Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 241, Easel 148

11:00 AM to 1:00 PM

SUMO and Cellular Stress: Responding to Ethanol

Heather Ruth Borror, Senior, Biochemistry, Applied Music (String Instruments)
UW Honors Program
Mentor: Richard Gardner, Pharmacology
Mentor: Cory Nadel, Pharmacology
Mentor: Amanda Bradley, Pharmacology, Molecular and Cellular Biology

SUMO (small ubiquitin-like modifier) is a protein post-translational modification that has wide ranging influence on protein function. Among its many roles, SUMO is known to regulate transcription, DNA repair, and maintain cellular homeostasis under environmental stress conditions. Changes in sumoylation patterns under environmental stress have been identified, yet the specifics of these pathways remain uncharacterized. By using mass spectrometry (MS), we have identified three yeast proteins that are sumoylated in response to high levels of ethanol: Top2, Smc5, and Smc6. These proteins play a major role in maintaining DNA stability during replication and are conserved through higher eukaryotes. We have tagged these proteins with epitopes identifiable by western blot by yeast transformation, and subjected cells with these tags to ethanol stress, which allowed us to verify the hits from the MS screen. This will allow us to begin further investigation of the role sumoylation plays on these proteins. Our next goals include generating sumo deficient mutants and observing the changes in protein function within the cell after exposure to ethanol stress. As these proteins are involved in DNA stability and repair, we are notably interested in the interactions with chromatin. Through this work, we seek to gain us a better understanding of the signaling underlying adaptation to environmental stress. This research has implications for human health as it allows us to understand how cells respond to toxic levels of a recreationally encountered substance.

POSTER SESSION 1

MGH 241, Easel 139

11:00 AM to 1:00 PM

Neuronal Protein Expression in Novel HdhQ200/200 Mouse Model of Huntington's Disease

Sofia Maria (Sofia) Simonton Siegel, Senior, Neurobiology
Mentor: Jessica Cao, Pharmacology
Mentor: Nephi Stella, Pharmacology

Huntington's Disease (HD) is a fatal neurodegenerative disease that results in neurological and motor impairments that worsen after onset over a period of 10-25 years. HD is caused by an expanded polyQ gene characterized by increased CAG repeats. Specifically, HD causes degeneration of medium spiny neurons, located in the striatum. We studied the pathogenesis in a novel HD murine model containing approximately 200 CAG repeats inserted in the mouse homolog of the human HD gene, Hdh. This HdhQ200/200 mouse more closely mimics the symptomology of HD than the current mouse models, which do not completely imitate the HD phenotype seen in humans. The HdhQ200/200 model also allows for improved analysis of gene involvement in HD development. Previous models show decreases in neuronal protein expression responsible for functions including glutamate transport, cAMP regulation, initiation of glycolysis, and other similarly ubiquitous functions such as anchoring synaptic proteins. In addition, the CB1R gene, which is a significantly known early marker for the onset of human HD pathology, also shows decreased expression in previous mouse models. Decreased expression of these proteins causes widespread neuronal dysfunction, characteristic of HD symptomology. Here, we examine neuronal protein expression in this novel HdhQ200/200 model using western blotting technique to see if these proteins are similarly down regulated, indicating that this model could be an alternate method of studying HD in mice. This would provide substantiating evidence that the HdhQ200/200 mouse is an appropriate model for HD, which would allow for more accurate research into HD pathogenesis and symptomology that could help current HD patients

POSTER SESSION 1

MGH 241, Easel 128

11:00 AM to 1:00 PM

Using Label Free Dynamic Mass Redistribution to Determine the Presence of α1ARs in Colorectal Cancer Cells

Diana Tram Anh Dinh, Junior, Biochemistry
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine

