Extracellular vesicles as vehicles for alpha-synuclein misfolding in Parkinson’s Disease

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Introduction

Parkinson’s disease (PD) is second only to Alzheimer’s disease as the most common neurodegenerative disorder. A shared feature among various neurodegenerative diseases, is the accumulation of specific protein species prone to aggregating into β-sheet rich amyloid fibrils. A well-supported model for the progression of PD—known as the prion model—proposes that the misfolding of α-syn propagates to naive cells in a virus-like manner. This spreading involves a poorly understood “seeding” process, in which monomeric α-syn converts to the pathological oligomeric form. What remains to be understood is whether “seeding” primarily occurs intracellularly, or perhaps whether this process occurs within extracellular vesicles. Furthermore, degradative pathways are compromised in PD, which includes the autophagy-lysosomal pathway (ALP) and chaperone mediated autophagy (CMA). Recent findings suggest that an inability to successfully degrade α-syn corresponds with an increase in extracellular α-syn. Since extracellular CMA and ALP proteins are associated with attempts at degrading toxic aggregates, overburdened cells might produce seedlings that contain all three markers: pathological misfolded α-syn, monomeric α-syn, and these degradative proteins.

Objectives

This study investigates aspects of the seeding process. Specifically, 1) does treatment with pathological α-syn promote the secretion of endogenous α-syn from cells? 2) if so, are non-classical secretory vesicles providing a vehicle for α-syn misfolding, and can they be identified?

Methods

Experiments involved human derived SH-SY5Y cells containing a dual split α-syn protein construct (DSP).
- This dual split construct consists of α-syn that is both tagged with a GFP fluorophore for immunofluorescence as well as the enzyme luciferase to quantify the amount of endogenous α-syn secreted from cells.

Day 0: Cells were plated onto fibronectin treated coverslips in 24-well plates.

Day 1: After achieving approximately 70% confluence, half of the cells were treated with exogenous fibrillar α-syn for 24 hours (containing an Atto-550 fluorophore for imaging).

Day 2: Cells were subsequently treated with either DMSO (drug control) or bafilomycin. Bafilomycin inhibits the lysosomal proton pump and subsequently the autophagy-lysosomal pathway.

Day 3 (18 hours later): Supernatant was collected and utilized in two ways:
- Luciferase assay was performed for each condition in order to quantify the amount of endogenous α-syn/ EVs secreted by the cells
- Remaining supernatant was fixed and stained for immunofluorescence imaging—with markers of degradative pathways included (e.g. hsc70)

Results

The circled tricolored puncta represents an extracellular vesicle containing three different markers. This example of triple colocalization—presumably resulting from a non-classical secretory pathway—has the potential to act as a seeding compartment between the pathological and monomeric α-syn.

In the figure below, SY5Y cells treated with only α-syn fibrils corresponded with an increased detection of RLU, indicating increased secretion of EVs containing endogenous α-syn. Notably, cells treated with both α-syn fibrils and bafilomycin resulted in a further increase in RLU detection compared to bafilomycin alone.

Discussion

The luciferase reporter assay results in the top figure suggest that the “pathological” exogenous α-syn is effective in causing cellular stress and promoting a non-classical secretory pathway. In other words, the fibrillar α-syn increased the amount of EVs. Moreover, an additive effect is seen upon inhibiting the ALP with bafilomycin—showing that the mechanism by which the pathological α-syn disrupts cells is not saturated by the drug’s robust effect. This suggests that in PD pathogenesis, pathological α-syn could be sufficient in increasing the number of extracellular seeding vehicles containing both misfolded and monomeric α-syn.

As seen in the plot below, the data supports the hypothesis of well-defined EVs containing both forms of α-syn along with degradative markers (e.g. hsc70). It was much more likely for the pathological α-syn to have colocalized with the hsc70 positive EVs than the hsc70 negative EVs. Only 0.5% of vesicles that were hsc70 negative were exogenous α-syn positive. Meanwhile, 12.3% of vesicles contained all 3 markers: fibrillar α-syn, monomeric α-syn, and hsc70.

Conclusions

- As hypothesized, exogenous fibrillar α-syn is capable of increasing endogenous α-syn secretion, presumably due to inhibited degradation.
- Furthermore, as predicted, these extracellular vesicles contain markers of a non-classical secretory pathway (e.g. hsc70).
- The dual split protein α-syn construct allowed for two sets of findings: 1) The luciferase reporter assay quantified the amount of α-syn containing EVs secreted, and 2) immunofluorescence demonstrated the degree of colocalization between the two forms of α-syn, as well as with degradative pathway markers.
- Identifying markers associated with misfolded α-syn secretion could pinpoint a possible pathway for therapeutic intervention. For example, upregulating the autophagic proteins closely associated with degrading the aggregates could provide a protective effect through improved function.

References


Acknowledgements

- Research was supported by the NIAID of the National Institutes of Health under award number: T35 AI125220
- Dr. Edward Campbell & members of the Campbell Lab including Kevin Burbidge, Jessica Mattick, Adarsh Dharan, Sarah Talley, and Edgar Ramirez.
- Student Training in Approaches to Research Program, Stritch School of Medicine