Functional Role of Alpha-Synuclein and Dysfunction in Parkinson’s Disease

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Parkinson’s Disease as a Neurodegenerative Disorder

- The 2nd most common neurodegenerative disorder after Alzheimer's Disease
- Loss of dopamine-producing neurons in the substantia nigra pars compacta
- Characterized by loss of motor function such as uncontrollable tremor, postural imbalance, slowness of movement, rigidity
- Limited Treatment
- Age is the greatest risk factor
  By 2050, the US will have 20%
  The population over 65
- No known prevention or cures

2012. Journal of the American Academy of Arts and Sciences, 141, 98-107
Alpha-Synuclein

- α-Syn is a 140 amino-acid, soluble, presynaptic protein
- Major constituent of Lewy Bodies
- Mutations: A53T, A30P, E46K, H50Q and G51D as well as duplications and triplications of the SNCA gene will lead to disease
- Has been shown to interact with membranes both in vitro and in vivo
- Physiological function of alpha-synuclein is unknown

Lewy Bodies in Post-Mortem Human Brain Tissue

November 3, 2000 The Journal of Biological Chemistry, 275, 34328-34334
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- Has been shown to interact with membranes both in vitro and in vivo
  - 7 imperfect 11-residue repeats, each containing a variant of the consensus 6-residue sequence KTKEGV helps bind to lipids in the membrane
- Do not know the function of alpha-synuclein

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The interplay of Mitochondrial Dysfunction, Alpha-Synuclein, and Calcium In Parkinson’s Disease

- Mitochondrial Dysfunction
  - Associated with Parkinson’s Disease
  - Calcium ($\text{Ca}^{2+}$) is integral to mitochondrial homeostasis
  - A change in the concentration of calcium could cause mitochondrial dysfunction

- Relation to Alpha Synuclein
  - Our preliminary results suggests alpha-synuclein over-inflates mitochondrial interactions with calcium stores in endoplasmic reticulum

The relationship between calcium, mitochondrial dysfunction, and alpha-synuclein may contribute to Parkinson’s Disease.

Procedure

- **Transfection:**
  - Introduce foreign DNA to mammalian RBL-2H3 cells
  - Used this method to produce cells that expresses a control protein and alpha-synuclein
  - Transfected with mito-GCamp6, a marker that tracks the movement of calcium

- **Experiment:**
  - Use an antigen and IgE that sensitzes the cells so that the respond
  - Then, used ionomycin to see the maximum response
Control Cells under the Microscope: Stimulation of Mitochondrial Calcium Response (high dose of antigen no alpha-synuclein)
Control cells (low dose of antigen; no alpha synuclein)

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Alpha-Synuclein (low dose of antigen)

Cell Number | 84
---|---
Average | 76.01

Relative Intensity vs Time
V70P and Syn-1-102

Looking at the mutant forms of alpha-synuclein may give further insight into normal function of wild-type alpha-synuclein

- **V70P**
  - Point Mutation where the valine is replaced by a proline affecting its structure
  - Makes it more difficult for alpha-synuclein to bind to membranes

- **Syn-1-102**
  - Truncated form of alpha-synuclein

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Mitochondrial Responses Based on Alpha-Synuclein Membrane binding properties

Control (0.5ng/ml)  V70P (0.5ng/ml)  Syn-1-102 (0.5ng/ml)  Alpha-Syn (0.5ng/ml)

n=205  n=127  n=93  n=84
Conclusion

- After stimulation with antigen, mitochondrial calcium increases
- Alpha-synuclein increases mitochondrial calcium uptake
- V70P mutant, which disrupts helical structure of alpha-synuclein, disrupts calcium intake into the mitochondrial
- Syn-1-102 shows that the N-terminus is sufficient to cause the mitochondrial calcium increases
- This dysfunctional effect of alpha-synuclein may be related to Parkinson’s Disease
- Future Directions:
  - Determine the mechanisms by which alpha-synuclein disrupts mitochondrial calcium regulation
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Implications and Future Applications

- Mitochondrial calcium homeostasis by enhancing endoplasmic reticulum-mitochondria interactions by binding to the ER

- If alpha-synuclein can potentially disrupt the concentration of calcium, it could cause the mitochondrial dysfunction seen with Parkinson’s Disease

- Next Big Questions:
  - How is alpha-synuclein getting calcium into the mitochondria?
  - How do the mitochondria and the ER communicate and regulate the concentrations of calcium in the mitochondria?
  - Does alpha-synuclein work in conjunction with something else to cause these effects?