Approximately 40% of over-the-counter drugs target G protein-coupled receptors (GPCRs), which are seven-transmembrane proteins that become activated when drugs, hormones, or neurotransmitters bind to the receptor. One class of GPCRs, known as adrenergic receptors (ARs), are highly targeted by the endogenous catecholamines, epinephrine and norepinephrine. ARs are comprised of three unique subtypes: α1ARs, α2ARs, and βARs. Focusing on the α1ARs, the different types are α1A, α1B, and α1D. In cancer biology, most research has focused on βARs since many people take medications that specifically target βARs. In my research, I focus on α1BARs due to a recent publication of The Hague Lab, which recently discovered α1BARs in colorectal cancer cells. Reading this publication led me to search for α1BARs in various cancer cell lines by using the Label Free Dynamic Mass Redistribution (EPIC-DMR) method. After adding specific drugs, the EPIC-DMR tracks any conformational change of individual cells to indicate the cellular response to the drugs. Using this method, I discovered α1ARs are present in HCT116 cells, a colorectal cancer cell line. In the future, I intend to determine the α1AR subtype by performing radio-ligand binding, using Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR), and looking at agonist and antagonist responses. This discovery could potentially play an important role in therapeutic drug development for many cancer patients.

POSTER SESSION 1

MGH 241, Easel 129

11:00 AM to 1:00 PM

Dynamic Mass Redistribution Analysis of U251 Brain Cancer Cells for α-1 Adrenergic Receptors

Yana Dmitrievna (Yana) Karlova, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine
Mentor: Dorathy-Ann Harris, Pharmacology

Adrenergic receptors, a family of G-protein coupled receptors, are a common target by pharmaceutical drugs for cardiovascular disease. With cardiovascular disease being the leading cause of death in the United States, adrenergic receptor targeting drugs are increasing in use. Phenylephrine has long been known to be an agonist of the specific α-1 adrenergic receptor. Past research shows that cancer cells do not contain α-1 adrenergic receptors. Although, using dynamic mass redistribution (DMR), when we introduced U251 human glioma cells to increasing concentrations of phenylephrine, we found evidence of a conformational change in the U251 cells. Other adrenergic receptor agonists (norepinephrine, isoproterenol, and clonidine) exhibited a similar response. This suggests that U251 cells may have α-1 adrenergic receptors. We hypothesize that U251 glioma cells exhibit a structural change in the presence of phenylephrine because they contain α-adrenergic receptors that recognize the ligand and trigger a cellular response. The effects of phenylephrine on U251 cancer cell viability and cell proliferation is still unknown, allowing for a possible future in generating therapies to combat cancer.

POSTER SESSION 2

MGH 241, Easel 139

1:00 PM to 2:30 PM

Antidepressant Effects of Inhibiting Lateral Habenula Output Pathways Using DREADD Technology

Paige Bartlett, Junior, Neurobiology
Mentor: John Neumaier, Psychiatry
Mentor: Kevin Coffey, Psychiatry and Behavioral Science

The lateral habenula (LHb) is a structure in the epithalamus that encodes aversive states and accumulating evidence indicates that the LHb exhibits hyperactivity during depressive-like states. Previously, we have shown that inhibition of the LHb using Gi-coupled designer receptors exclusively activated by designer drugs (DREADDS; hM4Di) can produce antidepressant effects. The LHb is known to project to several midbrain nuclei, including the ventral tegmental area (VTA), the rostral medial tegmental nucleus (RMTG) and the dorsal raphe nucleus (DRN). In this study, we seek to determine which lateral habenula output pathway may give rise to the previously observed antidepressant effect in rats. We are separately testing inhibition of the three output pathways of the LHb to the VTA, RMTG and DRN, using intersectional expression of a floxed hM4Di-DREADD in LHb neurons that project exclusively to these target regions. Using stereotaxic surgery, an AAV viral vector containing floxed-hM4Di is infused into the lateral habenula of a rat, while CAV2-CRE is injected into the respective target region. CAV2-CRE travels retrograde up the axons of neurons and produces the CRE-enzyme, allowing DREADD expression to be restricted to neurons that project from the LHb to each respective target region. After a recovery period, rats are either administered Clozapine-N-Oxide (CNO), which activates the DREADDS, or a control vehicle, and then undergo the modified forced swim test (FST), which is a validated behavioral measure of antidepressant effects in rats. High escape behavior in the FST indicates anti-depressant effects. After experimental testing, the rats are sacrificed and their brains examined histologically to confirm expression of the DREADDs within

each pathway. By identifying which LHb pathways confer an antidepressant effect, this research could lead to future treatments of depression that act directly on those pathways.

