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Modeling Lewy Pathology Propagation in Parkinson's Disease

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Abstract

Lewy bodies (LBs) and Lewy neurites (LNs) comprised of alpha-synuclein (α Syn), are intraneuronal inclusions that characterize Parkinson's disease. Although the association between the extent of Lewy pathology and clinical symptoms is well established, how these proteinaceous deposits originate and target selectively vulnerable cell populations is unknown. Our knowledge of their role in PD pathogenesis is also limited. Here, we summarize recent findings demonstrating this pathology can be experimentally transmitted between animals by misfolded forms of α Syn that are capable of initiating and inducing LB and LN inclusion formation through a self-propagating mechanism reminiscent of prions. "Seeded" LBs and LNs in animal models also spread to multiple connected nuclei in a predictable pattern, recapitulating a phenomenon observed during human PD progression, leading to the dysfunction and degeneration of afflicted neurons. These models provide new perspectives on how this and other misfolded proteins may contribute to neurodegeneration in human disease.

Keywords

Parkinson's disease; protein misfolding; propagation; alpha-synuclein

Introduction

A common feature of many neurodegenerative diseases is the accumulation of proteinaceous deposits in the nervous system. In Parkinson's disease (PD), the signature histopathological lesions are represented by eosinophilic inclusions within the cytoplasm or enlarged neurites of neurons, and are known as Lewy bodies (LBs) or Lewy neurites (LNs). Importantly, these inclusions are present in virtually all sporadic and familial PD brains (with rare exceptions) [1], an observation which suggests a role for LBs and/or LNs in the disease process. In agreement with this line of thought, post-mortem studies of PD brains indicate that the extent of Lewy pathology correlates with the nature and severity of clinical symptoms.

The precise neuroanatomical distribution of Lewy pathology has been studied by multiple groups, most extensively by Braak and colleagues who proposed prototypic disease stages defined by pathology and corresponding motor and non-motor symptoms [2]. According to this scheme, new brain regions are affected with each successive stage, while the pathology

in previously affected areas increase in severity. In early disease, (stages 1 and 2) pathology occurs primarily in lower brainstem nuclei, olfactory nuclei, and peripheral neurons. Pathology in the midbrain, including substantia nigra, does not occur until stages 3 and 4 when classical PD motor symptoms become apparent. Stages 5 and 6 are marked by involvement of neocortical regions and cortical LBs robustly correlate with dementia in PD [2-4] whereas LBs in the midtemporal cortex are linked to hallucinations. Cognitive impairment in PD may also be associated with co-morbid Alzheimer's disease [5]. Although the proportion of individuals that deviate from this pattern remains debated [6], observations by several groups nonetheless indicate that the majority of patients examined exhibit the stereotypic pattern consistent with LBs cumulatively afflicting neuron populations [5, 7, 8].

How does this idiosyncratic pattern of pathology develop in patients of a disease spanning years and even decades? Perhaps the most controversial aspect of the PD staging scheme proposed by Braak is that the progressive spread of LBs/LNs suggests the transmission of a pathogenic process/agent from diseased to healthy cells [2, 9]. Multiple rounds of transmission could then lead to the expansion of LBs/LNs through the nervous system. Interestingly, LBs/LNs are frequently detected in gastrointestinal, cardiac, as well as olfactory neurons, especially in early stages of PD, indicating that spread might occur over long distances and raising the possibility that the pathogenic culprit may be environmental or viral in origin [2, 9]. Further evidence pointing towards the spread of LBs/LNs comes from post-mortem studies of PD patients who received mesencephalic grafts showing the time-dependent formation of LBs in grafted neurons (cells that were presumably normal at the time of implantation) [10].

Misfolded α Syn is a transmissible pathological agent

The discovery that point mutations in α Syn cause autosomal dominant forms of PD led to the initial identification of this 140 amino-acid protein as a major component of LBs/LNs in a family of neurodegenerative disorders now known as synucleinopathies [reviewed in 11]. In contrast to its highly soluble state in healthy brains where it likely regulates synaptic vesicle release [12, 13], a considerable portion of α Syn recovered from PD brains is insoluble and shows various post-translational modifications including proteolytic cleavage, hyperphosphorylation, ubiquitination, nitrosylation and oxidation [14-16].

