fibrils into the mouse striatum spread through the brain and cause pathologic alterations including α-syn aggregation, axonal degeneration, dopaminergic neuronal death, and movement disorder [22••]. Recasens and coworkers [23•] reported that LB-derived α-syn can trigger the pathological conversion of endogenous α-syn in mice and monkeys. This conversion was associated with progressive PD-like neurodegeneration. These studies indicate that misfolded α-syn molecules serves as an initial "seeds" and "templates," that catalyzes the conversion of resident normal α-syn into the pathological isoform. The effects of α-syn seeds observed in normal mice and monkeys model the prion-like behaviors. Whether there are α-syn seeds or templates in sporadic PD brains is still unknown. Morphologic analyses reveal that there are different shapes and intensities of α-syn immunostaining in cells of sporadic PD brain. Some cells have α-syn immunoreactive granule-like seeds which are distributed within neuronal perikarya (Fig. 1a) in sporadic PD. The larger the α-syn granules become, the more monomeric α-syn surrounds them in the perikarya (Fig. 1b). When aggregation increases, the monomeric (cytoplasmic) α-syn is reduced (Fig. 1c, d). Finally, when the α-syn aggregation becomes excessive, the cytoplasm disappears and the aggregate appears to be localized within the extracellular space (Fig. 1d). Histochemical analysis verified that aggregated α-syn is thioflavin positive, but cytoplasmic α-syn is not (Fig. 2). However, whether the granule α-syn is high in β-pleated sheets and whether cytoplasmic α-syn is unfolded needs more study to elucidate.

Overall, it appears that abnormal α-syn seed deposition can occur early in neurodegeneration and is potentially a driving force in PD pathogenesis. Whether the seeding of α-syn induces the cell to express more α-syn or the cell with high

