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**Bioinformatics:** Shakirov

**Cancer Research:** Amin;Dasgupta**;** Koc; Salisbury; Santanam; Sollars; Valentovic; Varney

**Cardiovascular Research:** Bihl;Li;Pierre;Rorabaugh; Santanam; Tian; Wang

**Diabetes:** Bihl; Kim

**Drug Action, Metabolism, and Resistance:** Amin; Morgan;Santanam;Valentovic

**GI Research:** Arthur; Lu

**Genetic Research:** Kim; Shakirov; Sollars

**Infectious Diseases:** Bogomolnaya; Long; Varney; Yu

**Neuroscience/Sensory Research:** Grover; Mitra; Morgan; C. Risher; L. Risher

**Obesity Research:** Arthur; Kim; Salisbury; Santanam; Varney

**Renal Research:** Rankin; Valentovic

**Toxicology Research**: Rankin; Valentovic

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1. **Investigating the mechanism of drug resistance**

Cancer is the second leading cause of deaths in the United States accounting over 600K deaths per year. Intrinsic as well as acquired resistance to anti-cancer drugs are continuously posing challenges toward the success of cancer treatments. Understanding the molecular mechanism of drug resistance is the key to overcoming drug resistance and developing new treatment regimen using combinatorial approach. Keeping this objective upfront, my laboratory is utilizing *in vitro* cell culture and *in vivo* animal models of lung and head and neck cancers to better understand the molecular mechanism of resistance to targeted therapy. My laboratory has identified that some head and neck cancer cell lines overexpress p-Met downstream of Src family kinases (SFK) and these cell lines are resistance to apoptosis induced by the combination of erlotinib (EGFR inhibitor) and BKM120 (PI3K inhibitor). Addition of SFK inhibitor or Met inhibitor sensitizes these cells to apoptosis. The laboratory is currently exploring the downstream targets of SFK-Met signaling.

1. **Chemoprevention using natural compounds**

Chemoprevention means pharmacological intervention before the development of invasive cancer (full blown cancer) at a precancerous stage with the hope to slow down or reverse the carcinogenesis process. However, drug-associated toxicity is one of the major concerns in using drugs in prevention settings since the recipients of the chemopreventive drugs are normal subjects with high risk for developing cancer. Therefore, those agents with non-toxic or minimal side effects would be ideal candidates as chemopreventive agents. Because of their proven high safety margin through centuries of human consumptions as food or as traditional medicines, natural compounds present in fruits, vegetables and spices have drawn special attention for chemoprevention. With this objective in mind, my laboratory is investigating the potential of diet derived natural compounds such as green tea EGCG, luteolin, resveratrol, curcumin etc. for chemoprevention of head and neck cancer and lung cancer. The project includes testing of *in vitro* and *in vivo* efficacy as well as exploring their molecular mechanism of action.

These projects will provide opportunities to learn:

* Mammalian cell culture and maintenance
* Cell proliferation and cell death assays
* RNA and plasmid DNA isolation
* Gene expression analysis by PCR
* Protein expression analysis by western blotting
* Flow cytometry analysis
* Measuring protein and nucleic acid concentrations

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**Regulation of intestinal bile acid absorption in obesity.**

Obesity is known to increase the risk of many debilitating diseases, including cardiovascular and cerebrovascular diseases, non-alcoholic fatty liver disease, type 2 diabetes, and metabolic syndrome. Excess dietary energy intake, especially fat intake, is known to be one of the primary causes of obesity. In obesity, excessive dietary fat assimilation is known to be facilitated by increased bile acid (BA) levels in the intestine. Apical sodium-dependent BA cotransporter (ASBT; SLC10A2) located in the brush border membrane of absorptive villus cells in the terminal ileum is the major transporter responsible for BA absorption in the intestine. However, how ASBT may be affected in obesity is unknown. To date, our studies in *in vivo* obese rat models have demonstrated that ASBT is stimulated not only at the cellular level, but also along the crypt-villus and caudal-oral axes in small intestine. This increase of ASBT at three levels in the obese intestine, undoubtedly increases net BA absorption and subsequently likely contributes to enhanced fat absorption in obesity, indicating that altered ASBT regulation may be central to the pathogenesis of obesity. The ongoing research involves understanding the physiological and molecular regulation of intestinal bile acid absorption mediated by ASBT in obese intestine using *in vivo* rat models of obesity. Better understanding of the regulation of bile acid absorption that directly affects lipid absorption in obesity may result in novel and efficacious treatment modalities.

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The Bihl lab studies the role of extracellular exosomes (EXs), stem cell therapy, and the renin-angiotensin system in ischemic stroke, hemorrhagic stroke, and diabetic vascular complications. Our goal is to develop new therapeutical avenues addressing cerebrovascular diseases based on stem cells and their-released exosomes. The research approaches include transgenic mouse models in combination with animal surgeries, such as telemetric probe implantation for recording blood pressure and heart rate, minipump/microinjection for chronic/acute drug administration, and animal modeling for MCAO-induced ischemic stroke and brain injection for hemorrhage stroke.

