Distinct Synuclein Seeds in Parkinson Disease and Multiple System Atrophy

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Objective: To determine whether seeding activity of alpha-synuclein differs between Parkinson disease (PD) and Multiple system atrophy (MSA).

Background: There is growing evidence from both in vitro and in vivo studies that in many neurodegenerative diseases, including synucleinopathies, cell-to-cell transmission of a pathological protein occurs and may be a vehicle for spreading of pathology throughout the brain. This misfolded protein, or "seed", further templates misfolding of native protein within the cell. Pathologic proteins may exist in diverse conformations with distinct cellular and biochemical properties.

Methods: We have developed a system which combines the sensitivity of a fluorescent system with the quantitative power of flow cytometry. Our assay uses Fluorescent Resonance Energy Transfer (FRET), to detect small amounts of aggregated synuclein. We generated monoclonal cell lines that stably express synuclein fused to cyan or yellow fluorescent protein (syn-CFP/YFP). Upon aggregation, quenching of CFP by YFP produces spectroscopic changes which are readily detected and quantified by flow cytometry. We used this assay to test seeding activity in soluble and insoluble fractions of brain from PD and MSA.

Results: The FRET assay sensitively and specifically detects seeding from recombinant synuclein fibrils. It also robustly detects synuclein seeding activity in both PD and MSA. While insoluble fractions showed seeding activity in both diseases, only MSA showed robust seeding in the soluble fraction. Morphology of the seeded aggregates was also distinct between the two diseases.

Conclusions: Using a quantitative cell-based assay, we have found clear differences in synuclein seeding activity and aggregate morphology in MSA and PD. This work supports the idea of a conformational difference between the pathologic synuclein found in these two synucleinopathies.