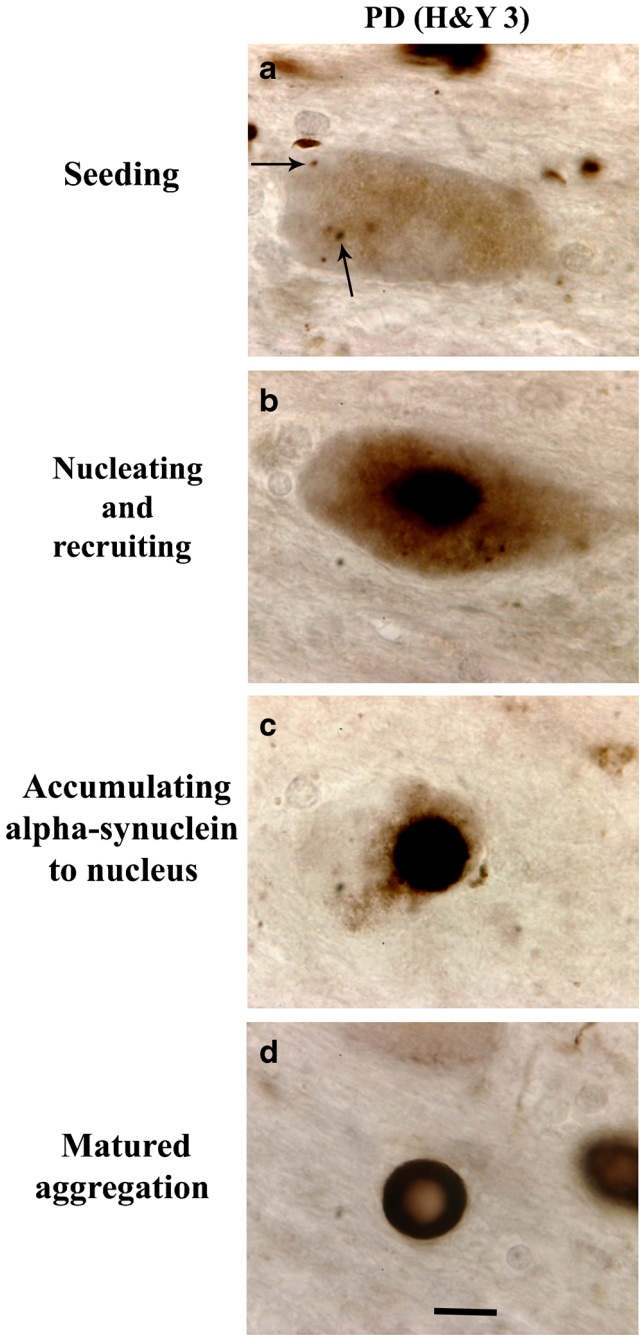

Fig. 1 Photomicrographs of neurons within the basal forebrain of Meynert from patient with Parkinson's disease (Hoehn and Yahr stage 3) illustrating the alpha-synuclein immunoreactivity. Alpha-synuclein (LB509) immunohistochemistry revealed several morphological features including granules within perikarya (**a**; *arrows*), nucleated inclusion surrounding cytoplasmic alpha-synuclein (**b**), inclusion enlargement with decrease of cytoplasmic alpha-synuclein (**c**), and typical Lewy body with disappearance of cytoplasm (**d**). *Bar*=5 μm (applies to all)

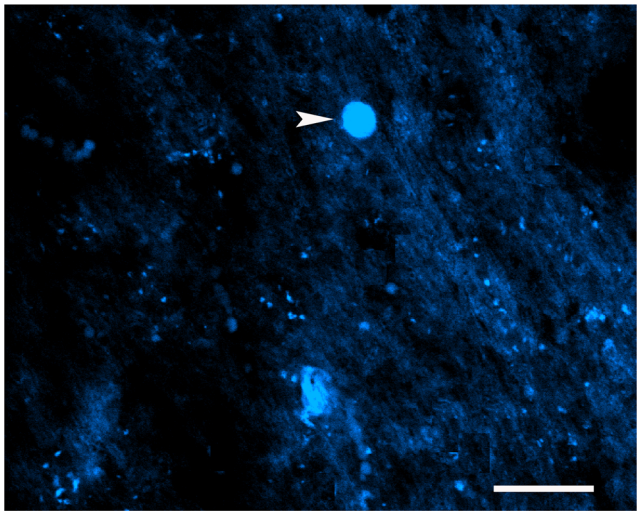

Fig. 2 Photomicrographs of the basal forebrain of Meynert from a patient with Parkinson's disease (same case as Fig. 1.) illustrating thioflavine-S staining inclusions. Note that thioflavine-S-stained aggregations displayed a mass (*arrowhead*). *Bar*=56 μm

levels of α-syn attracts the α-syn into the cell still needs to be investigated. Lee and coworkers used a transgenic mouse model of synucleinopathy (TgM83), to demonstrate that inoculation of young asymptomatic mice with α-syn preformed fibrils prepared from older symptomatic mice accelerated α-syn hyperphosphorylated at serine 129, aggregated α-syn, and significantly decreased survival time [39•]. These pathologic alterations were absent in α-syn knockout mice inoculated with the same brain homogenates prepared from older symptomatic transgenic mice. This indicates that endogenous α-syn plays a key role in the transmission of pathology from affected to unaffected sites and is consistent with a prion-like mechanism of disease.

The familial SNCA multiplication cases show a dose-dependent correlation of α-syn load to the PD phenotype [43, 44]. These pathologic PD features can be mimicked by α-syn overexpression in various models [45, 46]. Sporadic PD is an age-related disease. Consistent with this idea, α-syn protein levels are increased with aging in the nigral neurons and correlate with decreased tyrosine hydroxylase immunostaining [26]. Increases of α-syn protein levels are a critical condition for pathogen invasion. Laser-capture microdissection studies reveal a considerable increase of SNCA expression in surviving nigral neurons derived from PD brains compared with controls [47]. Several studies indicated that single nucleotide polymorphisms in the 5′-promoter and 3′-flanking regions of SNCA gene influence α-syn protein level that are associated with susceptibility to idiopathic PD [48–50]. Furthermore, genome-wide association studies identified SNCA as a common risk factor for PD [51, 52]. Recently two studies uncovered epigenetic regulation of SNCA gene expression. Methylation may be important in SNCA expression, as methylation within intron 1 suppressed SNCA transcriptional activity. Brains from patients with Lewy body diseases showed decreased methylation, suggesting regulation of SNCA expression [53, 54]. One study reported that sequestration of

the methylation factor Dnmt1 away from the nucleus by α-syn led to reduced methylation and enhanced SNCA expression, thus, creating a feed-forward loop of SNCA overexpression [55]. Whether seeded-α-syn sequestrates Dnmt1 away and induces SNCA overexpression needs further investigation.

