Topographical Propagation of α-synuclein Pathology in Parkinson’s Disease: Phenomenology and Hypothetical Mechanism

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ABSTRACT

Parkinson's disease is an age-related, slowly progressing neurodegenerative disorder characterized by abnormal deposition of aggregated α-synuclein in neuronal cell bodies (Lewy bodies) and neurites (Lewy neurites), as well as in glia. Based on semiquantitative assessment of Lewy pathologies in autopsy samples, a staging system was proposed indicating a highly predictable sequence of pathological progression. This staging system implicates a propagation of α-synuclein aggregation throughout the brain with an ascending pattern from lower brain stem to neocortex. The underlying mechanism for the pathological propagation is unknown. However, the recent discoveries on the secretion of neuronal α-synuclein and subsequent uptake of the protein by neighboring cells propose an interneuronal transmission of α-synuclein aggregates as a novel mechanism for the spread of Lewy pathology in PD. Elucidation of this mechanism is likely to identify novel therapeutic strategies that halt the progression of PD.

Key words: lewy body, protein aggregation, exocytosis, endocytosis

INTRODUCTION

Parkinson’s disease (PD) is a common age-related neurodegenerative disorder, first described by English physician James Parkinson. Clinically, PD is primarily defined by motor symptoms, such as muscle rigidity, resting tremor, and bradykinesia (Fahn and Sulzer, 2004). However, PD also shows a spectrum of non-motor symptoms, ranging from central nervous system dysfunctions (dementia, cognitive impairment, psychiatric disorders, loss of olfaction, etc.) to various autonomic dysfunctions (oily skin, urinary incontinence, weight loss, etc.) (Poewe, 2007). Pathological features of PD include the loss of dopaminergic neurons in substantia nigra and the occurrence of α-synuclein immunoreactive inclusion bodies, called Lewy bodies (LBs) and Lewy neurites (LNs), in the remaining neurons (Forno, 1996). Lewy pathology is not limited to PD, but also found in a variety of neurological disorders, including dementia with LBs (DLB) and the LB variant of Alzheimer's disease, which are now collectively referred to as synucleinopathies (Hardy and Gwinn-Hardy, 1998). Lewy pathology in PD and other synucleinopathies generally shows wide spread patterns affecting various regions of the brain. Recently, Braak and colleagues proposed
a staging system for assessing the progression of PD pathology, based on their observation that $\alpha$-synuclein-positive Lewy pathology shows a predictable sequence of pathological progression throughout the brain (Braak et al., 2003). In this review, we will summarize the current knowledge on the pathological progression in PD, and propose a hypothesis for the underlying mechanism of the progression based on the recent discoveries in extracellular $\alpha$-synuclein.

**CLINICAL OVERVIEW OF PD**

In PD, although not all patients display identical symptoms with identical progression, common motor symptoms of PD show a characteristic pattern of progression (Alves et al., 2005; Jankovic, 2008). The early motor symptoms include bradykinesia, tremors, postural deformities, and decreased facial expression (Jankovic, 2008). Bradykinesia, which refers to slowness of movement, is most commonly seen in PD patients. It is marked as a basal ganglia disorder, and renders slowness in daily-life activities and reaction times. Rest tremor, one of the common and easily recognized symptoms of PD, is present in the distal part of an extremity. As the disease progresses to more severe forms, the symptoms on one side of body begin to encroach on the other. More advanced PD patients show poor-balance ability, difficulty in initiating movements, and more severe rigidity. PD patients also show non-motor symptoms; autonomic dysfunction features from orthostatic hypotension, sweating dysfunction, sphincter dysfunction to erectile dysfunction; sleep disorders, especially the REM sleep disorder; cognitive and psychiatric abnormalities such as depression, apathy, anxiety, hallucination, and dementia (Poewe, 2007). Like the motor symptoms, the severity of the non-motor symptoms in PD patients tend to show progressive characteristics; the average MMSE score, which measures the state of cognitive condition, of PD patients showed a linear trend of decline from Braak stages 3 to 6 (Fig. 1).

**TOPOGRAPHICAL PROGRESSION OF LEWY PATHOLOGY**

PD pathology is characterised by $\alpha$-synuclein inclusions: LBs and LNs (Braak et al., 2003). While the mechanisms of lesion development are not yet understood, autopsy studies by Braak and colleagues showed $\alpha$-synuclein deposition in various brain regions following a predictable and systematic progression pattern (Braak et al., 2002; Braak et al., 2003) (Fig. 1). Brain samples from potential PD patients, presumably representing the earliest PD pathology, showed confined lesions in the two sites: the dorsal motor nucleus of the vagus nerve and the olfactory bulb (Braak et al., 2006). No more than a few LBs and LNs were found in the vagus nerve at this stage (Braak Stage 1). As the Lewy pathology in the dorsal motor nucleus worsens along the progression of the PD, this pathological feature then spreads to the substantia nigra. At the same time, previous damages in the lower brain worsen. Pathological progression after this point overlaps with AD pathology. Inclusion bodies start to appear in the transition zone between the allocortex and
the neocortex, which corresponds to the antero-medial temporal mesocortex. Specifically, numerous LBs were found in high order sensory organs such as entorhinal region and amygdala (Braak Stage 4). Sometime at this point, it is thought that PD is clinically manifested. After mesocortex, the pathology finally spreads to neocortex, with the clinical symptoms fully developed. Pathology of neocortex first appears in the prefrontal and high sensory area, and is later shown in the premotor and first order sensory association areas (Braak Stage 5 and 6). Although some studies reported the cases that did not comply with the Braak staging of PD pathology (Parkkinen et al., 2005; Parkkinen et al., 2008), this staging system has been confirmed by a number of autopsy studies (Jellinger, 2003; 2004).