**Project 1. The role of exosomes in strokes and diabetes.**

Exosomes (EXs) are small vesicles of cellular membrane released from almost all types of cells in response to physiological and pathological stimuli. EXs represent a novel way of cell-to-cell communication by transferring their molecular signatures (proteins and miRs) to target cells and tissues. Thus, extracellular EXs could be the novel therapeutic target or avenue for stroke; and could be the biomarkers for stroke and diabetes patients. These studies emphasize the protective effects of EXs derived from endothelial progenitor cells (EPC-EXs) on vascular cells and neurons. We have discovered the function of EXs from different origins by carrying different molecular signatures. We also have established the approach to identify the size, concentration, and origins of EXs by using the Nanosight Tracing Analysis system (NTA). Our recent study reported that EPC-EXs could provide the therapeutic effects on ischemic stroke by alleviating the acute injury and promoting long-term neurological function recovery. Further studies are needed to investigate the mechanisms that are related to their carried proteins and miRs, such as miR-126, miR-210, etc.

The approaches used for this project include *in vitro* cell culture and *in vivo* animal models. For the *in vitro* model, vascular cells (endothelial cells and smooth muscle cells) and neuronal cells (neurons and astrocytes) under hypoxia/reoxygenation condition (*in vitro* stroke model) or oxHb stimulation (*in vitro* hemorrhage stroke model) will be treated with a different type of EXs. For the *in vivo* model, we have middle cerebral occlusion (MCAO) surgery to induce ischemic stroke, and brain microinjection model to induce a hemorrhagic stroke. After surgery, the mice will be treated with different types of EPC-EXs. Neurological behavior will be tested before collecting the brain samples for further analysis. Moreover, we have human blood samples from stroke and diabetes patients. These samples will be used to identify the biomarker for the outcome of these diseases by using NTA.

**Project 2. The protective role of ACE2/Ang-(1-7)/Mas in strokes and ageing by counteracting the effects of ACE/Ang II/AT1.**

The renin-angiotensin system (RAS) participants in the pathogenesis of stroke, primarily through the actions of the vasoactive peptide angiotensin II (Ang II) and its pathway ACE/Ang II/AT1. Angiotensin-converting enzyme 2 (ACE2) is a homolog of ACE that is abundantly expressed in the cardiovascular-related areas of the brain and blood vessels. The primary function of ACE2 is to metabolize the deleterious Ang II into Ang-(1-7), a heptapeptide with vasoprotective actions. ACE2/Ang-(1-7)/Mas, a newly identified member of RAS, has been demonstrated to counteract the effects of ACE/Ang II/AT1. Therefore, activation of the ACE2/Ang-(1-7)/Mas pathway might represent a novel target and strategy for treating strokes. Our previous publications demonstrate that ACE2 and Ang-(1-7) protects the brain from ischemic and hemorrhagic stroke. These studies also discuss the protective effects of ACE2 on EPC function and how ACE2 improves the therapeutic efficacy in ischemic stroke. We recently found that ACE2 be carried by EPC-EXs and provide an additive beneficial effect on ageing cells by decreasing apoptosis and promoting cell viability. Further studies are needed to verify the protective effects of ACE2 on ageing animal models. In addition, the modulation of exercise on the balance of these two pathways and its implications in strokes will be studied.

The approaches for this project include *in vitro* cell culture and *in vivo* animal models as well. For the *in vitro* model, ageing cells induced by Ang-II treated with EPC-EXs w/wo ACE2 overexpression. For the *in vivo* model, we have the Renin-transgenic hypertensive mice. We also have mice with age over six-month-old. The mice will be treated with different types of EPC-EXs.

**Project 3. The role of exercise in vascular diseases by modulating the concentration and contents of exosomes.**

It is well-known that exercise induces beneficial impacts on vascular diseases and diabetes. We have confirmed that long-term exercise could provide beneficial effects on blood pressure, body weight, and diabetic metabolic parameters. Recent studies suggest that exercise could modulate the levels of circulating exosomes and miRs, which are known to be majorly carried by EXs. We have recently demonstrated that exercise could modulate the level and contents of circulating EPC-EXs. Further studies will focus on the effects of exercise on different organs, such as adipose tissue, vessels, and the brain through the communication of EXs. The correlations of the level and contents of EXs after exercise with the outcome of strokes and diabetes will also be explored.

The approaches for this project will test the effects of long-term exercise on different animal models, such as hypertensive and type-2 diabetic mice.

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Our research focuses on characterization of molecular mechanisms utilized by Gram-negative bacteria *Salmonella enterica* ser. Typhimurium and *Serratia marcescens* to survive host response and to develop better treatment options. In particular, we are interested in the following important questions:

**Project #1:** **Identification of natural functions of drug efflux pumps during infection**

Non-typhoidal *Salmonella enterica* serotypesincluding Typhimurium are the leading cause of bacterial food-borne enteritis in the United States. Until recently, *Salmonella* isolates were highly susceptible to most of the commonly used antibiotics but in the last decade the emergence of multidrug resistant *Salmonella* has been reported worldwide. *Serratia marcescens* is an opportunistic pathogen with increasing clinical importance. *S. marcescens* can cause meningitis, endocarditis, infections of airway and urinary tract, especially in immune-compromised patients. Efficiency of antibiotic therapy for these patients in some cases is extremely low due to the high intrinsic antibiotic resistance of *S. marcescens.* One mechanism for resistance of bacteria to antibiotics is through antibiotic efflux via multidrug efflux pumps. However, little else is known about the natural functions of these pumps during infection. We found that at least one pump called MacAB present in both bacterial species protects them against reactive oxygen species (ROS). We are interested in identification of natural substrates of this pump; how these substrates protect bacteria from ROS and how substrate production is regulated. Our studies will advance our understanding of the natural functions of bacterial efflux pumps beyond excretion of antibiotics and will aid to develop alternative strategies to control bacterial infections and augment conventional antimicrobial therapy.