Whether α-syn aggregation is beneficial or detrimental to neurons has been debated for over a decade. On the one hand, the α-syn aggregation is part of a physiologic response to sequester/deactivate a neurotoxic species [56, 57]. Mutations in α-syn reduce the number of vesicles available for dopamine storage, which results in an abundance of neurotoxic by-products such as dopamine-quinone, superoxide radicals and hydrogen peroxide, and an increased level of oxidative stress [56, 57]. These studies suggest that α-syn aggregation protects neurons. On the other hand, significant evidence points to aggregates occurring at the onset and during the progression of age-related neurodegenerative diseases [58, 59]. Formation of α-syn inclusions has been discovered as a causal factor in apoptosis, suggesting that α-syn aggregates are toxic at certain stages of Lewy body formation [22••, 60]. Furthermore, there are more α-syn inclusions in the substantia nigra in early stages of PD compared to later stages [46] suggesting that the number of α-syn inclusions is reduced by neuronal death. If α-syn aggregation is part of a physiologic response to sequester/deactivate a neurotoxic species, the number of α-syn aggregations should increase in the late stage of PD than the early stage of PD. Phosphorylation of serine 129 is the dominant pathological modification of α-syn in both familial and sporadic PD [61–64]. Approximately 89 % of α-syn deposited in Lewy bodies is phosphorylated at serine 129 [64]. In contrast, only 4 % or less of total α-syn is phosphorylated at this residue in the normal brain [65]. Lee and colleagues demonstrated that dephosphorylation of α-syn at serine 129 can reduce aggregation in the brain, enhance neuronal activity, increase dendritic arborizations, and decrease astroglial and microglial activation, as well as improved motor performance [66]. These data indicate that neurotoxic effects come from processes of α-syn aggregation. Morphologic studies demonstrate that nigral neurons with α-syn aggregation display significant decreases of transcription factors (Nurr1 and MEF2D) [67, 68], reductions of lysosome and proteasome function [69], and impairment of axonal transport and mitochondrial function [46, 70]. Abnormal α-syn binding transcription factor is the critical cause to result in neuronal phenotype loss. For example, α-syn fibril can bind one of the most important chromatin proteins called high-mobility group protein 1 (HMGB-1) [71]. In the nucleus, HMGB1 interacts with nucleosomes, transcription factors, and histones [72]. This nuclear protein organizes the DNA and regulates transcription [73]. HMGB1 supports transcription of many genes in interactions with many transcription factors. It is possible that α-syn fibril sequesters the transcriptional cofactor HMGB-1, thereby, in turn, contributing many neuronal phenotypic losses and the neurodegenerative process.

Alpha-synuclein is Transported to Different Brain Areas

It is well documented that the infection route of acquired prion diseases is via oral intake and what follows is an accumulation and amplification of prion infectivity in lymphoid tissues associated with the gut. Prions then spread to other lymphoreticular tissues, including the spleen, lymph nodes, tonsils, and appendix and to the enteric nervous system which lead to the eventual spread of prions to the central nervous system by retrograde axonal transportation [74–78]. After infection and replication within the central nervous system, there is centrifugal spread of prions via the peripheral nervous system by rapid anterograde axonal transport to other tissues and sites of secondary prion replication. This broad descriptive picture of infection is typical of ovine scrapie, chronic wasting disease, human variant Creutzfeldt-Jakob disease, and experimental ovine bovine spongiform encephalopathy [79].

The movement of abnormal α-syn is still debated, and one must not be confused by the use of words that are often incorrectly applied interchangeably. These words are the 4 Ts, transport, transfer, transmission, and templating. Transport usually occurs retrogradely with α-syn being uptaken by terminals and transported back to the cell soma, and no further. Transfer occurs across at least one synapse using the neuroanatomical connectome as its highway. Transmission implies spread, the movement of α-syn outside the normal neuroanatomical connectome. Finally, templating is not movement at all but the induction of newly synthesized α-syn in regions proximal and distal of α-syn aggregation.