PD AND $\alpha$-SYNUCLEIN

Even though the cause of PD is largely unknown, both environmental and genetic factors are thought to contribute to the development of the disease. Since 1997, several mutations have been identified in familial cases of the disease (Farrer, 2006). The first of such mutations was identified in the gene encoding neuronal protein $\alpha$-synuclein; two additional autosomal dominant missense mutations and several gene multiplication mutations in the same gene have been identified in familial PD afterwards. The link between $\alpha$-synuclein and PD was further strengthened by the finding that fibrilar aggregates of this protein are the major component of LBs and LNs in sporadic PD (Spillantini et al., 1998). Biochemical analyses indicated that $\alpha$-synuclein may form amyloid fibrils that are similar to the aggregates found in LBs (Conway et al., 2000). Furthermore, all the $\alpha$-synuclein missense mutations accelerated the aggregation of the protein (Cookson, 2005). The role of $\alpha$-synuclein aggregation in neurodegeneration was demonstrated when overexpression of wild type and mutant $\alpha$-synuclein led to neuronal loss and LB-like inclusion formation in animal models (Maries et al., 2003).

$\alpha$-synuclein is a member of the synuclein family which also includes $\beta$-synuclein and $\gamma$-synuclein (George, 2001). Human $\alpha$-synuclein consists of 140 amino acids and is characterised by highly conserved amphipathic N-terminal regions with seven repeats of the KTKEGV consensus motif and highly variable acidic C-terminal regions (Cookson, 2005). This protein also contains a central hydrophobic non-amyloid-$\beta$ component (NAC) domain, through which amyloidogenic intermolecular interaction might occur (Giasson et al., 2001). Biological function of $\alpha$-synuclein is not completely understood. However, its localization in the presynaptic nerve terminals and its ability to bind to lipid membranes indicate its role in neural transmission (Iwai et al., 1995; Davidson et al., 1998). Subtle, but significant changes in neurotransmission and synaptic vesicle recycling have been found in $\alpha$-synuclein knock-out mice (Abeliovich et al., 2000; Cabin et al., 2002; Liu et al., 2004; Yavich et al., 2004). The knockout studies also suggested its role in fatty acid metabolism (Castagnet et al., 2005; Golovko et al., 2005). Other studies suggested that $\alpha$-synuclein has characteristics of molecular chaperones (Kim et al., 2000; Souza et al., 2000).

EXOCYTOSIS OF $\alpha$-SYNUCLEIN

Despite the fact that $\alpha$-synuclein is typically a cytosolic protein, a small amount of this protein is constitutively secreted from various types of cells, including primary neurons (El-Agnaf et al., 2003; Lee et al., 2005; Sung et al., 2005). Although the mechanism of the secretion is not well understood, translocation of the protein into vesicles and subsequent exocytosis appear to be the primary mechanism responsible for the secretion of $\alpha$-synuclein (Lee et al., 2005). The identity of the vesicles is unknown as yet. However, electron microscopy and density gradient ultracentrifugation experiments showed that the vesicles containing $\alpha$-synuclein have morphologies and sedimentation properties similar to the dense core vesicles (Lee et al., 2005). The critical questions to be addressed include how the $\alpha$-synuclein proteins to be secreted are selected and what are the vesicles involved and how the exocytosis is regulated.

Analyses of human cerebrospinal fluid (CSF) and blood plasma confirmed the presence of extracellular $\alpha$-synuclein, suggesting that the secretion of $\alpha$-synuclein occurs in human (Borghi et al., 2000; El-Agnaf et al., 2003). Although whether the level of extracellular $\alpha$-synuclein changes in disease is
embroiled in controversy (Borghi et al., 2000; El-Agnaf et al., 2003; Lee et al., 2006; Tokuda et al., 2006), measurement using the ELISA specific for oligomeric forms of \( \alpha \)-synuclein showed that oligomeric \( \alpha \)-synuclein levels in blood plasma and post-mortem CSF from PD patients have elevated significantly compared to control group (El-Agnaf et al., 2006).