**Project #2: Defining the role of secreted DUF1471-containing proteins in adaptation of bacteria to different environments**

Bacteria are able to successfully exist in ever-changing environment. For a quick adaptation to a new niche, bacteria rely on secondary metabolites, peptides and secreted proteins. These molecules can participate in a number of important biological processes: signal transduction within population, production of new compounds (for example, antibiotics), formation of biofilms, and also play an important role in virulence. Gram-negative bacteria from *Enterobacteriaceae* family secrete in the environment a number of proteins containing DUF1471 domain with unknown function and a similar structure. The physiological role of these proteins in maintaining of bacterial viability remains unexplored. We hypothesize that bacteria utilize DUF1471-containing proteins as a network of signals to accelerate adaptation to a new environment. Our studies will be focused on identification of DUF1471-containing proteins needed for survival during infection and during antibiotic exposure.

WV-INBRE participants will receive training in standard microbiological techniques, molecular cloning, generation of mutants, DNA and protein analysis, and animal handling.

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The following projects are available in my laboratory:

1. **Anti-cancer activity of nicotinic antagonists in lung cancer:** Smoking bears a strong correlation to the development of a type of lung cancer called lung adenocarcinoma. In our laboratory we study the signaling pathways of how nicotine and NNK (components of cigarettes) promote the growth of lung cancer. Specifically, students working on this project will examine whether compounds which block the effect of nicotine can be useful for lung cancer therapy. Other techniques the students will learn are (i) to measure the effects of nicotine on the growth of human lung cancer cells (ii) the measure the anti-cancer activity of compounds (that inhibit the effects of nicotine) in human lung adenocarcinoma.
2. **Capsaicin and small cell lung cancer:** Capsaicin is the major active ingredient ofchilli peppers. Preliminary data in our laboratory shows that capsaicin can inhibit the growth of human small cell lung cancer cells. We are interested in investigating molecular pathways contribute to this process. If you are interested in this project, you will learn (i) to perform specific assays to determine whether capsaicin can cause cell death in human small cell lung cancer cells (ii) to examine the biochemical mechanisms underlying this growth-inhibitory activity of capsaicin.

**TECHNIQUES:**

The techniques that are routinely performed in our laboratory:

1. Cell culture techniques
2. Preparation of lysates, nuclear, membrane and cytosolic fractions
3. Assays to study cell growth and cell cycle progression
4. Detection of proteins using Western Blotting
5. Measurement of tumor angiogenesis.
6. Animal studies: anti-cancer studies on nude mice models

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**Systems genetics and recombinant inbred mouse panels for discovery of the mechanisms driving drug addiction**

Drug addiction is a critical public health issue with genetic and environmental causes for which the underlying biological mechanisms remain largely unknown. To uncover these mechanisms, the Dickson Lab uses construct-valid behavioral techniques within the context of a systems genetics approach. Systems genetics using experimental mouse populations enables discovery of novel genetic and genomic mechanisms influencing disease by associating genetic and phenotypic variation. The intravenous drug self-administration paradigm is the gold-standard of volitional drug use assessment in rodents due to its ability to index drug taking and seeking at many stages of drug use including initiation, maintenance, and relapse. Through integration of a systems genetics approach and construct-valid behavioral techniques such as intravenous drug self-administration, novel and unexpected genetic mechanisms underlying the complex psychological phenotype of drug addiction and behaviors that predict drug use and addiction can be discovered.

Students in the Dickson lab can expect to learn about:

* Systems genetics as an approach to biological discovery
* The importance of genetic diversity in the laboratory mouse in the context of systems genetics
* The use of recombinant inbred mouse panels in the context of systems genetics
* Intravenous drug self-administration as an approach to identify biological and psychological mechanisms driving addiction

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***Central nervous system consequences of prenatal opioid exposure.***  Fetal exposure to opiate drugs due to maternal drug use has both immediate and long-term consequences due to effects on the central nervous system. Immediate consequences include neonatal abstinence syndrome (NAS), which is caused by drug withdrawal after birth. Symptoms of NAS may include hyperexcitability, high-pitched cry, tremor, diarrhea, vomiting, sweating, rapid breathing, feeding and sleep problems, and seizures. The symptoms of NAS reflect disturbances in nervous system development and function as a consequence of opiate exposure. Infants exposed prenatally to opiates also have a long-term risk for neurobehavioral problems lasting at least through childhood. The mechanisms through which prenatal opiate exposure produces these adverse consequences are still poorly understood. We are investigating the hypothesis that prenatal opiate exposure alters the development of central nervous system white matter through effects on oligodendrocyte differentiation and myelin formation. The resulting deficits in CNS white matter may contribute to both immediate symptoms of NAS and longer-term alterations in the nervous system and behavior.

***Methods and instrumentation.*** Students participating in this project will have the opportunity to learn animal handling, techniques for *in vitro* analysis of neuron function (dissection of CNS tissue samples, electrophysiological measurement of neural functions), techniques for measuring protein expression (Western blotting, enzyme-linked immunosorbent assay or ELISA), and techniques for analysis of emotional and cognitive behaviors in laboratory animals. For tissue preparation and analysis of neural function, we use *in vitro* incubation chambers, micromanipulators, amplifiers, stimulators, and oscilloscopes; data is collected and analyzed using software running on personal computers. For Western blotting and ELISA we use gel electrophoresis equipment, chemiluminescence, a digital imager, and a plate reader. Animal behavior is recorded and analyzed using a computer-based animal tracking and measurement system. Students will use standard lab equipment (scales, pH meter, osmometer, pipettors, sonicator, centrifuge, etc) for preparing solutions, reagents, and samples.

