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COMMENTARY

The importance of developing strain-specific models of neurodegenerative disease

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Over the last four decades, it has become increasingly clear that the misfolding and accumulation of a small number of proteins cause most, if not all, neurodegenerative diseases [8, 16, 19, 20]. In the case of a Parkinson's disease (PD) patient, misfolded α-synuclein aggregates to form Lewy bodies (LBs) and Lewy neurites (LNs) in the brain. While the field of neurodegeneration has made important advances toward understanding how protein misfolding gives rise to disease, there are still a number of important questions that remain about the disease process. In this issue of *Acta Neuropathologica*, Loria et al. established important new models of synucleinopathy to address questions about the cell-to-cell movement of α-synuclein in the brain and investigate the role astrocytes play in α-synuclein spread and degradation [13].

In this new report, the authors fluorescently tagged wild-type human α-synuclein fibrils, which were first used to study cell-to-cell transmission of the protein in mouse primary cultures [13]. After pre-loading neurons or astrocytes with fibrils, Loria et al. found both cell types efficiently transferred the tagged fibrils to astrocytes; however, astrocyte-to-neuron transfer of α-synuclein fibrils was inefficient. Combined with the lack of detectable endogenous α-synuclein expression in astrocytes, these findings suggest astrocytes likely play a key role in removing misfolded α-synuclein from the brain, and therefore, are less susceptible to developing intracellular aggregates. These initial observations were replicated using ex vivo organotypic mouse brain slices co-cultured with primary neurons or astrocytes pre-loaded with tagged fibrils, which suggests astrocytes play a critical role in sequestering pathogenic α-synuclein in PD patients.

The novel application by Loria et al. of the organotypic brain slice to investigate the cellular transfer of α-synuclein fibrils establishes a new model that will prove to be important for studying α-synuclein trafficking in the disease state. Moreover, these studies represent the first model to look at cellular transfer of α-synuclein in the presence of all glial cell types. Recently, 3D cultures using differentiated SH-SY5Y cells were used to study neuron-to-neuron transfer of α-synuclein [6]. The studies reported by Loria et al. expand upon this idea and use an organotypic brain slice as the "receptor" cells in this modified version of a pulse-chase experiment. In evaluating the role of astrocytes using this system, the authors identify a previously undescribed ability of astrocytes to rapidly degrade α-synuclein fibrils. This observation, as well as the inability of α-synuclein to transfer from astrocytes to neurons, indicates astrocytes may be critical for preventing or reducing robust neuronal spreading of the pathogenic protein, and suggests astrocytes may be an important new therapeutic target to explore for synucleinopathy patients. However, while these studies are likely to launch a new area of investigation in PD research, they also demonstrate some of the challenges that arise in studying neurodegeneration.

The remarkable ability of a small number of proteins to give rise to multiple diseases, each with unique symptoms, progression, and neuropathology, is thought to occur as a result of the protein misfolding into a distinct conformation. The idea that each conformation causes a different disease forms the basis of the strain hypothesis [20].

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The concept of strains was first proposed to explain the diversity of diseases caused by the misfolding of the prion protein (PrP), which includes Creutzfeldt–Jakob disease, fatal familial insomnia, Gerstmann–Sträussler–Scheinker syndrome, and kuru [1]. The idea has since been applied to β-amyloid, as different pathologies have been observed in patients with familial mutations and have also been found in transgenic mice inoculated with homogenate prepared from deceased patient tissue [28]. In addition, this idea has also been applied to the proteins tau [10, 22, 30] and α-synuclein [9, 21, 29, 31]. In the case of α-synuclein, the protein misfolds and aggregates into both LBs and LNs in PD patients [18, 24] and glial cytoplasmic inclusions (GCIs) in multiple system atrophy (MSA) patients [23]. Evidence shows that the molecular pathways, glial cell involvement, and the cell types that are affected by MSA and PD can differ substantially, supporting the idea that distinct α-synuclein strains are responsible for these two diseases [7].