In addition, α Syn in LBs/LNs exist as β -sheet rich amyloid fibrils, a structural arrangement shared by proteins that accumulate in several other major neurodegenerative diseases such as Alzheimer's disease, polyglutamine disease, and prion diseases. Both extracellular and intracellular amyloids are potent catalysts for the misfolding of their cognate proteins by acting as conformational templates for the corruption and recruitment of endogenous proteins into amyloid fibril aggregates [17].

We previously used cationic liposomes to introduce small fragments of recombinant α Syn fibrils into α Syn-expressing cell lines, and show that this led to the conversion of cellular α Syn into LB-like inclusions [18]. Subsequently, we demonstrated that an analogous process occurs in mouse primary neurons, where synthetic fibrils seed the formation of insoluble inclusions that share many of the same biochemical markers as human LBs such as

phosphorylation of α Syn at serine129, and the recruitment of ubiquitin and heat shock proteins. Interestingly, efficient pathological seeding can be achieved with non-transgenic neurons and in the absence of a delivery agent [19], suggesting that endogenous α Syn levels are sufficient to support LB/LN formation and that α Syn fibrils are readily internalized by neurons.

***In vivo* transmission of Lewy pathology**

Based on these findings, we hypothesized that misfolded α Syn could similarly induce Lewy-like pathology when introduced *in vivo*. Transgenic mice overexpressing human α Syn bearing the A53T familial PD mutation (M83 line) spontaneously develop α Syn pathology in multiple regions of the central nervous system (CNS), most notably brainstem and spinal cord where mutant α Syn expression is highest in this line. Normally, appearance of this pathology occurs within 8-16 months of age, coinciding with a variety of behavioral and motor phenotypes and death shortly thereafter [20]. To determine whether pathology-bearing CNS homogenates from these symptomatic mice could efficiently accelerate α Syn aggregation *in vivo*, we stereotactically injected this material into the dorsal striatum and cortex of young, healthy M83 mice. Both we and others showed that homogenates prepared from symptomatic animals led to the formation of α Syn-rich inclusions in the recipient animals at ages well before the first signs of pathology typically appear in non-injected animals [21, 22].

The observation that homogenates from asymptomatic transgenic mice lacking detectable α Syn inclusions failed to accelerate pathology in recipient M83 mice suggested that misfolded α Syn within LBs/LNs found in sick donors might be the active pathogenic agent. To test this hypothesis, we inoculated healthy M83 mice with recombinant human α Syn that had been assembled into amyloid fibrils *in vitro*. These synthetic fibrils elicited a nearly identical distribution of LB/LN-like pathology, demonstrating that misfolded α Syn alone is sufficient to initiate pathology. Interestingly, inclusions were also observed in astrocytes, which express the mutant α Syn in this line of transgenic mice, indicating that non-neuronal cells are capable of supporting α Syn pathology when exposed to this agent [22].

Surprisingly, this paradigm of α Syn-seeded pathology can be extended to non-transgenic mice. Specifically, synthetic α Syn fibrils targeted to either dorsal striatum, hippocampus, or substantia nigra all lead to the formation of LBs/LNs in wildtype mice of a variety of background strains, indicating that α Syn overexpression is not a prerequisite for pathological seeding *in vivo* and that misfolded α Syn readily propagates in young healthy animals [23, 24]. Moreover, inclusions in both transgenic and wildtype mice are positive for the amyloid dye thioflavinS, and antibodies to ubiquitin and misfolded/phosphorylated α Syn, thus displaying the key markers seen in human LBs/LNs and in the cell models above. Interestingly, fibrils comprised of full-length murine α Syn appear to induce pathology more rapidly than human α Syn fibrils, consistent with previous *in vitro* studies that minor sequence variations as found between species influence efficient nucleation [24]. This ability to induce LBs/LNs formation through *in vitro* and *in vivo* seeding models has provided some new insights into a few fundamental questions regarding α Syn pathology.

Transmission of α Syn along neuroanatomical pathways

How α Syn pathology spread between cells? Examination of the CNS from both transgenic and wildtype mice following inoculation with misfolded α Syn reveal that LB/LN formation occurs initially at the site of injection [22, 23]. However, α Syn pathology disseminates over time to additional regions that project to or receive connections from the original injection site. In M83 mice, homogenate or fibrils injected into the striatum and cortex develop considerable pathology in thalamus and brain stem but also in frontal cortical regions, where α Syn accumulation is typically not observed in non-injected symptomatic animals [22]. Intriguingly, these animals also show LBs/LNs in multiple nuclei located at considerable distances from and contralateral to the injection sites and lacking direct input/output were also affected (e.g. spinal cord and deep cerebellar nuclei). Abundant α Syn deposits were present along intermediary white matter tracts suggesting that pathology propagated along axonal fibers. Despite the direction of this propagation, it remains to be determined if tertiary neurons develop pathology through the trans-synaptic spread of misfolded α Syn.