According to Braak's model, vulnerable brain regions are affected in a predictable sequence, progressing in a stereotypical caudal-rostral pattern starting from the lower brainstem and in the rostrocaudal direction from the olfactory bulb [80]. Braak's pattern suggests that in the central nervous system, α-syn pathology starts from the dorsal motor nucleus of the vagus and spreads to rostral structures allowing a six-point staging system [80–82]. Braak's pattern supports the hypothesis "gut-to-brain" propagation of Lewy pathology [83–86]. PD patients have gastrointestinal symptoms including reduced salivation, dysphagia, impaired gastric emptying, constipation, and defecatory dysfunction [87]. Furthermore, viral expression of α-syn within the vagus nerve results in transfer to the nucleus of the desmuslin in the dorsal motor nucleus (DMN) of X and to more rostral brain sites, suggesting that

the gut could be the nidus and the DMN of X is a portal for α-syn entrance to the CNS [88]. Constipation may precede the development of somatic motor symptoms of PD for several years. α-syn pathology has been observed in the peripheral autonomic nervous system and the dorsal motor nucleus of the vagus in PD and incidental Lewy body disease [84, 86]. In contrast, phosphorylated α-syn histopathologic analyses reveal that the most abundant α-syn pathology occurs in the spinal cord, followed by the paraspinal sympathetic ganglia, the vagus nerve, the gastrointestinal tract, and endocrine organs in subjects with PD and LBD. A rostrocaudal gradient of phosphorylated α-syn was distributed in the gastrointestinal tract [89] advocating a "brain-to-gut" spread pattern. A recent study demonstrated that rats displayed delayed gastric emptying 4 weeks after 6-OHDA microinjection into the substantia nigra. In 6-OHDA-treated rats, there was a decreased expression of choline acetyltransferase and nNOS immunoreactivity in the dorsal vagal complex neurons. This study indicated that the delayed gastric emptying in a 6-OHDA rat model of PD may be caused by neurochemical and neurophysiological alterations in the brain-gut axis. Hyposmia is another early symptom of PD [90–92]. Doty reported that olfactory dysfunction has been detected in ~90 % of early-stage sporadic PD cases [93]. The α-syn pathology was observed in the olfactory bulb and anterior olfactory nucleus in PD and incidental Lewy body disease [94–96], suggesting that pathogenic α-syn spreads to the brain by nose-brain axis. Luk and colleagues reported that α-syn fibrils can transmit cell to cell in a prion-like fashion [22••]. A single injection of α-syn fibrils into the striatum of normal mice causes PD pathology to be transported from the striatum to the cortex and substantia nigra with additional transfer limited to a few cells in the olfactory bulb. Lewy pathology accumulation results in progressive loss of dopamine neurons in the substantia nigra pars compacta, reduced dopamine levels in the striatum, and the induction of motor deficits. The evidence from this study demonstrates that exogenously applied α-syn fibril can infiltrate surrounding cells and transport to other brain regions directly connected to the dorsal striatum including cortex and substantia nigra. Preformed fibrils are not required for this pathology to occur as Lewy body extracts can be transported or templated following intrastriatal injections of Lewy body extracts, a procedure that induced nigrostriatal degeneration [23•]. Indeed this paper, as well as our preliminary data [97], demonstrates a similar phenomenon when injected into the striatum of nonhuman primates. Taken together, the pathogenic α-syn could happen all the time to multiple brain sites, but in most individuals, brain cells are able to degrade aggregates at the nucleation stage. It is only in these cells that lose the ability to degrade abnormal protein; the conformational inversion of α-syn to rich β-sheet fibrils will take place. For example, intranasal delivery of lipopolysaccharide (LPS) resulted in α-syn aggregation in the olfactory bulb, due to lysosom and proteasom dysfunction by LPS. Furthermore, it is only in the brain area with accumulated abnormal α-syn that the pathogenic α-syn will be taken and transported to the connected brain areas, as too much pathogenic α-syn will be scavenged by microglia cells.