POSTER SESSION 2

MGH 241, Easel 138

1:00 PM to 2:30 PM

The Effect of Altering Serotonergic Neuron Excitability during Stress on Ensuing Emotional Behavior

Amit Galitzky, Junior, Biochemistry
Mentor: John Neumaier, Psychiatry
Mentor: Alec Gibson, Psychiatry and Behavioral Sciences

Serotonin is a key player in the regulation of anxiety, fear, and stress. While acute stress is associated with increased activity in serotonergic neurons, chronic stress is characterized by the transition to decreased serotonergic neuron activity, the desensitization of serotonin autoreceptors, and the development of maladaptive emotional behaviors. We hypothesize that chronic stress causes dysregulation of serotonergic neuron excitability, and that these adaptations affect emotional responses to future exposure to stressful and hedonic stimuli. The goal of this study is to determine if alterations to serotonergic neuron excitability can alter the effects of stress on subsequent emotional behavior. We predict that reducing or intensifying the excitability of serotoninergic neurons will decrease and increase maladaptive behavioral responses to chronic stress, respectively. We expose experimental groups of wild type and transgenic mice expressing either excitatory or inhibitory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to chronic stress. The mice undergo 5 consecutive days of social defeat stress, during which they receive injections of saline or clozapine-N-oxide (the ligand for DREADDs). Following chronic exposure to stress, behavioral responses are measured through sucrose preference, social interaction, and immobility during forced swim tests. Mice expressing inhibitory DREADDs are anticipated to exhibit greater hedonic responses (increased sucrose preference), improved sociability, and decreased immobility during forced swim, compared to wild-type mice. Mice expressing excitatory DREADDS are anticipated to show decreased sucrose preference, decreased sociability, and increased mobility during forced swim. By altering the activity of serotonergic neurons, we can potentially modify the emotional and behavioral effects of stress. This study could provide new insight into the role of the serotonergic system in behavioral responses to chronic stress, and yield new therapeutic targets for conditions such as depression and post-traumatic stress disorder.

SESSION 2I

MCNAIR SESSION - GOING MOLECULAR

Session Moderator: Ray Malfavon-Borja, OMAD

MGH 254

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Trajectory of Change in sST2 Following LVAD Implantation

Sarah Therese Florig, Junior, Biochemistry, Portland State University
McNair Scholar
Mentor: Beth Habecker, Oregon Health & Science University
Mentor: Quin Denfeld, Oregon Health & Science University

Heart failure (HF) is a serious medical epidemic, and the number of those suffering from end-stage, advanced HF continues to rise along with the use of long-term left ventricular assist devices (LVADs) as treatment. Despite improvements in LVAD technology and clinical management, patient response remains significantly heterogeneous with variable predicted outcomes based on measurement of traditional biomarkers. Soluble suppression of tumorigenicity-2 (sST2) is upregulated in HF due to myocyte stretching and fibrosis during disease progression; this protein, as a novel biomarker, may provide insight into pathophysiologic changes that occur following LVAD implantation and potentially help predict patient response. More comprehensive patient selection would reduce the use of unfavorable and expensive surgery, saving money, and lives of those suffering from HF. Thus, we aim to quantify changing levels of plasma sST2 in adult patients who have undergone LVAD implantation. Plasma samples were collected from 100 individuals prior to surgical implantation of a LVAD and at 1-, 3-, and 6-months post-operatively. Levels of sST2 will be measured in all available plasma samples using commercially-available quantitative sandwich monoclonal ELISA kits (Critical Diagnostics, San Diego, CA). Data will be analyzed using repeated measures ANOVA and modeled using growth modeling approaches, as appropriate. We anticipate that plasma sST2 levels will significantly decrease from pre-LVAD to 6-months post-LVAD in this cohort of patients. Moreover, we anticipate discovering differing trajectories of sST2 change that will help differentiate who will do better or worse following LVAD implantation. Our findings may support use of sST2 as a clinically relevant biomarker in HF patients following LVAD implantation. Incorporating sST2 into the model of response to LVAD implantation could lead to improved outcome prediction to LVAD implantation.

