Neurodegenerative Disorders: From Molecular Mechanisms to Targeted Therapies

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Relevant Disclosures

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SAB Member, Intellia Therapeutics, Cambridge, MA

SAB Member, The Silverstein Foundation, New York, NY

SAB Member, Prevail Therapeutics, New York, NY (acquired by Eli Lilly)

SAB Member, AcureX, Palo Alto, CA

SAB Member, Leal Therapeutics, New York, NY

SAB Member, Brenig Therapeutics, San Diego, CA
Convergent and Divergent Pathways in Neurodegeneration

*Potentially Shared:*
- Accumulation of aggregation-prone proteins
- Neuro-inflammation
- Systemic (e.g. gut microbiome, systemic immunity, etc)
- Environmental/epigenetic factors (head trauma, toxins, etc)
- Age-dependent mechanisms

*Disease Specific:*
- Preferential degeneration of neuronal subtypes in different diseases

*Example of Parkinson’s disease:*
- Preferential degeneration of midbrain DA neurons--the interplay of cell-autonomous and non-cell-autonomous mechanisms
- Development of therapeutic approaches to target specific and shared pathways underlying familial and sporadic PD
## Genetic clues to the pathogenesis of PD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primary Toxic Effect of Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PINK 1</td>
<td>Mitochondrial degradation pathways</td>
</tr>
<tr>
<td>LRRK2</td>
<td>Vesicle trafficking, lysosomal dysfunction</td>
</tr>
<tr>
<td>Parkin</td>
<td>Mitochondrial degradation pathways, synaptic dysfunction</td>
</tr>
<tr>
<td>DJ-1</td>
<td>Mitochondrial dysfunction</td>
</tr>
<tr>
<td>ATP13A2</td>
<td>Lysosomal dysfunction</td>
</tr>
<tr>
<td>VPS35</td>
<td>Retromer, Lysosomal dysfunction</td>
</tr>
<tr>
<td>SNCA</td>
<td>Mitochondrial and lysosomal dysfunction</td>
</tr>
<tr>
<td>GBA1</td>
<td>Lysosomal dysfunction</td>
</tr>
<tr>
<td>SYNJ1</td>
<td>Synaptic dysfunction</td>
</tr>
<tr>
<td>Auxillin</td>
<td>Synaptic dysfunction</td>
</tr>
<tr>
<td>Synaptogenin-1</td>
<td>Synaptic dysfunction</td>
</tr>
</tbody>
</table>

Wong et al, *Nat Med*, 2017
Singleton et al, *Neuron*, 2017
Rare lysosomal disorders are linked to neurodegeneration

Lysosomal Storage Disease → Protein target → Protein aggregation pathology → Neurodegenerative disease

Gaucher Disease
- Glucocerebrosidase
- A-N-Acetylglucosaminidase
- Lewy Body
- Parkinson’s disease

Niemann-Pick Disease
- NPC
- Tangles
- Plaques
- Lewy Body
- Alzheimer’s disease

Sanfillipo B
- A-N-Acetylglucosaminidase
- Tau NFT
- Fronto Temporal Dementia

Sidransky et al., *Lancet Neurol*, 2014
Burbulla et al., *Mov Disord*, 2021
Kudo et al., *PNAS*, 2009
Gaucher’s and Parkinson’s Disease

Gaucher’s disease (GD) is caused by mutations in GBA1 that lead to deficiency of lysosomal glucocerebrosidase (GCase) and accumulation of sphingolipid substrates, esp glucosylceramides.

Mutations in GBA1 are linked to increased risk of Parkinson’s disease and Lewy body dementia (DLB) suggesting that α-syn may be involved.

Sidransky et al., NEJM, 2010
Nalls et al. JAMA Neurol, 2013
GCase deficiency and α-synuclein accumulation constitute a bi-directional feedback loop

- Mutations in GBA1 lead to decreased lysosomal GCase activity and accumulation of lipid substrates
- Accumulated sphingolipids contribute to accumulation of α-synuclein
- Elevated α-synuclein interferes with trafficking of mutant or wild-type GCase

Mazzulli et al., Cell, 2011
**Prediction**

The feedback loop leads to decreased activity of wild-type or mutant GCase in any cell that accumulates α-synuclein.

**Caveat**

This mechanism does not explain preferential vulnerability of midbrain dopaminergic neurons in PD.
Parkinson’s disease—preferential vulnerability of midbrain dopaminergic neurons

- Degeneration of DA neurons in SNc leads to Bradykinesia, Tremor, Rigidity

Surmeier et al., *Nat Rev Neurosci*, 2017
Nalls et al., *Lancet Neurol*, 2019
Vulnerability of dopaminergic neurons: the role of neuromelanin

- Neuromelanin normally accumulates with age in nigral dopaminergic neurons
- Neuroprotective role - neuromelanin is a chelator for metals, oxidized products
- No neuromelanin present in rodent brain

Sulzer et al., *J Neurochem*, 2008
Zucca et al., *Neurotox Res*, 2013
Experimental Approach

