Molecular Pathogenesis of Parkinson’s Disease and Therapeutic Strategies

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Pathology of Parkinson’s Disease

Control  

Parkinson  

Lewy body

\(\alpha\)-Synuclein IHC of Lewy bodies
In Vitro Fibrillization of α-Synuclein

WT 300 μM
4 months

A53T 100 μM
1 month

A30P 300 μM
4 months

Conway, Biochemistry 39:2552, 2000
Staging PD: Pre-Symptomatic and Symptomatic Phases

Braak et al, Cell Tissue Res. 318:121, 2004
α-Synuclein Seeding and Propagation

Commonalities of Misfolded Proteins and Hyper-phosphorylated Aggregates in Synucleinopathies and Taupathies

<table>
<thead>
<tr>
<th>Tau</th>
<th>Neurofibrillary tangles</th>
<th>p-tau</th>
<th>p-α-synuclein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloid plaque</td>
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</tbody>
</table>

![Image showing Tau and p-τ aggregates with arrows indicating localization]
Consequences of Increased $\alpha$-Synuclein Levels in Neurons

- Misfolding and aggregation
- Permeabilization of synaptic vesicles leading to dopamine leakage
- Oxidative stress
- Disruption of vesicular trafficking between the endoplasmic reticulum (ER) and the Golgi, causing ER stress
- Interference with autophagy
- Impaired proteasome function
- Interaction with other proteins

Reducing $\alpha$-synuclein levels can be beneficial
Reducing $\alpha$-Synuclein Levels as a Therapeutic Strategy

- **Reduce production**
  - Inhibit transcription
  - Inhibit translation

- **Enhance clearance**
  - Autophagy
  - Proteasome
MicroRNA

- Small noncoding RNA molecules
- Regulate gene expression post-transcriptionally
MicroRNA-7 Reduces $\alpha$-Synuclein Protein Levels and Protects against its Toxicity

**Protein Level**

- Relative $\alpha$-Syn Expression ($\alpha$-Syn/b-actin)
- Pre-miR-7 (nM): 0, 40, 80
- * P < 0.01

**Cell Death**

- % Cell Death
- Vector, $\alpha$-Syn-3’UTR

Junn et al, PNAS, 106(31): 13052, 2009
α-Synuclein Phosphorylation as a Therapeutic Target in PD and DLB
Misfolded α-Synuclein is Phosphorylated in α-Synucleinopathies

Human DLB

LB509 Anti-p-Ser129

Fujiwara et al NCB 4:160, 2002

Mice

WT α-Synuclein\textsuperscript{Tg}

Anti-p-Ser129

Lee...Mouradian, J. Neurosci. 31: 6963, 2011
$\alpha$-Synuclein Phosphorylation Promotes its Fibrillization in vitro

Fujiwara et al NCB 4:160, 2002
Therefore,

Decreasing the Phosphorylation State of

\( \alpha \)-Synuclein is a Plausible

Therapeutic Strategy
Casein Kinase I & II
GRK1, 2, 5, and 6
Calmodulin-dependent Kinase II
Polo-like kinase 1, 2, and 3

Serine

PP2A

P-Serine

Kinase

Phosphatase
PP2A B55α is the Major Ser/Thr Phosphatase for α-Synuclein


PP2A Holoenzymes

B

Bα

B′

B″

C

catalytic

A

scaffold

PP2A (nM)

0 12 25 50 100 200

AB55αC

AB′αC

AB′γIC

AB′′C

p-S129 α-Syn

Total α-Syn

Methylation Affects PP2A-B55α Holoenzyme Assembly

Methylated PP2A is more effective in de-phosphorylating α-Synuclein

Dephosphorylate specific Phospho–proteins
An Approach to Promote PP2A Activity

Carboxyl Methylated PP2A

LCMT-1

PME-1

Demethylated PP2A

Deprophosphorylates specific target proteins

Inactive
EHT Keeps PP2A Methylated leading to De-Phosphorylation of α-Synuclein

PP2A Demethylation Inhibitor
EHT Modulates PP2A Methylation and Reduces α-Synuclein Aggregation in α-Syn Transgenic Mice

- Inhibits PP2A demethylation
- Reduces α-synuclein S129 phosphorylation
- Reduces α-synuclein oligomers

EHT Treatment Improves the Neuropathology of α-Synuclein Transgenic Mice

<table>
<thead>
<tr>
<th></th>
<th>Control-WT</th>
<th>Control</th>
<th>EHT (0.01%)</th>
<th>EHT (0.1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Syn</td>
<td>CX</td>
<td></td>
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<tr>
<td>p-Syn</td>
<td>HP</td>
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<tr>
<td>MAP2</td>
<td>CX</td>
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<tr>
<td>GFAP</td>
<td>CX</td>
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</table>

What drives hyper-phosphorylation of pathogenic proteins in α-synucleinopathies and tauopathies?
PP2A is De-Methylated in α-Synucleinopathies

Dysregulation of PP2A Methylating Enzymes in α-Synucleinopathies

PP2A is DeMethylated in Tauopathies

- Controls
- PSP
- AD

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<tr>
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<th>PSP</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me-PP2A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deMe-PP2A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total-PP2A</td>
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<td></td>
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<tr>
<td>β-actin</td>
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</tbody>
</table>

B

C

D

E

PP2A Methylating Enzymes are Dysregulated in Alzheimer and PSP

Dysregulation of PP2A Methylation Leads to Hyper-Phosphorylation of \( \alpha \)-Synuclein & tau

\( \alpha \)-Synucleinopathy / Tauopathy

- PP2A
- PME-1
- LCMT-1
- \( \alpha \)-Syn
- Tau
- PP2A-Me
- Kinases
- \( \alpha \)-Syn-P
- \( p \)-tau

\*EHT\*
Summary

- Considerable molecular similarities exist among neurodegenerative diseases of aging
- Protein misfolding and fibrillization are considered pathogenic
- Increased levels of these proteins and their hyper-phosphorylation accelerate their misfolding
- Both these factors are tractable therapeutic targets for disease prevention and disease modification