Insights into the Role of α-Synuclein as an Epigenetic Mediator in Environmental Parkinson’s Disease

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Human allele-specific methylated regions have increased resilience to rotenone exposure in α-Synuclein knockdown neurons.

Utilizing Next Generation Sequencing (NGS) and molecular assays to identify mechanisms of rotenone toxicity in a Parkinson’s disease model.
Parkinson’s disease in the United States

- Parkinson’s disease affects 1/100 people over the age of 60 in the US.
- Motor symptoms include: tremor, slowness of movement (bradykinesia), rigidity of limbs, postural instability
- Diagnosis involves:
  - presence of at least 2 out 4 motor symptoms
  - imaging tests (CT, PET, MRI, DATScan)
- Treatment involves treating symptoms and replacing dopamine.
Parkinson’s disease is caused by the death of dopaminergic neurons.

Parkinson’s disease is the result of gene-environment interactions.

- Gene-Environment Interactions alter expression of neuronal genes.
- Altered gene expression changes neural activity and behavior.
Rotenone targets the electron transport chain.
Rotenone and Parkinson’s disease

Farming and Movement Evaluation Study:

"Users of rotenone were 2.5 times more likely to develop Parkinson's as nonusers and this association was consistent even when exposure was truncated as many as 15 years before diagnosis."

Other family-based nested case control studies found that children living in environments with pesticide application also have increased risk of developing early-onset Parkinson's disease.
DNA methylation in Parkinson’s disease

- Altered DNA methylation in whole blood and post mortem brain tissues of patients.
- DNA methylation patterns in PD can be predictive of disease phenotype, stage, and symptoms.
- Targeting epigenetic machinery alters risk of developing Parkinsonism in PD models.
- There is a lack of studies on epigenetic changes due to environmentally driven PD.
We used NGS and molecular assays to accomplish these three aims:

1. To evaluate changes in DNA methylation patterns at vulnerable allele-specific methylated regions in human cells exposed to rotenone.

2. To examine the association of oxidative stress induced α-Synuclein to DNA methylation changes in rotenone treated neuronal cells.

3. To investigate resilience of α-Synuclein knockdown neurons to DNA methylation changes at vulnerable regions of the genome.
Rotenone causes widespread changes in gene expression via mRNA sequencing.

Human neuronal cell line SH-5YSY was treated with Rotenone 200nm for 24h.
Global DNA Methylation is reduced by dot blot method and CpG methylation is reduced 5% in whole genome.
Allele specific methylated regions (ASMs) are vulnerable to environmental exposures.
Rotenone changes the expression of brain-specific genes at allele-specific methylated regions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log2FC</th>
<th>FDR</th>
<th>Region Type</th>
<th>Function/Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH3</td>
<td>-1.62</td>
<td>3.31E-09</td>
<td>intragenic</td>
<td>myosin protein; cell movement and transport</td>
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<tr>
<td>PPFA4</td>
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<td>neurotransmitter release synaptic function</td>
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<td>GIPR</td>
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<td>gastric inhibitory peptide; insulin release</td>
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<td>neurofilament; synaptic function</td>
</tr>
</tbody>
</table>

Freeman et al. 2020 Epigenetics and Chromatin
These brain-specific ASMs were also hypomethylated in rotenone treated cells.

Freeman et al. 2020
Epigenetics and Chromatin
CTCF is an insulator that binds a specific motif regulates chromatin organization.

CTCF is dependent upon DNA methylation patterns at binding sites.

Environmental factors can target epigenetic patterns at CTCF binding sites and cause long range changes in genome stability.

Rotenone targeted allele-specific methylated regions with CTCF transcription factor binding motifs.
Predicted allele-specific methylated regions at CTCF binding sites in prominent Parkinson’s disease genes were hypomethylated in response to rotenone.

Freeman and Wang 2020
Frontiers in Genetics.
Reduced global DNA methylation was associated with increased CTCF binding in neurons exposed to rotenone.
2. To examine the association of oxidative stress induced α-Synuclein to DNA methylation changes in rotenone treated neuronal cells.

- Exposure
- α-Syn
- Altered DNA methylation and gene expression

- Its aggregation into Lewy bodies is a prominent feature in Parkinson's disease.

- Both genetic and environmental factors induce the formation of Lewy bodies.

- Humans that express more copies of α-Syn have a linear increased risk of Parkinson's disease.

- Mice without α-Syn are resistant to developing Parkinson's disease motor phenotypes.

https://www.ks.uiuc.edu/Research/synuclein/
2. To examine the association of oxidative stress induced α-Synuclein to DNA methylation changes in rotenone treated neuronal cells.

Hypothesis: Exposure induced α-Synuclein sequesters DNMT1 in cytoplasm, thereby depleting DNMT1 in the nucleus and further altering DNA methylation patterns.

Desplats et al, 2011, JBC
Rotenone increases intracellular ROS and damages mitochondria.

**DCFDA Fluorescence Assay**

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>Rotenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold Change ROS</td>
<td></td>
<td></td>
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<tr>
<td>Intracellular ROS</td>
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</table>

**Resazurin Absorbance Assay**

<table>
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<tr>
<th></th>
<th>DMSO</th>
<th>Rotenone</th>
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<tr>
<td>% Viable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Viability</td>
<td></td>
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</tbody>
</table>
Rotenone increases α-Synuclein accumulation by 1.5 fold. (p<0.05)
Roterone induced α-Synuclein interacts with DNMT1 in whole cell lysate.

Roterone treated SH-5YSY cells were co-immunoprecipitated with α-Synuclein antibody and probed for DNMT1 with Western.
Rotenone reduced global DNA methylation patterns and altered ASM expression.
3. To investigate resilience of α-Synuclein knockdown neurons to DNA methylation changes at vulnerable regions of the genome.

Hypothesis: α-Synuclein knockdown neurons will be resistant to rotenone induced damage by rescuing DNMT1 maintenance.
α-Syn knockdown did not have reduced mitochondrial viability or increased ROS.
α-Syn knockdown did not have decreased mitochondrial number.
1. α-Syn knockdown did not have decreased mtDNA.
α-Syn knockdown maintained even after rotenone exposure.
α-Syn knockdown increased nuclear levels of DNMT1.
α-Syn knockdown increased nuclear levels of DNMT1.
4 α-Syn knockdown enriches the expression of genes involved in the cellular defense response.

Number of genes differentially expressed per treatment group.

p<0.001
α-Syn knockdown reverses DNA hypomethylation observed at allele-specific methylated regions.

**A**

*DLK1: chr14:100808189-100809386*

**B**

**DLK1**

Delta = 0.33  
Pvalue = 8E-10

**GNAS: chr20:58851492-58852993**

Delta = 2.3  
Pvalue = 3E-07
Conclusions

- Rotenone promotes chromatin opening at vulnerable regions of the genome and enhances binding of structural reorganizers like CTCF.
- Rotenone-induced α-Syn sequesters DNMT1 from the nucleus and interferes with maintenance of global DNA methylation patterns.
- Large scale structural changes create instability in the DNA and ultimately impact neuron function and survival.
Significance to Public Health

• Provide a mechanism for gene-environment interactions in Parkinson’s disease.

• Provide insights into the pathophysiology for idiopathic Parkinson’s disease and potentially other “aggregate” diseases like dementia or Alzheimer’s.

• Provide potential targets for the development of biomarkers for screening and diagnosis of disease.

• Provide potential targets for better pharmaceutical interventions.
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