- Reprogramming of patient fibroblasts to iPSC and differentiation of iPS cells into DA neurons from genetic and idiopathic PD for time-dependent analysis of pathological phenotypes
- Gene editing by CRISPR/Cas9 for genotype-phenotype studies

![Graph showing time points and associated phenotypes]

- Age (days): 0, 50, 100, 150, 200
- Phenotypes:
  - Mitochondrial dysfunction
  - α-synuclein accumulation
  - Synaptic dysfunction
  - Lysosomal dysfunction

![Images showing control, SNCA-trp, and GD conditions]

- SSEA1, OCT4, DAPI
- TRA 1-81, NANOG, DAPI
Vulnerability of dopaminergic neurons: the role of neuromelanin and oxidized dopamine in genetic forms of PD

Burbulla et al., Science, 2017
Accumulation of oxidized dopamine in neurons from patients with other genetic forms of PD

Burbulla et al., Science, 2017
Oxidized dopamine and neuromelanin not detected in mouse models of PD

iPS-derived DA neurons from mice

<table>
<thead>
<tr>
<th>Day 40</th>
<th>Day 70</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>DJ-1 KO</td>
<td>DJ-1 KO</td>
<td>DJ-1 KO</td>
</tr>
</tbody>
</table>

Brain samples from 12 months old mice

Burbulla et al., Science, 2017
Downstream effects of oxidized dopamine – reduced GCase activity in midbrain dopaminergic neurons

Modification of cysteine on GCase by oxidized DA reduces its enzymatic activity

Enzymatic activity

Mass spectrometry
Recombinant enzyme + DA

Rescue with mitochondrial antioxidant

Burbulla et al., Science, 2017
Summary 1

- Oxidized dopamine and neuromelanin were detected in human but not mouse dopaminergic neurons.

- Mitochondrial oxidant stress and dysfunctional synaptic vesicle endocytosis contribute to increased oxidized dopamine in PD patient neurons.

- Oxidized dopamine inhibits wild-type GCase in PD patient neurons.

- The effects of oxidized DA at least in part explain preferential vulnerability of nigral DA neurons in PD.
Accumulation of oxidized DA and/or a-synuclein leads to decreased activity of wild-type GCase: therapeutic implications

Hypothesis

Direct targeting of glucocerebrosidase may improve pathogenic phenotypes in dopaminergic neurons
Development of GCase modulators for synucleinopathies


Jianbin Zheng
Activation of wild-type GCase lowers oxidized dopamine and α-synuclein in multiple forms of PD

Burbulla et al., Sci Transl Med, 2019
Ysselstein et al, Nature Comm, 2019
Allosteric GCase modulators increase wild-type GCase activity in DA neurons from patients with various forms of PD.

These modulators improved lysosomal dysfunction, lowered oxidized dopamine, α-synuclein and sphingolipids in patient neurons.

Activation of wild-type GCase may serve as potential therapeutic target for diseases that exhibit decreased GCase activity (e.g. sporadic PD, LRRK2-PD, progranulin-FTD, 84GG-GBA1, etc).

Burbulla et al., Sci Transl Med, 2019
Valdez et al, Hum Mol Genetics, 2020
Ysselstein et al, Nature Comm, 2019
Unexpected finding: a novel pathway for direct mitochondrial and lysosomal crosstalk

Yvette Wong

Wong et al, *Nature*, 2018
Increased mito-lyso contact duration and decreased axonal mitochondria in GBA1-PD

Ongoing Work: Other Functions of Mito-Lyso Contacts

① From lysosomes to mitochondria: Ca^{2+} (Peng et al., *PNAS*, 2020)

② Mitochondrial fission (Wong et al, *Nature*, 2018)

③ From mitochondria to lysosomes?

Wesley Peng
Robert Coukos
Convergence of mitochondrial, lysosomal and synaptic pathways in cell-autonomous dysfunction of human dopaminergic neurons in PD

- PINK1, Parkin, DJ-1
- oxidant stress

- oxidized DA
- α-Synuclein

- Synapse
  - Auxillin, SYNJ1, Parkin
  - SVE

- Mitochondria
- Lysosome
  - LRRK2, Park9, GBA1
  - lysosomal proteolysis

References:
- Burbulla et al., Science, 2017
- Wong et al. Nature, 2018
- Nguyen & Krainc, PNAS, 2018
- Ysselstein et al, Nature Comm, 2019
- Burbulla et al., Sci Transl Med, 2019
- Wong, Peng et al, Dev Cell, 2019
- Kim et al, Nature Comm, 2021
- Wong et al., Nat Med, 2017
- Mazzulli et al., Cell, 2011

Singleton et al., Neuron, 2017; Bingol et al., Nature, 2014; Sliter et al., Nature, 2018
Ongoing Work: Is cell-autonomous dysfunction of dopaminergic neurons modified by microglia?

1. To identify key mediators of microglial activation in patient-derived microglia

2. Co-cultures of iPS-derived neurons, astrocytes, microglia to assess the role of glia in modifying pathogenic phenotypes in DA neurons

3. Therapeutic targeting of such inflammatory mediators of neuronal damage
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