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**Examining the effect of flavors on vaping-related behaviors**

While nicotine is the primary addictive component of all tobacco and vaping products, flavor additives have now been found to alter the neurons that are critical for addictive behaviors. As the use of electronic cigarettes continue to grow, it is critical that we understand how all constituents of vaping e-liquids effect the neurons that mediate addiction. The Henderson lab directly studies how nicotine and flavors trigger addiction. We use mice that are trained to use vaping devices to model human smoking and vaping behaviors. In these experiments we directly study how combining flavors with nicotine can increase drug reinforcement and vaping initiation. We then conduct follow-up experiments to examine changes in neurobiology and neurophysiology. These include the use high-powered fluorescence microscopy to examine structural changes in the dopamine neurons that play a major role in addiction neurocircuitry and electrochemistry to examine functional changes in the release of dopamine in the brain. Together, these experiments allow us to determine how entire brain circuits are modified by vaping constituents and trigger changes that reinforce vaping-related behaviors. For more information, visit the Henderson lab website: [www.hendersonlab.org](http://www.hendersonlab.org)

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**Genetics of Obesity, Type 2 Diabetes, and Hyperlipidemia**

My research interest is in understanding the etiology and pathogenic mechanisms underlying type 2 diabetes, obesity, and hyperlipidemia, which have strong implications for cardiovascular diseases (CVD). Type 2 diabetes is the most common form of human diabetes, accounting for over 90% of cases and obesity at such epidemic proportions creates serious public health problems. The prevalence of atherogenic dyslipidemia including hypercholesterolemia has increased considerably. Atherogenic dyslipidemia is causally linked to the development and progression of atherosclerotic CVD. There is substantial evidence demonstrating that genetic factors are strongly involved in the development of type 2 diabetes, obesity, and hyperlipidemia, and I have focused my attention on the link between gene dysfunction and these diseases and its interaction with diets. As an internship project in our laboratory for the WV-INBRE Summer Research Program, I propose to study candidate genes and pathways for diabetes, obesity, and hyperlipidemia loci identified in a genetic mouse model and their interactions with diets. This study will ultimately provide ready targets for the disease therapies in humans. Experimental methods involved in this internship research will include enzyme-linked immunosorbent assay, colorimetric assay, polymerase chain reaction (PCR), western blot analysis, and real-time quantitative PCR. DNA, RNA and protein will need to be isolated from mouse tissues. Instruments involved in this project include gel electrophoresis, western blotting apparatus, microplate readers, spectrophotometer, imaging system, thermal cyclers, EchoMRI, and comprehensive lab animal monitoring system.

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SUMMER RESEARCH PROJECTS

Ovarian cancer, worldwide, is the most common cause of gynecologic cancer death. Primary treatment consists of a combination of surgical and platinum-based therapy. Despite success in attaining remission in many cases of ovarian cancer, over half of women with ovarian cancer experience of recurrence with chemoresistance and metastasis, specifically in high grade serous ovarian cancer (HGSOC). Interestingly, mitochondrial dysfunction is emerging as one of the major contributors of aggressiveness and chemoresistance in HGSOC due to its central role in energy metabolism.

Energy requirements for tumor growth in epithelial high-grade serous ovarian cancer (HGSOC) are fulfilled by a combination of aerobic glycolysis and oxidative phosphorylation (OXPHOS). Although reduced OXPHOS activity has emerged as one of the major contributors to tumor aggressiveness and chemoresistance, up-regulation of mitochondrial antioxidant capacity has been shown to be required for matrix detachment and colonization into the peritoneal cavity to form malignant ascites in HGSOC patients.

To evaluate modulation of OXPHOS in HGSOC tumor samples and ovarian cancer cell lines, we will perform:

1. Proteomic analyses of proteins involved in mitochondrial energy metabolism and biogenesis and formation of reactive oxygen species (ROS) by immunoblotting and flow cytometry, respectively.
2. Cell culture studies using drugs directed against mitochondrial targets such as those involved in transcription and translation machineries.

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**Project 1. Examine the mechanistic role of thymidine phosphorylase in thrombosis.**

Our recent study revealed, for the first time, that thymidine phosphorylase (TYMP), an enzyme in the DNA salvage pathway, plays important mechanistic roles in facilitating platelet activation and thrombosis (Li et al. Circ Res. 2014, Belcher et al. ATVB 2021). Thrombosis, namely, clot formation in the vascular system, is a fatal complication for many diseases, which causes myocardial infarction, stroke, or pulmonary embolism. Various anti-platelet and anti-thrombotic drugs have been developed; however, in addition to that they cause severe side effects, such as bleeding, some patients do not respond to those drugs well. Therefore, developing novel mechanism-mediated anti-platelet and anti-thrombotic therapy is a top priority. We believe that TYMP is a promising target because TYMP deficiency significantly inhibits arterial thrombosis but does not disturb systemic hemostasis. Furthermore, TYMP inhibitor has been approved by the FDA for clinical use, which makes it possible to be repositioned as a novel and systemically safe anti-platelet drug. For this, it is first necessary to elucidate the detailed mechanistic pathways of TYMP in platelet activation and thrombosis. Our hypothesis is that *TYMP plays an important mechanistic role in platelet activation via signaling pathways involving platelet glycoprotein VI (GPVI) and G-protein coupled receptors (GPCRs)*.