In the field of neurodegenerative diseases, synthetic or recombinant protein fibrils have widely been incorporated into cell and animal models to better understand the role of a specific protein in disease onset and progression. Using this approach with α-synuclein, investigators have made progress toward understanding the effect of protein aggregation on a neuron [26, 27], how α-synuclein is trafficked in a cell [25], and how protein misfolding spreads between neurons [5, 6, 25] or other cell types [13]. Importantly, while specific aggregation protocols can induce distinct conformations of α-synuclein fibrils with differing effects on model systems [2, 17], it remains unclear if the majority of α-synuclein fibrils used in research are comprised of one conformation or multiple conformers. Furthermore, it is unknown if the fibril conformations are representative of the α-synuclein

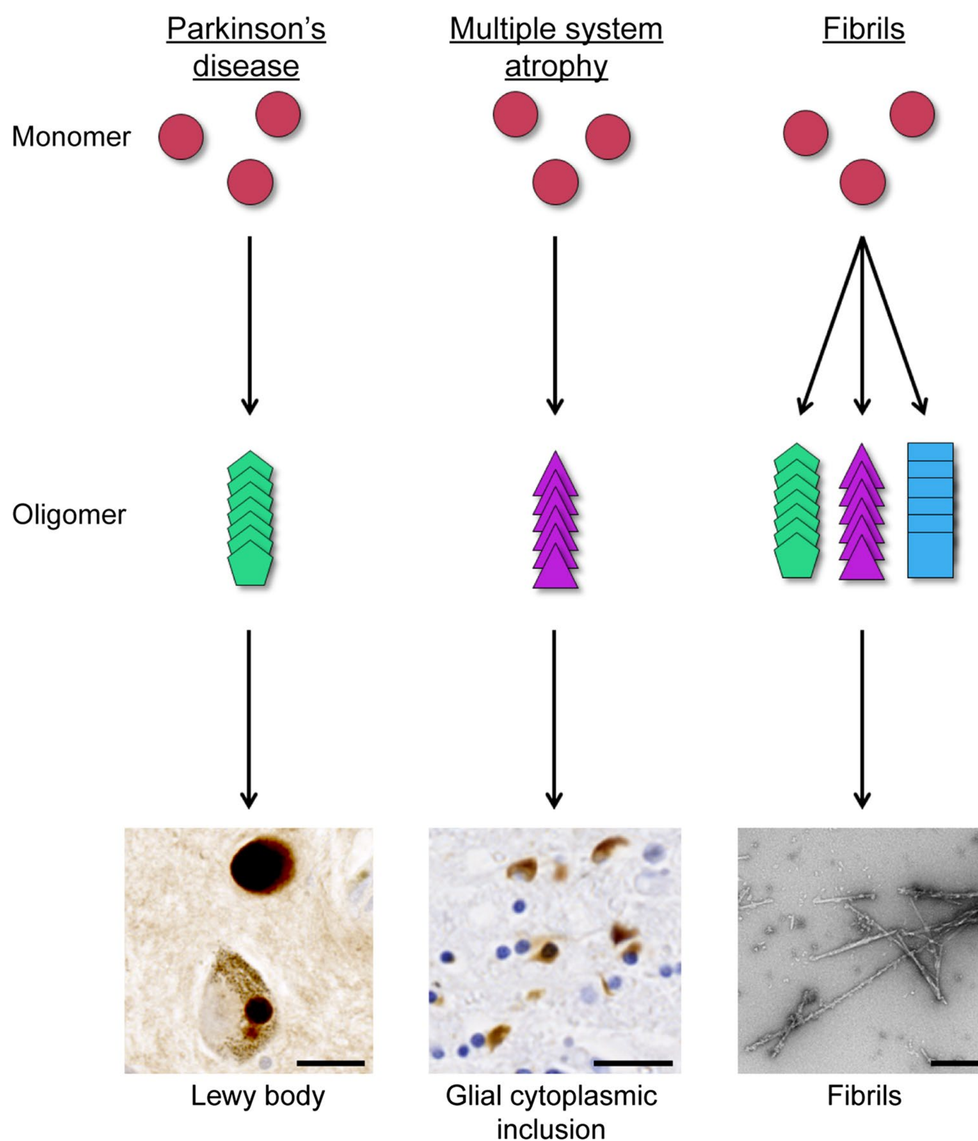

Fig. 1 Applying the strain hypothesis to misfolded α-synuclein. In the disease state, monomeric α-synuclein misfolds and aggregates into Lewy bodies (LBs) and Lewy neurites (LNs) in Parkinson's disease (PD) patients or glial cytoplasmic inclusions (GCIs) in multiple system atrophy (MSA) patients. It is hypothesized that the distinct conformation α-synuclein misfolds into is responsible for determining which disease a synucleinopathy patient develops. Recombinant or synthetic α-synuclein monomer is often used to form fibrils to mimic α-synuclein in PD and MSA patients in research models. However, it is unknown if the fibrils conformers are representative of α-synuclein in PD or MSA. Moreover, it is possible the fibrils may contain both disease-causing conformations, as well as neither conformation. Neuropathology *scale bar* 25 µm. Fibril *scale bar* 250 nm

strains that give rise to PD or MSA, and thus whether they are indicative of disease (Fig. 1). As a result, the use of fibrils in research may not effectively model human disease and may instead be a study of disease-associated proteins.

For the field of neurodegeneration to continue making progress toward understanding degeneration of the central nervous system, the models used must reflect and encompass key aspects of disease as well as differentiate between the diseases caused by the same protein. A central facet of PD is the transmissibility of misfolded α-synuclein from one neuron to the next [11, 12, 25]. It is clear from the Braak staging of PD that α-synuclein pathology progressively spreads from one neuron to the next through synaptically connected brain regions [3, 4]. This systematic spreading should be replicated in the models used to study PD, particularly in models designed to elucidate the mechanism(s) of cell-to-cell spreading.

