Isolation and Transfection of Brain Derived alpha-synuclein and Its Toxicity on Neurons

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Background

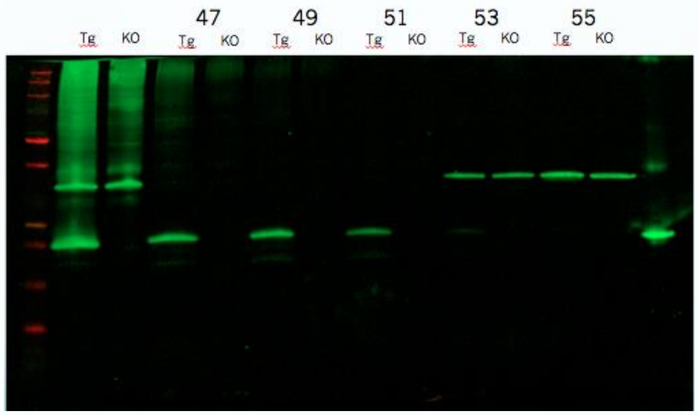
Encoded by the *SNCA* gene, alpha-synuclein,αSyn, is a soluble protein mainly found in the pre-synaptic terminals of neurons. Past research has shown that αSyn does play a role in the pathophysiology of Alzheimer's disease. Research by Larson et al showed that levels of αSyn more strongly correlated with cognitive impairments than similar levels of Aβ and tau[1]. Additionally, in mice overexpressing human αSyn, they showed an elevation of αSyn induced memory deficits similar to the observed deficits in Alzheimer's mouse models[1]. There are several different oligomeric forms of αSyn, but what remains unknown is if one oligomeric form is more toxic than another.

Methods

9.3 month I2.2+ and 9.3 month alpha-synclein knock-out mice brains were extracted using a 4-step extraction process. Extracellular, intracellular, and membrane associated lysates were isolated from the brains. 250ug of the extracellular and intracellular lysates from both the I2.2, and alpha-syn knock out brains were all run separately through a Size-Exclusion Chromatography (SEC) apparatus. After separating all of the fractions from the SEC, the fractions were run on 10-20% gels, and the nitrocellulose membranes were revealed with 4D6 and IgGm-800 antibodies in order to see what forms of alpha synuclein were isolated. Fractions containing alpha-syn monomer (~15kD) and dimer (~30kD), as well as the corresponding fractions from the knock out brain lysate to use as controls, were delivered via Chariot™ into the primary neurons. An LDH assay was performed to assess the toxic effect on the cells.

Data & Results

As seen in the Figures 1 and 2 below, alpha-synuclein from mouse brain lysate was successfully separated into fractions containing solely monomer or dimer. 4 extracellular fractions, 36, 42, 48, and 54, of the I2.2 lysate were introduced into primary neurons. The same fractions of the extracellular alpha-synuclein knock out mice were also introduced to primary neurons as a control. Primary neurons were incubated with each fractions for 0, 2, 4, and 6 hours. Toxicity was assessed at each time point. As seen in Figure 3 below, after a period of 6 hours, the alpha-synuclein was most toxic to the cells. Additionally, fractions 36 and 42, both containing alpha-synuclein monomer were more toxic to the cells than 48 and 54, which contained multiple species and dimer, respectively (Figure 2).



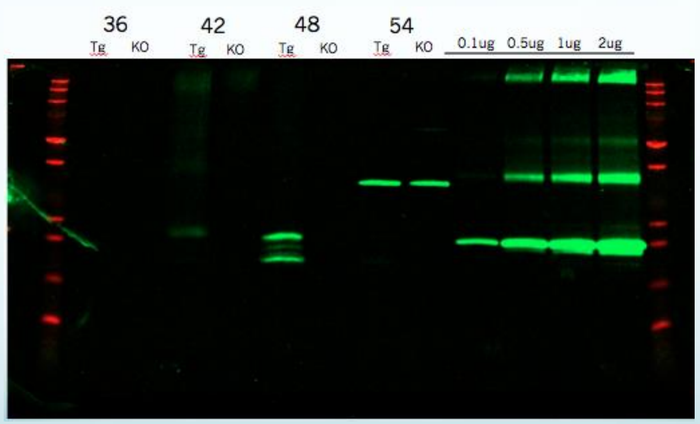


Figure 1 (Top). Odd intracellular fractions 47-55 of both the transgenic (I2.2) and asyn-knock out (KO) were run on a gel and revealed with 4D6, a mouse specific alpha synuclein antibody.

Figure 2 (Bottom). Even fractions 36, 42, 48, and 54, that were applied to cells, of 9.3M extracellular I2.2 & asynKO SEC Fractions and synthetic alpha-synuclein standards were run on a gel. Different amounts of the synthetic standard were used to determine the amount (in ug) of alpha synuclein protein in each fraction.

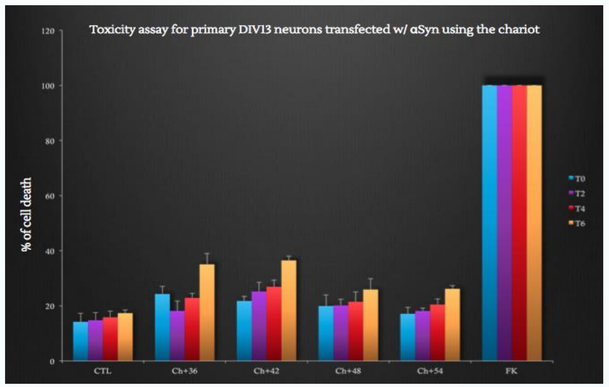


Figure 3[2]. LDH Assay of primary neurons. This graph shows the percentage of cell death over four periods of time for each of the fractions 36, 42, 48, and 54, and a control.
Figure 3 made, and toxicity experiment performed by Fatou Amar, a coworker in the Lesne Lab

Conclusions

Based on the results of the *in vitro* introduction of monomeric and oligomeric alpha synuclein species into primary neurons we have shown that it is the monomeric species, and not the oligomeric forms of alpha-synuclein that has the most toxic effect on neurons.

1. M. E. Larson *et al.*, Soluble -Synuclein Is a Novel Modulator of Alzheimer's Disease Pathophysiology, *J. Neurosci.* **32**, 10253–10266 (2012).
2. Amar, F. *Figure 3.* Lesne Lab (2014).