Other issues still need to be addressed such as how the α-syn fibril enters and exits cells. The neuron has an ability to engulf large intact proteins. In general, extracellular protein binds to a membrane receptor and forms receptor-ligand complexes, a process that involves the formation of membrane-bound vesicles [98]. Cellular membranes create "endosomes" with a highly acidic pH that tends to dissociate the ligand [99, 100]. The receptor is then recycled back to the cell membrane for reuse, while the ligand is passed along to a lysosome for degradation. Natural α-syn binds to membranes containing acidic phospholipids [101] and proteins such as synphilin-1 [102]. Whether the α-syn fibrils can bind to membranes or synphilin-1 is unknown. Synphilin-1 is, like α-syn, a neuronal terminal protein of unknown function [103] and also accumulates in Lewy bodies [104]. The synphilin-1 was first identified as an α-synuclein ligand by yeast 2 hybrid screening [105]. It binds to the synaptic vesicles, and this interaction is inhibited by α-synuclein [103]. The interaction between α-syn and synphilin-1 is mediated by the N-terminal 65 residues of α-syn [105]. Interestingly, α-syn mutation (A30P) caused an increase in the interaction between the synuclein aa 1–65 fragment and the synphilin-1 central domain [106] while A53T reduced the interaction [102]. The positive effect of the A30P mutation indicates that the interaction requires a different N-terminal α-syn structure than the α-helical used for vesicle binding. There is a need for further study of these protein interactions. Several studies report that neurons can release α-syn by exocytosis [107, 108]. Interestingly, lysosomal inhibition causes SH-SY5Y cells transfected with the human α-syn gene to release α-syn to culture media [107], suggesting that lysosomal dysfunction plays a role in α-syn transmission. In coculture of SH-SY5Y cells with human α-syn gene and primary astrocytes, α-syn was released from the neurons and transferred to astroglial cells, causing inflammatory responses [108].

Inflammation and α-Syn

Emerging evidence has indicated that neuroinflammation contributes to PD [46, 109–111]. PD onset and progression are characterized by inflammation and immune abnormalities, including the activation of microglia and expression of pro-inflammatory molecules [46, 112]. However, several questions still remain: (1) does α-syn inclusions cause microglia activation and neuroinflammation? (2) does neuroinflammation trigger α-syn misfolding? and (3) does neuroinflammation

promote cell-to-cell transfer of α-syn as well as the seeding and development of new α-syn aggregates?

Clearly, α-syn inclusions can cause microglial activation and neuroinflammation [46, 113–117]. The extrusion of α-syn aggregates including Lewy bodies and Lewy neurites from dying nigral neurons activates microglia which in turn phagocytize aggregated α-syn, activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and release chemokines and oxygen free radicals [115]. Free radicals damage the cellular membrane, including the mitochondrial membrane, and DNA causing the mitochondria to stop producing energy which in turn leads to cell death [118]. These studies demonstrate that α-syn aggregation is a primary cause of inflammation in PD. Interestingly, substantial evidence demonstrates that microglia are activated prior to frank neuron death in mouse models of PD [117, 119]. The α-syn is released early from cells with α-syn overexpression and acts on microglial cells to release pro-inflammatory molecules, such as tumor necrosis factor (TNF)-α and IL-1b, which are detrimental to dopamine neurons [107, 114, 120, 121]. Injections of α-syn to mouse substantia nigra result in a robust microglial activities, pro-inflammatory cytokines and NFkB expression, and neuronal death [122]. These data clearly suggest that α-syn exerts a pivotal role in promoting neuroinflammation and neurodegeneration.

Conversely, several studies supported this hypothesis that inflammatory factors can modulate α-syn. First, α-syn is nitrated during oxidative stress responses [123, 124], and the nitrated α-syn enriches intraneuronal inclusions in PD brain [125]. Secondly, inflammogens such as lipopolysaccharide (LPS) can promote α-syn aggregation and lead to dopaminergic neurodegeneration both in the brain and in the gastrointestinal system [110, 126, 127]. An intraperitoneal injection or chronic nigral infusion of relatively high doses of LPS led to time-dependent degeneration of nigral dopaminergic neurons in rodents [128–131].

Dopaminergic cell loss following LPS takes several months to occur, suggesting that a chronic neuroinflammation influences progressive neurodegeneration [128, 129]. Injection of LPS to intracerebral ventricle not only induces astrocytosis activity, dopaminergic neuron loss, and motor defects but also leads to phospho-α-syn expression, a reflection of parkinsonian pathogenesis [132]. In addition, neurodegeneration and α-syn aggregation can be observed 21 and 30 days after a single nigral LPS injection but not in TNF knockout mice [126]. This study verified that inflammation can promote formation of α-syn aggregation. Furthermore, intranasal delivery of LPS every day for 5 months resulted in progressive hypokinesia, selective loss of dopaminergic neurons, reduction in striatal dopamine content, as well as α-syn inclusions in the substantia nigra [133]. These studies defined that LPS delivery into mice induces PD-like pathogenesis and symptoms and mimic the progressive changes of PD including the aggregation of α-syn. Whether the α-syn inclusions induced by LPS are the rich β-pleated sheets fibrils needs to be verified. Whether LPS directly leads to dopaminergic neuronal death or indirectly downregulates lysosomal and proteasomal functions to cause α-syn accumulation is still unanswered.