In this project, we will use basic laboratory techniques including cell lysate preparation, protein concentration quantification, Western blotting and immunohistochemistry, as well as platelet aggregation assay, flow chamber assay, among others. The intern will have opportunities to participate in platelet isolation, stimulation, cell culture, cell lysate preparation, measurement of protein concentration, Western blot assays, etc.

**Project 2. Examine the mechanistic role of thymidine phosphorylase in obesity and atherogenesis.**

Dysregulated lipid metabolism and chronic inflammation have been recognized as key contributors to the development of obesity and atherogenesis. All of these are risk factors for thrombosis, which leads to myocardial infarction and stroke. TYMP is an enzyme in the pyrimidine salvage pathway. Our recent study revealed, for the first time, that TYMP possesses signaling functions and is essential for platelet activation and thrombosis, suggesting a novel function of TYMP in the cardiovascular system. TYMP is present in the lipid rich core of human atherosclerotic lesions, yet its function remains unknown. By searching Gene Expression Omnibus, we have found that TYMP is potentially correlated with the development of obesity, which is supported by our preliminary studies. We will continually clarify how TYMP regulates lipid metabolism, obesity, and atherogenesis.

In addition to the basic laboratory techniques mentioned in project 1, students involved in this project will have opportunities to learn histological study including tissue sectioning, staining, and quantitative analyses etc.

**Project 3. Examine the mechanistic role of thymidine phosphorylase in the development of severe COVID-19.**

As mentioned in project 1 and 2, we have found that thymidine phosphorylase (TYMP) plays important mechanistic role in platelet activation, thrombosis, as well as in the development of obesity. Obesity is one of the high-risk cohorts for developing severe COVID-19, which is also characterized by combination of thrombotic events. Studies from different groups, including our lab, have demonstrated that TYMP is significantly increased in the plasma and in the lungs of COVID-19 patients, and the increase of TYMP is positively correlated with the disease severity. Studies conducted in our lab also demonstrated that TYMP was significantly increased by SARS-CoV-2 spike protein and its receptor binding domain, and TYMP inhibition attenuated SARS-CoV-2 spike protein enhanced thrombosis. We hypothesize that the SARS-CoV-2 spike protein-increased TYMP expression plays an important mechanistic role in the development of severe COVID-19.

Students involved in this project will have opportunities to evaluate platelet function, thrombosis, lung inflammation, western blotting of inflammatory markers and signaling transduction molecules, etc., using tissues harvested from animal models as well as in vitro cell culture.

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Since 2013, my lab at Marshall University has focused on discovering new treatment strategies for multi-drug resistant infections. We are currently investigating the use of disulfiram (Antabuse) and other FDA-approved drugs as antibiotic adjuvants for the treatment of vancomycin-intermediate *Staphylococcus aureus* (VISA) infections. It was discovered that disulfiram reduces the MIC of vancomycin in VISA to levels observed in vancomycin-susceptible MRSA. Mechanistic studies suggest that disulfiram and its primary metabolite disrupt energy production in MRSA and cause perturbations in bacillithiol-mediated redox homeostasis. Following discovery that disulfiram decreases intracellular bacillithiol levels, we are now evaluating its potential therapeutic application to counteract the resistance mechanism employed by Gram-positive and Gram-negative bacteria to inactivate fosfomycin. Researchers will learn techniques to evaluate antimicrobial synergy between disulfiram and other agents using the checkerboard assay and time-kill studies. Hands-on experience using plate readers and flow cytometer may also be employed for additional studies on pharmacodynamic interactions.

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**Peroxisomes and metabolic liver disease**

Chronic alcohol and high fat diet consumption may cause metabolic liver disease designated alcohol-related liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), respectively. Both ALD and NAFLD range from simple steatosis (fatty liver) to steatohepatitis (liver inflammation), fibrosis and cirrhosis, and even liver cancer. Fatty liver is benign, but it is sensitive to developing to advanced liver disease like fibrosis, cirrhosis and liver cancer. Impaired fatty acid oxidation is one of major reasons for the development of fatty liver. Fatty acids are mainly oxidized in mitochondria, but they can also oxidize in peroxisomes. Usually, very long chain or side chain fatty acids are metabolized in peroxisomes, and the resultant short chain fatty acids will be further oxidized in mitochondria. Peroxisomal fatty acid oxidation is regulated by a transcriptional factor peroxisome proliferator activated receptor α (PPARα), and PPARα agonist WY-14,643 can induce peroxisome proliferation, which enhance peroxisomal fatty acid oxidation, and ameliorate alcoholic fatty liver. We are examining how peroxisome proliferation influence the development of metabolic fatty liver in mouse model.

In this project you will learn about scientific experiment design, data collection and analyses, and result interpretation. You may practice the following techniques: general protein quantification, specific protein detection by immunohistochemistry staining, Western blot analysis, or enzyme-linked immunosorbent assay (ELISA), liver disease judgement by biochemical assays (serum levels of ethanol, lipid, glucose, and ALT) using spectrophotometers and histopathology (liver section H&E staining) using microscopes.

