Research Question:

Is the neuronal chaperone proSAAS capable of decreasing α-synuclein-mediated cytotoxicity?

Abstract:

In Parkinson’s Disease, abnormal aggregation of α-synuclein is toxic to dopaminergic neurons and results in serious neuronal dysfunction, impairing motor skills. Recent research suggests that molecular chaperone proteins can be cytoprotective against neurodegenerative biochemical processes including protein aggregation. ProSAAS is a widely expressed secretory chaperone in the brain. We hypothesize that proSAAS is cytoprotective against the aggregation and fibrillation of α-synuclein in Parkinson’s Disease. The purpose of this research is to determine the ability of proSAAS to decrease α-synuclein cytotoxicity. Full length and truncation constructs of proSAAS were affinity purified and tested for cytoprotective effects. Addition of recombinant proSAAS to SH-SY5Y neuroblastoma cells overexpressing α-synuclein rescued the α-synuclein-mediated cytotoxic effects. To see if protection was specific for α-synuclein cytotoxicity, proSAAS was tested against other forms of cytotoxicity. ProSAAS had no effect on cytotoxicity due to oxidative stress (H₂O₂) or to endoplasmic reticulum stress (Tunicamycin). We conclude that proSAAS cytoprotection is specific for α-synuclein-mediated cytotoxicity. Ongoing research is being performed to determine the specific protein sequence responsible for cytoprotection, using purified truncated constructs of proSAAS, as highly conserved amino acid residues 160-180 have been shown to be critical in blocking in vitro fibrillation.

Background:

Parkinson’s Disease is the second most common neurodegenerative disease in the United States. It primarily affects the motor system, and symptoms result from dopamine loss and neuronal cell death in the substantia nigra. A key characteristic of Parkinson’s Disease is abnormal α-synuclein protein aggregation into Lewy Bodies in neurons. α-synuclein is a 140 amino acid natively unfolded protein believed to have a functional role in synaptic transmission regulation. Proteostasis is a molecular process by which chaperones prepare or fix unfolded and misfolded proteins for translation or degradation. However, through environment stressors, accumulation of errors, and mutations, proteostatic mechanisms break to fail and proteins like α-synuclein are more prone to misfolding, aggregating and fibrillating. When the protein forms fibrils, it adapts toxic behavior which can cause proteasome disruption, mitochondrial dysfunction, and ER-Golgi traffic blockage. Probable therapeutic targets are secretory chaperones that block α-synuclein aggregation. The focus of this project is on the widely expressed neuronal secretory chaperone proSAAS. Preliminary data shows that proSAAS colocalizes with α-synuclein in Lewy bodies in the substantia nigra. Further, proSAAS can block α-synuclein fibrillation in vitro. In this project, we produced proSAAS deletion mutants to test for efficacy at blocking α-synuclein toxicity in cell culture.

Methods:

In order to prepare purified protein, recombinant proSAAS expression vectors were grown in bacteria. After inducing for protein expression with IPTG, the bacteria was incubated for 18 hours at 27°C and then centrifuged. Cell pellets were disrupted by addition of Bug Buster and lysosyme. Benzoate nuclease and PMSE were then added to break down DNA and inhibit proteases, respectively. The lysate was incubated while rocking for 15 minutes, and then centrifuged at 12,500 RPM for 20 minutes. The new sample supernatant was then affinity purified using fast protein liquid chromatography (FPLC), and collected fractions were analyzed by SDS-PAGE. Fractions with bands matching our desired proSAAS construct molecular weight were then dialyzed into 0.1 M acetic acid, concentrated by 10 kDa cutoff centrifugal filters, and diluted in 5 mM acetic acid for use in experiments.

ProSAAS residues 160-180 are critical to preventing α-synuclein aggregation. Experiment of α-synuclein fibrillation inhibition by proSAAS 21 kDa, deletion constructs, carbonic anhydrase (control), and alpha-crystallin (positive control). Each point is presented by the mean ± SEM, n=6 (*p<0.05, **p<0.01, ***p<0.001, two-way ANOVA, vs. carbonic anhydrase control).

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ProSAAS blocks α-synuclein mediated cytotoxicity. SH-SY5Y neuroblastoma cells were transfected with α-synuclein expression and LacZ control vectors. After 24 hours, they were split into a 96 well plate and treated with proSAAS, acetic acid buffer, and ovalbumin. After 48 hours, the percentage of surviving cells was measured through the WST-1 assay. Cell survival increased from 75% to 85% after proSAAS addition. Each point is represented by the mean ± SEM (*p<0.05, **p<0.01, two-way t-test).

Conclusions:

- ProSAAS residues 160-180 are essential to its anti-aggregant function.
- ProSAAS is effective at reducing α-synuclein cytotoxicity in SH-SY5Y cells.
- ProSAAS cytotoxicity is specific for α-synuclein toxicity in SH-SY5Y cells, as it has no effect on toxicity from ER or oxidative stress.

Future Studies:

- Does proSAAS interact with α-synuclein in the extracellular space to prevent cytotoxicity?
- Does proSAAS block α-synuclein oligomerization intra- and/or extracellularly?
- Does proSAAS have an effect on an increased fibrillation α-synuclein A53T mutant?
- Create a finer map of proSAAS functional domains.

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