Whether the neuroinflammation promotes cell-to-cell transfer of α-syn and seeding and development of new α-syn aggregates is still unknown. Transport of α-syn from the parkinsonian brain to grafted neuron clearly occurs in an inflammatory environment. And interestingly, elevated levels of α-syn oligomers are seen in the cerebrospinal fluid from PD patients [134]. Increased levels of CSF α-syn oligomers are also found in patients with synuclein-based dementia [135]. The soluble oligomers and early fibrils of α-syn are the pathogenic species that input to neurons and lead to neurodegeneration [136–138]. Whether the increased levels of CSF α-syn are associated with neuroinflammation is unknown. There are several hypotheses: (1) cell with α-syn overexpression can secrete α-syn [139], (2) activated microglia cells promote α-syn release from cell with α-syn accumulation [140], and (3) release of α-syn can occur from the compromised blood-brain barrier that is damaged by activated microglia and inflammatory factors such as free radicals. It is possible to seed soluble oligomers and early fibrils from cell to cell when the blood-brain barrier is incomplete. Positron emission tomography to measure brain uptake of [11C]-verapamil, which is normally extruded from the brain by P-glycoprotein in the endothelium cells of the blood-brain barrier, demonstrated a significantly elevated uptake of [11C]-verapamil (18 %) in the midbrain of PD patients relative to controls [141, 142]. This is the evidence supporting a dysfunctional brain barrier as a causative mechanism to spread pathogenic α-syn in PD brain.

Conclusions

Most neurodegenerative diseases have a long prodromal phase without clearly clinical symptoms. There is a long prodromal period between the first emerged pathologic protein aggregation and the first clinical symptoms. The prodromal period is a potent therapeutic opportunity. How long is the prodromal period in neurodegenerative disease? No one knows. Experimental evidence from mouse PD model shows that the prodromal period was 30 days between the injection of fibrillated α-syn into the striatum and motor deficits emerged [22••]. It is possible that abnormal protein aggregation is taking place all the time, but that in most individuals, brain cells are able to degrade aggregates at the nucleation stage, thus, preventing their stable formation and propagation. With aging, the lysosomal and proteasomal functions are downregulated [69]. The formation of α-syn aggregation processes several steps including uptake and/or release of misfolded α-syn,

forming nucleation or granule, recruiting monomer α-syn and conformational conversion from unfold to β-sheets. Therapeutic approaches can target early three steps. First, immunotherapy or compounds inhibit cell-to-cell transmission or uptake and release of misfolded α-syn through their sequestration in the extracellular space. This could interfere with the spreading mechanisms of α-syn. Second, enhancements of lysosomal and proteasomal functions digest granule or seed α-syn within intracellular cytoplasm. Third, increase of the methylation factor Dnmt1 in the nucleus reduces SNCA expression to hinder recruiting monomer α-syn. In addition, α-syn fibril binds synphilin-1, and both aggregated in the Lewy body suggesting that synphilin-1 is a ligand for α-syn and promotes α-syn aggregation. Sequestration of the synphilin-1 can prevent α-syn fibril into cells and aggregation.

Although α-syn aggregation appears to be central to the development and progression of PD pathology, inflammation also plays an important role in this disease. Overactivated microglia cells in PD phagocytize aggregated α-syn, activate NADPH oxidase, and released chemokines and oxygen free radicals. Free radicals cause α-syn nitration and aggregation, damage the cellular membrane, and impair the brain barrier [142]. The increased levels of α-syn oligomers in CSF are considered as leaking from compromised blood-brain barrier [143]. It is also possible that neuroinflammation promotes the prion-like transfer of α-syn cell to cell in limited locations by increasing its release and uptake of α-syn inclusions. However, further studies are needed to support this hypothesis. If correct, it will open up new avenues for early detection and therapeutic intervention. Anti-inflammation can slow down the progression of PD by reducing the underlying inflammation and mitigating its effects on cell-to-cell α-syn transfer.

Compliance with Ethics Guidelines

Conflict of Interest Yaping Chu declares no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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