**Dr. Swarup Mitra**

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The main theme of my research laboratory is to examine sex specific epigenetic and circuit plasticity underlying heroin induced neuroadaptations. There are three primary questions that we intend to examine under this theme.

**1) Identify the role of dorsal striatal projections underlying heroin induced behavioral plasticity**

The striatum forms a critical neural substrate underlying habitual behaviors and is often implicated in reinforcement learning and the development of compulsive habits, a behavioral characteristic of heroin addiction. Habit formation has mostly been attributed to the DS and time-dependent intensification of habit formation is thought to occur due to neuroadaptations in the DS. Dorsal striatal (DS) sub-structures (dorsolateral striatum-DLS and dorsomedial striatum-DMS) receive inputs from several essential brain areas including the thalamic nuclei (TN) and prefrontal cortical (PFC) regions. Selecting and generating appropriate actions for execution of a behavior involves integrating associative and sensory information. The DS, TN, and the PFC structures form a key hub where such integration occurs. We will examine how projections from TN and PFC substructures to DLS and DMS mediate heroin-induced behavioral plasticity.

**2) How does controllable versus uncontrollable stress influence heroin relapse**

Stress plays an important role in drug use and relapse. Specifically, daily life stress during abstinence is linked to increased craving for heroin in individuals with OUD, which is predictive of higher risk of relapse. Most importantly, emotional response over a stressful experience is an important determinant of behavioral adaptation to future stressful events. Modulation of stress responses to stressful experiences is a common pathology observed in several psychiatric diseases. The control dimension has most often been examined by using an escapable (controllable) versus non-escapable (non-escapable) foot shock paradigm. While it is well established that the DS mediates stimulus-response learning, the role of DS in influencing freezing or escape responses to aversive stimuli (foot shock) is less understood. We will examine how projections in DS substructures (DMS and DLS) modulate aversive learning in a controllable versus non controllable stress behavioral paradigm, and how pairing such stressors influence drug-taking and relapse assays.

Sex differences are an essential determinant of neuroadaptations underlying addiction and stress mechanisms and warrants further exploration. Ovarian hormones regulate DS plasticity through its receptors present on the DS neurons. We will therefore examine questions 1 and 2 between males and females. We will utilize retrograde tracing followed by immunofluorescence and in-situ-hybridization to characterize PFC and TN projections to DS substructures in heroin addiction and or stress: We will then chemogenetically activate or inhibit these circuits to examine their role in these behaviors. Finally, we will deplete ovarian hormones in females through ovariectomy and examine the sex-specific circuit plasticity underlying stress and or heroin exposure.

**3) Identify the neuronal circuit specific transcriptional and epigenetic changes that contribute to maladaptive plasticity during heroin relapse and or stress.**

Opioids are thought to alter transcriptional and chromatin processes in brain reward regions modulating synaptic plasticity underlying the addicted state. Experiences and cues associated with learning have been observed to induce distinct neural gene expression patterns in a temporal and pulsative manner, which is analogously referred to as the genomic action potential (gAP). Mechanistically, gAP has cascading consequences that influence long term functioning required for behavioral adaptations, which is achieved through governing of sex and cell-type-specific transcriptome and the translatome. Such gAP-mediated transcriptional and translational changes over a period of time enable experience-dependent modification of the synaptic circuitry to catalyze behavioral adaptations. There is emerging evidence that gAP underlies experience-dependent neuroplasticity through immediate early genes (IEG). However, the characteristics and functions of such neuroadaptations within specific neural substrates and between sexes in stress and opioid induced plasticity remain unclear. We will examine time-dependent changes in IEGs in the DS in rodents that will undergo heroin self-administration, relapse and stress assays. This will be addressed through biochemical approaches, including RNAscope and CatFish. To determine cell-type specific (i.e., the D1 and D2 expressing medium spiny neurons) IEG changes in the DMS and DLS, we will utilize RiboTag capture using Cre-inducible RiboTag in transgenic D1 and D2 cre lines. RiboTag will allow isolation of ribosomal RNA for sex and cell-type-specific quantification of IEGs. We will then do viral manipulation of IEGs in specific cell types in DMS and DLS will enable dissecting the role of D1 and D2 MSN in the DMS and DLS in stress, drug-taking and relapse. To examine the patterns of genomic enrichment of IEGs, we will use Chromatin immunoprecipitation (ChIP) followed by RNA-seq to examine patterns of gene expression that may be related to IEG binding.

Summer Program participants will work closely on one of the research questions and get trained on preclinical models of substance use disorder, biochemical approaches, and imaging techniques. Data obtained during the training duration will be analyzed and formulated into poster presentations, grant proposals, and manuscripts for publications.

