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The Prion Hypothesis in Parkinson Disease

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Parkinson disease (PD) is a neurological disorder characterized by the selective degeneration of dopaminergic neurons within the substantia nigra. Motor deficits of patients with PD are characterized by the rest tremor, bradykinesia, and rigidity; however, patients with PD show additional features including postural instability, falls, freezing of gait, and dementia during disease progression.[1] Although the precise pathogenesis remains unclear, genetic and postmortem studies have demonstrated that aberrant aggregation of α-synuclein may have a crucial role.[2] Aggregated α-synuclein may be associated with dysfunction of various cellular mechanisms such as synaptic transmission, maintaining integrity of cytoskeleton, protein degradation, mitochondrial energy production, and management of oxidative stress.[3] This review will discuss prion-like spread pattern of α-synuclein pathology in PD.

The interneuronal spread α-synuclein pathology

Braak et al., in their seminal study of the staging of PD pathology, used α-synuclein immunohistochemistry staining in large number of autopsy cases. Based on α-synuclein neuritic pathology ("Lewy neurites"), they have suggested a six-staged scheme in which the pathology begins first at olfactory bulb and dorsal motor nucleus of the vagus (DMV), and gradually follows an ascending course, cumulating in widespread α-synuclein pathology at the later stages, involving cortical regions.[4] Propagation of PD pathology in the Braak staging scheme would suggest that α-synuclein pathology may transfer between neurons, and spread of α-synuclein aggregates may be a driving factor of PD progression.[5]

The first evidence that Lewy pathology spreads from cell-to-cell came from clinical trials of PD patients who received donor neuronal grafts.[6,7] During these trials, PD patients showed similar progression of parkinsonian motor deficits compared to those who did not receive neuronal grafts. In the autopsy study of these patients, dopaminergic neurons from healthy donors exhibited Lewy pathology. The number of grafted neurons with Lewy pathology was positively correlated with duration from neuronal graft to autopsy. In 16 year post-grafting case, up to 30% of cells had α-synuclein pathology, whereas 3-5% of grafted neurons revealed Lewy pathology in 10 year post-grafting case. These results raise 2 different possibilities that i) α-synuclein pathology might directly come from patients' neurons with Lewy pathology, or ii) PD brain might provide a microenvironment prone to aggregation of α-synuclein (e.g., inflammation).[5]

Since these clinical observations, *in vivo* and *in vitro*

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studies have shown that α-synuclein aggregates spread intra- and inter-neuronally.[8-10] Brain extracts from PD patients have induced pathology and neurotoxicity when inoculated into the brains of mice and non-human primates.[11] In animal and cell culture models, pathogenic fibrils generated from purified α-synucleins, termed with preformed fibrils (PFFs), seed pathology and induce neurotoxicity along with neuronal circuits.[12] Once in the cell, PFFs are localized to endocytic vesicles, particularly lysosomes[13], and can escape into the cytosol possibly by disruption of lysosomal membrane.[13-15] PFFs in the cytosol can seed further aggregation of endogenous α-synuclein via a permissive templating mechanism.[16] Newly formed fibrils can then undergo exocytosis, and bind and spread to adjacent cells. Correspondingly, locally injected PFFs in animal models produce widespread Lewy pathology along connected neural circuits.[12,17,18]

The stereotypical spreading pattern in Braak staging scheme presupposes the existence of specific neuronal interconnections. Studies employing neuro-anatomical tracers have demonstrated the existence of relevant neuronal interconnections.[19] The DMV is connected to the raphe nuclei and locus coeruleus (LC), which are in turn connected to the substantia nigra.[20, 21] No direct mono-synaptic pathways have been found between DMV and nigra[20], which could conceivably introduce a time lag for the α-synuclein propagation from lower brain stem to mesencephalon. At the initial formulation of the Braak staging scheme, the importance of early olfactory bulb pathology was questioned because few incursions into related olfactory regions were seen. However, it has been shown that the nigra and LC are directly connected to the olfactory bulb.[22, 23] Indeed, a study using high throughput mapping of barcoded mRNA demonstrated that 23% of LC neurons directly innervate the olfactory bulb in mice.[24]

Prion disorders and PD: is PD pathology initiated in peripheral part?