It is not known if the fibrils used in the studies presented by Loria et al. [13] are predictive of the α-synuclein conformation in PD patients. Moreover, the fibrils also introduce an additional variable that excludes pathological transmission or self-templating in their model systems. It is known that a species barrier reduces the templating efficiency between mouse and human α-synuclein—inoculating mice or mouse cells with human fibrils requires more time to induce α-synuclein pathology than when mouse fibrils are used [14, 15]. However, using mouse fibrils to infect mouse cells removes the human disease aspect of the model system. In the reported studies, primary cells from wild-type mice were incubated with human α-synuclein fibrils [13]. In using human α-synuclein fibrils to maintain the disease relevance of the model, the authors introduced a species barrier to the experiments, reducing the likelihood of self-templating occurring during a 72-h assay. Recognizing that the spreading of α-synuclein pathology may impact transmissibility and degradation of the protein, future studies using the innovative culture models reported by Loria et al. should combine the organotypic brain slices from transgenic mice expressing human α-synuclein with patient-derived α-synuclein to confirm these initial findings and pursue additional research questions about the role of glial cells in PD.

An important metric for validating any model is determining how consistent it is with data that have been collected from human tissue. For example, an analysis of brain tissue from PD patients who received fetal tissue transplants more than 10 years before death found that LBs spread from the host neurons to the grafted tissue [11, 12]. This is consistent with Braak staging of PD, showing that patients progress from exhibiting autonomic dysfunction to emotional and cognitive disturbances as LBs spread from the brainstem into the cortices [3, 4]. The use of co-cultures and organotypic brain slices by Loria et al. to understand cell-to-cell transmission of α-synuclein fibrils is an important new method for understanding the role glial cells play in the disease process [13]. Furthermore, the authors show for the first time that α-synuclein can be transferred from astrocyte-to-astrocyte, but not astrocyte-to-neuron which may be important for thwarting more rapid disease progression. However, they also observed poor neuron-to-neuron transfer of α-synuclein fibrils after 72 h, suggesting the fibrils may not be representative of α-synuclein from PD patients.

Studying neurodegeneration requires the use of imperfect models to make sense of the complexities at play during the breakdown of the central nervous system. Working hand-in-hand with neurologists and neuropathologists to develop and refine cell and animal models can help minimize limitations, though they will nevertheless exist. The knowledge gleaned from our studies explains what is happening in model systems, which may or may not be indicative of what is happening in the human brain. When translating the results of cell or rodent studies to human patients, recognizing the importance of strains will facilitate progress as the field of neurodegeneration makes strides toward identifying critical processes underlying disease etiology and progression.

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