**Daniel Morgan, PhD**

Associate Professor and Associate Vice Chair

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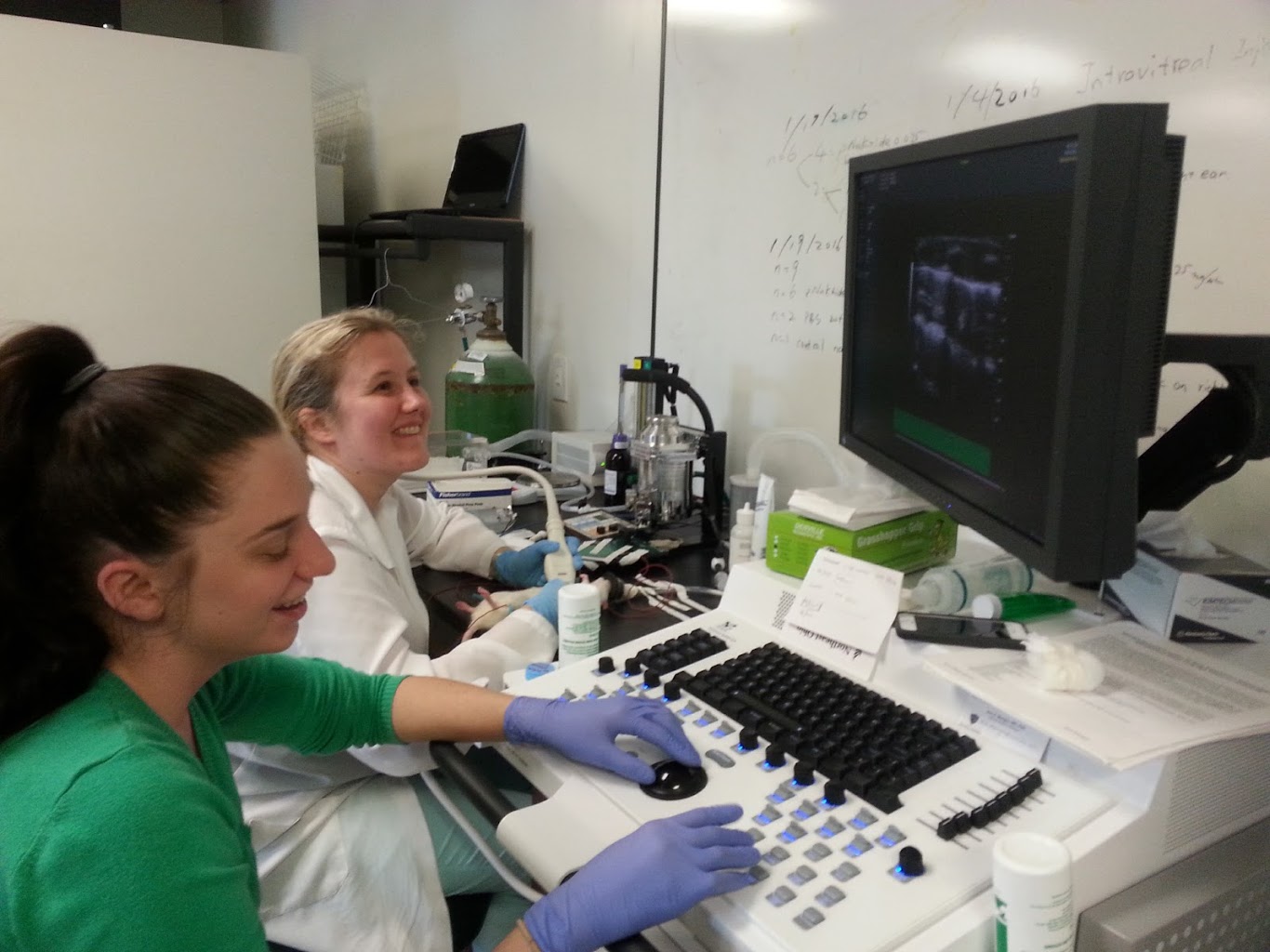
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1. The primary focus of my laboratory is to understand the mechanisms responsible for endocannabinoid signaling in human health and disease including cannabinoid tolerance, drug addiction, and metabolic homeostasis. Currently my group is funded by NIDA to assess the mechanisms responsible for cannabinoid tolerance (DA044999). This work involves assessing the contribution and molecular mechanisms of c-Jun N-terminal kinase (JNK) signaling in tolerance for different cannabinoid drugs. We are also actively engaged in work to understand the mechanisms responsible for sex differences between male and female mice in the response and tolerance to cannabinoids. This work involves using different strains of mutant mice that express either desensitization or internalization-resistant forms of the cannabinoid type 1 receptor. Members of my group commonly use methods in behavioral pharmacology to assess acute, inflammatory, and chronic pain in mice and molecular pharmacological approaches to assess cannabinoid receptor function and signaling. These pain testing approaches include the tail-flick and hotplate tests to measure acute pain, the formalin test to measure inflammatory pain, and the von Frey, Hargraeve's, and acetone tests to measure mechanical and thermal sensitivity in mice with chronic pain from chemotherapy exposure or nerve injury. We also use the elevated plus maze, forced swim test, conditioned place preference, and ultrasonic vocalizations to measure affective components of chronic pain. Lab members use molecular approaches such as qRT-PCR, Western Blotting, radioligand binding, and agonist-stimulated G protein activation to probe receptor expression and function.

2. A second project that we are interested in involves understanding the role of neuropeptide signaling in the regulation of pain and motivated behaviors such as drug addiction and feeding. This work focuses on assessing the role of small neuropeptides derived from a protein precursor protein called proSAAS.  ProSAAS-derived peptides have been shown to signal through two recently deorphanized G protein-coupled receptors, GPR171 and GPR83, to modulate body weight, feeding behavior, morphine tolerance and reward, and anxiety behaviors. Our current work involves examining acute, inflammatory, and chronic pain as well as morphine tolerance in mutant mice lacking proSAAS.