POSTER SESSION 3

MGH 241, Easel 142

2:30 PM to 4:00 PM

***c-Fos* Studies in a Mouse Model of Leigh Syndrome**

Jennifer Manyu (Jennifer) Wong, Senior, Biology (Physiology)
Mentor: Franck Kalume, Neurological Surgery and Pharmacology, UW/ Seattle Children's

Leigh syndrome (LS) is an infantile necrotizing brain disorder associated with progressive neurological deterioration of the central nervous system (CNS), and is caused by the loss of *Ndufs4*. The *Ndufs4* gene codes for the iron-sulfur protein 4 subunit of Complex I (NADH dehydrogenase) in the electron transport chain. The absence of *Ndufs4* causes deficiency in Complex I, which negatively impacts mitochondrial energy production and results in symptoms associated with LS. One of the symptoms LS patients experience is seizure activity. Seizures can be caused by inhibition of inhibitory neurons, resulting in hyperexcitibility of neurons. In this study, we sought to identify brain regions that are involved in the generation of seizure activity in a mouse model of LS using *c-Fos* immunocytochemistry. *c-Fos* proteins are activated by seizures, and therefore are treated as metabolic markers for tracking seizure pathways. The LS mouse model used is homozygous (Hmz) Ndufs-floxed crossed with Gad-Cre mice, which selectively removes the *Ndufs4* gene in GABAergic inhibitory interneurons. We induce thermal seizures in LS mice using a heating lamp. Sham animals, of the same corresponding genotype, are processed in the same protocol but are not exposed to the heating lamp. The mice are perfused 90 minutes after the start of seizure activity, and the brain tissues are extracted and fixed with PFA. Fixed brains are sliced, and the slices are stained and imaged on a confocal microscope to map out sites of *c-Fos* immunoreactivity. We anticipate that our results will show that Hmz Ndufs/Gad-Cre(+) mice with thermal-induced seizures will express elevated levels of *c-Fos* in the hippocampus, thalamus, and cortex. The results will provide insights on potential treatment drugs targeted at these specific brain regions in LS patients.

POSTER SESSION 3

MGH 241, Easel 141

2:30 PM to 4:00 PM

Influence of Cardiorespiratory Coupling on the Risk of Sudden Unexpected Death in a Mouse Model of Dravet Syndrome

Sandy Liang, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Franck Kalume, Neurological Surgery and Pharmacology, UW/ Seattle Children's

Sudden unexpected death in epilepsy (SUDEP) is the most common type of death in people with intractable epilepsies, including Dravet syndrome (DS). DS is a treatment-resistant infantile-onset epilepsy syndrome with comorbidities of cognitive impairment and premature death. DS is caused by a heterozygous loss-of-function mutation in SCN1A, the gene encoding the α subunit of the type I voltage-gated sodium channel $Na_v1.1$. Cardiovascular dysfunctions have been identified as the main causes of SUDEP. Recent studies have indicated that changes in cardiorespiratory coupling can indicate signs of disease and predict disease susceptibility, such as schizophrenia. We used the established mouse model of DS, which carries a global knock out of Scn1a, and conducted an examination of cardiac and respiratory functions. We recorded video recordings, electroencephalogram (EEG), electrocardiogram (ECC), whole body plethysmography, and LabChart Software 8.0 (AD Instruments) in freely moving DS and wild type (WT) control mice. We then identified and characterized the defects in cardiorespiratory coupling strength associated with SUDEP risk in the DS mice. We hypothesize that cardiorespiratory coupling of DS mice, compared to the WT mice, is disturbed and results in increased complexity between the heart rate and respiration. Findings from these studies may indicate that cardiorespiratory coupling parameters can be used as biomarkers of susceptibility to sudden death in intractable epilepsies and in other severe neurological disorders.