Diverse Biochemical and Immunofluorescent Characteristics of Proteopathic alpha-Synuclein in PD and MSA

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Abstracts will be considered for both poster and platform presentations

Movement disorders

Objective: Determine whether biochemical differences exist in alpha-synuclein found in Parkinson's disease (PD) and multiple system atrophy (MSA).

Background: In synucleinopathies such as PD and MSA there is growing support for the idea that different conformations of alpha-synuclein exist. In prior studies we found alpha-synuclein seeding ability is present in both PD and MSA brain extracts using a cell-based FRET assay. However, there were quantifiable differences in seeding ability from soluble fractions from MSA and PD brains, and inclusion morphology within biosensor cells overexpressing alpha-synuclein was also distinct. We utilized biochemical and microscopy-based techniques to determine whether differences exist in pathologic alpha-synuclein in these two diseases.

Methods: Brain tissue was serially extracted from two different regions from patients with PD and MSA to yield buffer-soluble and detergent-insoluble fractions. Immunoprecipitation was performed with multiple antibodies to different epitopes in alpha-synuclein to determine a binding profile in PD vs MSA samples. We also utilized immunoblot, immunoprecipitation with commercial and novel alpha-synuclein antibodies, limited proteolysis, and immunofluorescent-staining methods to assess aggregated forms of alpha-synuclein in samples from these synucleinopathies.

Results: The antibodies used in this study were able to bind a form of alpha-synuclein capable of seeding synuclein aggregation in the cell-based assay robustly from MSA samples, but only minimally from PD samples. Immunoblot studies showed high levels of alpha-synuclein in both soluble and insoluble brain fractions, but aggregation ability as measured on the FRET assay did not correlate with total synuclein levels or phosphorylated synuclein levels. Immunofluorescence did show that aggregated synuclein inclusions within biosensor cells have differential morphology but that inclusions in both conditions co-localize with a marker for the amyloid conformation (X34) and phosphorylated alpha-synuclein (PSer129).

Conculsions: Biochemical differences in pathologic alpha-synuclein in PD and MSA support an underlying conformational difference in the aggregated state. Further immunoblot and immunofluorescent characterization suggests that seeding differences seen in soluble forms of PD vs MSA are not necessarily driven by phosphorylated forms of synuclein. Different conformations or strains of alpha-synuclein would argue for diverse therapeutic approaches to these two diseases should pathologic alpha-synuclein truly spread in a prion-like manner.