Further support that pathological spread follows neuronal projections is provided by the observation that α Syn injections into either dorsal striatum or somatosensory cortex produce distinct global patterns of pathology, indicating that the location of the originating misfolded α Syn dictates the route of LB/LN expansion. The observation that pathology preferentially affects neurons sharing direct connections with the fibril injection site also applies to wildtype mice [23]. For example, dorsal striatal fibril injections resulted in prominent α Syn pathology in substantia nigra pars compacta (unilateral), cortical layers 4/5 (bilateral), and amygdala (bilateral). Inclusions were also detected in select neurons that lack direct projections to the injection site, such as mitral cells in the olfactory bulb. The contrasts in LB/LN distribution with M83 animals injected at identical locations likely stem from differences between endogenous and transgenic α Syn expression patterns. Nonetheless, these findings demonstrate that pathological spread is associated with connectivity, and are also consistent with recent reports that α Syn is secreted and taken up by a variety of CNS cell types, the mechanisms for which have been reviewed extensively elsewhere [25].

α Syn inclusions are detrimental to neurons

Is the accumulation of α Syn inclusions toxic or simply a marker of disease? An important observation from these experiments is that acceleration of pathology in the transgenic M83 mice leads to a dramatic reduction in the survival, brought on by the onset of behavioral impairments similar to those observed in aged non-injected animals [22]. This phenotype appears within a narrow time frame and both homogenate- and fibril-injected animals succumb to disease within 4 months following inoculation, regardless of age at the time of injection. Although the extent of LBs/LNs in inoculated wildtype mice is more restricted compared to that of transgenic animals, affected areas also show clear signs of dysfunction and degeneration. Most notably, dopaminergic neurons in the substantia nigra ipsilateral to the injected striatum progressively degenerate following α Syn inclusion formation, leading to loss of dopamine innervation to the striatum and reduction in motor function and coordination [23]. Thus, exposure of the CNS to small quantities of misfolded α Syn can initiate neurodegeneration even in intact animals. Significantly, injection of either diseased

CNS homogenate or α Syn fibrils into α Syn null mice do not result in LBs/LNs nor any detectable phenotype further supporting that LBs/LNs directly contribute to the observed impairments.

Implications for human synucleinopathies

The above *in vivo* findings provide additional evidence substantiating the so-called “Braak hypothesis” that a transmissible agent is responsible for the spread of pathology seen in PD. The observations demonstrating α Syn pathology can spread between considerable distances within the CNS and that misfolded α Syn, the key protein component of Lewy pathology, is capable of self-propagating in neurons presumably extend to peripheral nervous system as well. Studies showing that altered α Syn species are elevated in cerebrospinal fluid of PD patients [26, 27] and that homogenates isolated from brains of PD and dementia with Lewy body patients induce pathology in both transgenic and wildtype animals [24] further suggest that seeding-competent α Syn species are present in human disease. The recent identification of conformational strains of pathological α Syn that elicit distinct pathologies *in vivo* [28], also provides a possible explanation for the histopathological and symptomatic diversity among synucleinopathies.

These observations augment a converging hypothesis among major neurodegenerative disorders that transmission of the disease protein plays a critical role in progression [29]. Although there is no current evidence that they are transmitted between individual organisms through conventional means, the ability of α Syn, β -amyloid, tau, and other proteins have increasingly elicited comparisons with prions. The ease and rapidity in which expansion and spread of these proteins occur (especially for α Syn) suggests the ability to inhibit transmission should have an exponential effect in protecting downstream connected populations and it will be interesting to see how pioneering clinical trials for α Syn immunotherapy fare. Finally, although the relationship between α Syn pathology and cellular dysfunction are now more apparent, understanding of how synucleinopathies arise will require the identification of both the location and molecular nature of the primordial pathologic seed. Moreover, the underlying events and pathways ultimately leading to degeneration still need to be defined. Thus, elucidation of the physiological function of α -Syn should provide a better understanding its role in disease.

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