Since only a small amount of cellular \( \alpha \)-synuclein is translocated and secreted through vesicles with the majority of the proteins remain in the cytoplasm, vesicle entry of this protein must be a selective process. Although the mechanism of this selection is unknown, our recent study suggests that misfolded/damaged proteins are preferentially translocated into vesicles and subsequently discarded from cells by exocytosis (Lee et al., unpublished data). The accumulation of misfolded \( \alpha \)-synuclein in specific vesicle populations predicts the aggregation of this protein within the vesicles. In fact, \( \alpha \)-synuclein aggregation preferentially occurs in vesicles, and these aggregates are exocytosed from cells (Lee et al., 2005). Although physiological and pathological functions of extracellular \( \alpha \)-synuclein is still in mystery, its potential role in the initiation and progression of PD has been proposed from its ability to induce neuronal death in culture and neuroinflammatory responses from glial cells (Zhang et al., 2005; Klegeris et al., 2006; Su et al., 2007; Thomas et al., 2007; Klegeris et al., 2008; Lee, 2008).

HYPOTHETICAL MECHANISM OF PATHOLOGICAL PROPAGATION IN PD

PD is a progressive disease with its pathology beginning and advancing in a highly predictable sequence throughout the CNS (Braak et al., 2003). Given the consistent progression pattern, it may not be unreasonable to consider the possibility of progressive propagation of pathogenic events through the affected areas. How the pathogenic events are propagated is unknown. One possible mechanism for the propagation might involve a spatial progression from one brain region to another of the factors that affects the basic physiology of neurons and surrounding glial cells. Alternatively, the pathological process, such as \( \alpha \)-synuclein aggregation, might directly propagate from affected neurons to adjacent neurons or to distant neurons through synaptic connections. The latter possibility was recently supported by the studies showing the neuron’s ability to take up extracellular \( \alpha \)-synuclein (Sung et al., 2001; Ahn et al., 2006; Lee et al., 2008b). Extracellular \( \alpha \)-synuclein aggregates are taken up by the receptor-mediated endocytosis, followed by endosomal trafficking and lysosome-mediated breakdown (Lee et al., 2008b). Both neuronal and glial cells are capable of this endocytic internalization and degradation of \( \alpha \)-synuclein aggregates (Lee et al., 2008a). Although the normal fate of the internalized \( \alpha \)-synuclein appears to be the lysosomal degradation, following the prolonged exposure to extracellular \( \alpha \)-synuclein aggregates, the internalization of these aggregates may cause the accumulation of the protein in LB-like inclusion forms (Lee et al., unpublished data). During the accumulation of these aggregates, the externally derived aggregates interact with the intrinsic cytoplasmic \( \alpha \)-synuclein, suggesting a seeding-induced amplification of the aggregates (Lee et al, unpublished data). These studies provide strong experimental evidence for direct cell-to-cell propagation of \( \alpha \)-synuclein aggregates.

Propagation of pathogenic events by exogenous pathogens has been well documented in prion disorders. In prion disorders, pathogenic prion aggregates can “infect” cells and nucleate the polymerization of normal cellular proteins (Caughey, 2000). Alzheimer’s disease may be operated by a similar pathogenic propagation mechanism. Administration of brain homogenates prepared from Alzheimer’s patients or a transgenic mouse model of the disease caused earlier and more severe plaque pathology and neurodegeneration in an animal model of AD (Meyer-Luehmann et al., 2006). Although no such experiments have been performed in models of PD, secretion of \( \alpha \)-synuclein aggregates into the extracellular space and subsequent uptake of these proteins by adjacent neurons implicate interneuronal transmission of \( \alpha \)-synuclein aggregates and the prion-like aggregate seeding, which may constitute the mechanistic basis for the pathological propagation (Fig. 2). This hypothetical mechanism may also explain the recent findings of host-to-graft propagation of \( \alpha \)-synuclein inclusions.
in long term mesencephalic transplants in PD patients (Kordower et al., 2008; Li et al., 2008). However, how the internalized α-synuclein aggregates interact with the intrinsic α-synuclein proteins and whether the aggregates can be amplified by this mechanism remain to be addressed. These questions should be addressable using the transgenic animal models and tissue culture models expressing human α-synuclein.

CONCLUSION

PD is a chronic disease with progressive manifestation of a wide spectrum of symptoms, which may be attributed to damages of various regions in CNS as well as in autonomic nervous system. Analyses of autopsied brains showed a highly predictable pattern of α-synuclein pathology progression. Recent advances in α-synuclein biology enabled us to formulate a testable hypothesis for the mechanism of direct cell-to-cell propagation of α-synuclein aggregates. The proposed mechanism involves interneuronal transmission and seeded amplification of α-synuclein aggregates, reminiscent of prion propagation (Fig. 2). This mechanism provides an entirely novel way of looking at the progress of the disease and permits the identification of new therapeutic targets. The new model proposed in the paper may also explain some of the unexpected, yet significant, experimental findings, such as the protective effects of α-synuclein immunization in a transgenic mouse model (Masliah et al., 2005). Validation of this hypothetical model provides the critical basic knowledge that will allow the mechanistic approaches to the immunization therapy in the future. Finally, the model proposed here may amount to one of the fundamental principles that are commonly applied to neurological diseases, including Alzheimer’s disease, which also feature spatiotemporal progression of protein pathologies.

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