UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

Poster Session 1

MGH 241, Easel 146

11:00 AM to 1:00 PM

Insights from Worms? Developing a *Caenorhabditis elegans* Model for BRCA1 Breast Cancer Variants

Natali Michelle Shumlak, Senior, Biochemistry
Mary Gates Scholar
Mentor: Rachel Klevit, Biochemistry
Mentor: Mikaela Stewart, Biochemistry

Even though *Breast Cancer type 1gene* (*BRCA1*) was the first gene to be definitively linked to hereditary breast and ovarian cancer, much is still unknown about how *BRCA1* influences cancer risk. What is known is that certain *BRCA1* variants confer an increased risk of early onset breast, ovarian, prostate and peritoneal cancer, but there are also many patients with variants of unknown significance that have yet to be characterized. Current methods for variant characterization require arduous genetic manipulations of mammalian cell lines, which could be simplified if a homologous model organism was used. A *BRCA1* homolog has been identified in the nematode, *Caenorhabditis elegans*, indicating that the nematode could be a useful *BRCA1* model system. My preliminary results indicate that activity of BRCA1, the gene product of *BRCA1*, is conserved in the worm homolog as both proteins expressed functionally homologous ubiquitin-ligase activity when studied using purified components. In order to investigate the conserved residues in the *C. elegans* homolog that lead to the conserved activity, I am comparing the interactions of both BRCA1 homologs with the E2 enzyme (required for ubiquitin-ligase activity). Using site-directed mutagenesis, I am producing mutations at the hypothesized E2 binding site on the worm homolog that I predict will inhibit ubiquitin-ligase activity by preventing E2 binding. The impact of these mutations are being studied through biochemical ubiquitin-ligase assays using purified components. A nematode-based *BRCA1* model would allow for hypotheses developed through in vitro or in cellulo studies to be tested in a whole organism, significantly expediting variant characterization. Understanding the importance of the conserved residues between the homologs will identify key regions of BRCA1 where patient variations may be more likely to pose a risk.

Poster Session 2

MGH 241, Easel 137

1:00 PM to 2:30 PM

A Study of Red Blood Cell Derived Exosomes from Blood Plasma

Sabrina Xie, Sophomore, Pre-Sciences
Mentor: Jing Zhang, Pathology
Mentor: Tessandra Stewart, Pathology, Pathology

The presence of protein biomarkers in specific human tissues can be indicative of diseases such as Parkinson's Disease (PD). One such protein, α-synuclein (α-syn) commonly exists in brain and blood and can be transmitted between different cell types in secreted membrane vesicles called exosomes. The blood levels of α-syn are very high, and α-syn originally from red blood cells (RBCs) could be transported in exosomes. In order to study α-syn in RBC exosomes, we have collected α-syn-containing exosomes from RBCs by culturing fresh blood samples. However, RBCs degrade quickly, meaning that in the past, RBC exosomes could only be collected from fresh samples on the same day as collection. So the purpose of this work is to save time and resources from culturing exosomes by determining if they can be pulled down from previously frozen blood plasma. RBC derived exosomes could be targeted by the RBC membrane specific cell surface protein CD235a and using antibody coated superparamagnetic beads to pull down the desired exosome from samples. We hypothesize that the technique of immunoprecipitation is able to make a pure preparation of RBC exosomes which can be use for studying exosome α-syn from RBCs.

Poster Session 2

MGH 241, Easel 136

1:00 PM to 2:30 PM

Inhibitory Effect of *Acer Saccharum* Extracts on aSyn Fibrillation in Dementia with Lewy Bodies

Veena Pillalamarri, Senior, Biology (General)
Mentor: Jing Zhang, Pathology
Mentor: Tessandra Stewart, Pathology, Pathology

Aggregation of alpha-synuclein is known to be a critical step in the development of Parkinson's disease and Dementia with Lewy bodies . Dementia with Lewy bodies and Parkinson's

disease are both chronic, neurodegenerative diseases that affect motor movements and cognitive development. The aggregation of alpha-synuclein induces neurotoxicity, which contributes to disruptive synaptic behavior which results in the neurodegenerative disease. Misfolded protein structures are a result of the accumulation of aggregated aSyn that form fibrils. Recent studies have found that plant extracts from plants with longer lives have inhibitors that could decrease fibrillation of alpha-synuclein. Previous research from our lab included screening of extracts from different plants. We found that some of the plant extracts that contain molecules smaller than 3kDA had anti-aggregation activity. Acer Saccharum extracts had results that showed a similar inhibitory effect. In the present study, we used this extract to treat brain tissue of Dementia with Lewy bodies and a healthy control on tissue slides. Treatments included testing with Phosphate Buffered Saline as a control as well as using multiple concentrations of the Acer Saccharum extract. We applied Thioflavin T Fluorescence assay to quantify and analyze the relative amount of alpha-synuclein to the experimental conditions. Based on previous research, we expect the fluorescence intensity to be lower for tissue treated with the extract, especially for the higher concentration. Elucidating the inhibitory effect the plant extract has on fibrillation of aSyn can potentially lead to pharmacological developments for future therapies for Parkinson's disease and Dementia with Lewy bodies Disease patients.

POSTER SESSION 2

MGH 241, Easel 164

1:00 PM to 2:30 PM

Mortalin Prevents α-Synuclein Up-regulation and Oligomerization in Astroglia Under Manganese-Induced Toxicity

Dakota Lynn (Dakota) Hammrich, Recent Graduate, Biology (Molecular, Cellular & Developmental)
Mentor: Tessandra Stewart, Pathology, Pathology

Parkinson's disease (PD) is a progressive, neurodegenerative disease with a global and national prevalence of four million people and one million people affected respectively. Though PD can be clinically characterized by dyskinesia, resting tremors and rigidity, it remains largely idiopathic biochemically. PD patients can be pathologically characterized post-mortem however, by the presence of Lewy bodies in the Substantia Nigra pars compacta (SNpc) of the ventral midbrain. While the major protein constituent of Lewy bodies is known to be α-Synuclein (α-Syn), the pathogenesis of α-Syn fibrillation in the SNpc remains elusive. One mitochondrial chaperone protein, mortalin, is thought to play a part in PD pathogenesis for its anti-apoptotic effects, role in stress responses, and through its regulation of transcription factor p53. We previously found mortalin to be down-regulated in the corpus striatum of manganese (Mn) miners, as well as primary murine glial cells. In the study at hand, we evaluated the connection between mortalin and p53 and the resultant effect on α-Syn oligomerization in astroglia with Mn-induced neurotoxicity. Using primary astrocyte cultures, western blot analysis, immunofluorescence and confocal microscopy, we evaluated this possible connection and its implications for α-Synucleinopathy. Within Mn-induced neurotoxic conditions, we saw decreases in total p53 of cortical and striatal mortalin knock-down astrocytes and increases in the density of cytosolic oligomeric α-Syn of these cells. Notably, we found that overexpressing mortalin under the same conditions decreased the density of oligomeric α-Syn dose-dependently. Taken together, these data suggest that the interaction of mortalin with p53 could have a role in α-Syn up-regulation, resulting in oligomerization within Mn-induced neurotoxicity. Further study is necessary to elucidate mortalin's role in α-Synucleinopathy and Lewy Body pathology more precisely, but this work illuminates an important protein-protein interaction and provides implications for understanding PD pathogenesis and for developing therapies against α-Syn fibrillation in Parkinsonian diseases.