WRIGHT STATE UNIVERSITY | BIOMEDICAL SCIENCES PhD PROGRAM

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DISSERTATION DEFENSE

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PhD Candidate

"Mitochondria-dependent cellular toxicity of α-synuclein modeled in yeast"

Wednesday, April 24th, 2019

9:30 a.m.

NEC Auditorium (Room 101)

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α-synuclein is a small lipid-binding protein primarily expressed in the brain. Lewy body is a characteristic hallmark of Parkinson's Disease (PD) and is primarily composed of the misfolded α-synuclein protein aggregates. Abnormal protein aggregates of α-synuclein is also found in several other neurodegenerative disorders, Dementia with Lewy body, multiple system atrophy and Alzheimer's disease. Increased copy numbers of the SNCA gene encoding the α-synuclein protein leads to familial PD. Consistent with dosage-dependent α-synuclein proteinopathy, overexpression of SNCA has been repeatedly shown to induce abnormal aggregation of α-synuclein and cell death in both neurons and animal models. Interestingly, the aggregation and toxicity of α-synuclein can be faithfully recapitulated in a simple eukaryote, the budding yeast *Saccharomyces cerevisiae*. Using yeast as a model, several hundreds of yeast genes have been found to suppress or enhance the toxicity of the α-synuclein protein, revealing complex cellular processes involved in α-synuclein proteinopathy. Mammalian homologs of the identified yeast suppressor genes were shown to exert similar protective effect in neuronal cells and animal models, supporting the existence of conserved cellular mechanisms pertinent to α-synuclein proteinopathy in yeast. Here, we hypothesize that previous genetic screens using yeast might not have uncovered the full spectrum of modifier genes of α-synculein toxicity. This is because that all the yeast models of SNCA expression employ the *GAL1* promoter, which is activated in galactose, a fermentable carbon source for yeast. Although the strength of the *GAL1* promoter is suitable for studying dosage-dependent toxicity of α-synculein, yeast does not require all mitochondrial function, such as oxidative phosphorylation, to grow on galactose. Accumulating evidence supports a causal role of mitochondrial defects in neurodegenerative disease. The dispensable mitochondria in yeast models of α-synuclein grown on galactose represent a critical missing context of the previous modifier screens. To address this limitation, we designed an overexpression system that would allow us to study the role of mitochondria in the regulation of abnormal misfolding, aggregation and cellular toxicity of α-synuclein. Specifically, we developed a cell-based gene-expression switch employing a constitutive active mutant transcription factor, $GAL3^c$. $GAL3^c$ induces the *GAL1* promoter in cells grown in media containing other carbon source, such as raffinose. In addition, two conditions that have been associated with neuronal cell death and survival, calorie restriction and nitrogen starvation, also allow gene activation from the *GAL1* promoter by $GAL3^c$. Finally, this expression system allows us to express SNCA under mitochondria-dependent respiratory growth condition, using glycerol-ethanol as the carbon source. With this novel overexpression method, we show that α-synuclein is more prone to form cytoplasmic aggregation and to induce cell death under respiratory growth as compared to grown on galactose. We further screened a yeast overexpression library containing ~15,000 human gene clones to identify genetic suppressors of α-synuclein toxicity under respiratory growth. We found 87 such suppressors. Eight of those human-gene suppressors appear to have mitochondria-related functions. Among those eight, four 14-3-3 protein isotypes, which have been previously linked to PD, suppress the toxicity of α-synuclein only under respiratory growth. Their suppressor effect appears to be independent from blocking cytoplasmic aggregation of α-synuclein. Our study provides a new platform to identify condition-specific genetic modifiers. The identified respiratory growth condition-specific human-gene suppressors support mitochondria-dependent genetic buffering mechanisms of α-synuclein proteinopathy. Validation of these genetic modifiers in other neuronal and animal models may reveal new pathways involved in PD.