Based on direct transport of α-synuclein aggregates along neuronal interconnectivity, several literatures have suggested that PD may have similar characteristics with true prion disorders such as Creutzfeldt-Jakob disease (CJD). After oral injection with bovine spongiform encephalopathy-associated prion in cattle, sheep, or hamsters, the prion aggregates are seen in the abdominal ganglia at first, and subsequent spreading of prions are observed in the intermediolateral cell column (IML) and DMV.[19] In addition, animal models have revealed that prion aggregates are observed in efferent pathway before afferent, that is, coeliac-mesenteric ganglia (CMG) and IML are affected before the dorsal root ganglia, and the DMV before the nodose ganglia.[25,26] Therefore, the initial spreading of prions in these animal models is highly similar with the hypothesized route of α-synuclein propagation in PD.[19]

If prion-like spread of aggregated α-synuclein pathology were a main factor of PD progression, PD pathology could initiate from a peripheral part of body, and spread into central nervous system (CNS). Indeed, α-synuclein pathology can be observed outside of CNS such as nervous plexus of gastrointestinal (GI) tract, heart, salivary gland, and skin.[19] In PD, it has been suggested that α-synuclein pathology may preferentially originate in the terminals of neurons with long, non-myelinated, hyper-branched axons.[27, 28] This proposition, at least partly, explain why autonomic nerves in GI tract are prone to formation of Lewy pathology, as these axons are among the longest and most hyper-branched in the body. In mice model, gastric lavage with rotenone was shown to induce α-synuclein aggregation and secondary spreading to the CNS.[29] A study examined archived GI tissues from PD patients prior to diagnosis has observed phosphorylated α-synuclein aggregates in 56% (22/39 cases) of prodromal PD subjects.[30] In the similar context, subjects underwent total vagotomy or appendectomy have lower PD risk.[31,32] Based on these results, it has been suggested that PD pathology may initiate in peripheral part, especially in autonomic nerves of GI tract, and propagate into the CNS.

Factors promoting the spread of α-synuclein pathology

However, at least currently, evidences showing prop-

agation of α-synuclein aggregates are insufficient to conclude that prion-like spread is a driving factor of PD progression. PFFs and PD brain extracts can induce PD-like pathology in animal models. However, in many of these animal models, the severity and distribution of pathology appears to plateau some time follow the inoculation. Inoculation into the olfactory bulb of mice results in local Lewy pathology and eventually (i.e. up to 12 months following injection) pathology in interconnected brain regions.[17] However, a similar follow-up study included animals sacrificed 18 and 23 months post-PFFs injection, and these animals showed a reduction of pathology at the later time points.[18] An important weakness of Braak staging as an explanation of spreading behavior is that the distribution of pathology could be explained by selective vulnerability of specific neurons to pathology.[5]

Some literatures have suggested that additional factors may affect the severity and breadth of propagation of seeded Lewy pathology in animal models. A study using M83 transgenic α-synuclein over-expressing mice have observed robust Lewy pathology by 180 days post-intrastriatal PFFs injections.[33] In contrast, much more limited propagation was seen following similar injections into wild-type mice.[34] Mutations in the α-synuclein gene (*SNCA*), specifically those that increase α-synuclein expression, promote α-synuclein spreading behavior by increasing the amount of endogenous substrate for intercellular permissive templating to occur.[35] Disruptions of lysosomal storage may be another factor for propagation of α-synuclein pathology. The autophagy lysosome inhibitor bafilomycin A1 appears increases toxicity in several models of synucleinopathy.[36,37] Mutations of GBA1, the gene that encodes for the lysosomal enzyme beta-glucoceribridase (GBA), are associated with high risk of developing typical PD.[38] GBA was also identified as modifier of α-synuclein pathology using a high-throughput cell screening technique.[39]

Conclusions

Is the phenomenon of α-synuclein pathology spread relevant to PD? Although α-synuclein pathology can spread via cell-to-cell pattern, it is still unclear whether spreading phenomenon may be the main driving factor in PD. Regardless, factors promoting progressive spread of α-synuclein pathology should be investigated. Future interesting studies may include elucidating genetic and environmental factors controlling spreading behavior, long-term studies looking at how/if a hypothetical endogenous α-synuclein seeds spread and characterizing the spread and pathogenicity of defined α-synuclein fibril conformers.

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