3. Finally, a third project involves assessing the impact of decursinol, the active anti-inflammatory and antinociceptive natural product component from the Korean Angelica Gigas Nakai plant, in acute, inflammatory, and chemotherapy-evoked chronic pain. Current work on this project involves assessing whether tolerance develops to the pain-relieving effects of decursinol and also whether co-administering this natural compound with chemotherapy might prevent development and "chronification" of neuropathic pain.

**Dr. Sandrine V. Pierre**



*Echocardiographic assessment of rodent cardiac function by Dr. P. Marck and undergraduate fellow A. Bryant.*

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The Pierre lab studies specific intracellular pathways involved in the integrated response of the myocardium to hemodynamic and metabolic disturbances. Our goal is to develop new paradigms to therapeutically address cardiovascular diseases based on the Na/K-ATPase signaling complex. We examine these issues by combining techniques of molecular and cell biology with *ex-vivo* (biochemistry and cell physiology, isolated heart perfusion, primary cardiac cell cultures, histology) and *in-vivo* assessments of cardiac function in genetically altered mice (echocardiography, measurement of blood pressure by tail-cuff and telemetry, cardiac and vascular catheterization). In the interdisciplinary environment provided by MIIR, interns are exposed to the pre-clinical models and key techniques that are currently available to cardiac and vascular physiologists and pharmacologists.

**Project 1. Cardioprotection by Na/K-ATPase ligands in acute myocardial infarction**

**Rationale**: In addition to pumping ions, Na/K-ATPase interacts with neighboring membrane proteins and takes part in signaling complexes to send messages to various intracellular organelles. We believe that understanding these pathways and targeting the Na/K-ATPase receptor function will lead to novel interventions for the treatment and prevention of ischemia and reperfusion injury.

**Method**: the INBRE fellow will learn the isolated Landendorff-perfused mouse heart preparation and expose it to novel compounds targeting the Na/K-ATPase cardioprotective signaling pathway. This includes analysis of contractile function in real time and assessments of activation of the Na/K-ATPase cardioprotective pathway biochemically. The effectiveness of promising compounds will be further tested *in vivo* following experimentally-induced acute myocardial infarction (AMI)*.* Mice will be subjected to an acute occlusion of the left descending anterior artery (LAD) for 30 min, and cardiac function and remodeling will be monitored after 1 and 2 weeks of reperfusion. In addition to functional echocardiographic assessments, the fellow will conduct morphometric and histological studies as well as biochemical (western blot) and qPCR evaluation of fibrosis, inflammation, and hypertrophy markers.

**Project 2. Role of α1 Na/K-ATPase in adverse cardiac remodeling and heart failure**

**Rationale**: Heart failure (HF), a chronic incurable illness, is the common end-stage of heart diseases caused by an array of highly prevalent conditions such as hypertension and coronary heart diseases. A greater and broader protection must be achieved to face the unmanageably high HF morbidity and mortality rates amidst the exploding incidence and prevalence of the condition worldwide. Targeting the Na+/K+-ATPase receptor function may lead to novel interventions

**Method**: Using our newly developed model of cardiac-specific KO of Na+/K+-ATPase α1, we will assess the role of Na+/K+-ATPase α1 in the development of hypertrophy, fibrosis and heart failure in mice subjected to Angiotensin II infusion by osmotic minipumps. In addition to functional echocardiographic assessments, the students will conduct morphometric and histological studies as well as biochemical (western blot) and qPCR evaluation of fibrosis, inflammation, and hypertrophy markers.

**Dr. Gary O. Rankin**

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The following projects are available in my laboratory:

**Project #1:** Chloroanilines are commonly used chemical intermediates in the manufacture of dyes, drugs, agricultural herbicides and fungicides and thousands of other products. Exposure to a chloroaniline can result in a number of toxicities including toxicity to the blood, liver and kidney. This project seeks to determine the chemical species (parent compound or metabolite) responsible for liver and kidney damage and the mechanism by which toxicity occurs.

**Project #2:** Halogenatedbenzenes and phenols are common intermediates in the synthesis of a wide range of commercial products and appear as environmental pollutants in many parts of the world. Many of these compounds and/or their metabolites target the kidney and can induce kidney injury. This project will examine the nephrotoxicity induced by these compounds, examine structure-toxicity relationships as well as mechanisms by which these important chemicals harm the kidney.

**Assays and Instrumentation:** Projects that will investigate nephrotoxicity will use in vitro assays that involve isolation of rat kidney cells, measurement of enzyme release from treated and control cells, and potentially, the measurement of cellular ATP levels and other mitochondrial functional parameters. Additional techniques may involve Western blotting, quantifying urinary contents (protein, glucose), and measuring blood urea nitrogen and glucose levels Instrumentation will primarily involve the use of balances, centrifuges and UV-visible spectrophotometers. High pressure liquid chromatography and thermocycler use is also possible.

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Recently, much progress has been made towards understanding how neurons, the cells responsible for the processing and transfer of information in the central nervous system (CNS), interact with non-neuronal brain cells. However, we have still only begun to scratch the surface about how non-neuronal cells contribute to the structural and functional maturation of the neuronal junctions known as synapses. Our work focuses on identifying and elaborating the genes, molecules, and signaling pathways that are crucial for linking non-neuronal cells with the synaptic structures that have been shown to be severely disrupted in nearly all known neurodevelopmental and psychiatric disorders. The long-term goal of our research is to contribute to novel therapeutic strategies to prevent or repair the impaired synaptic connectivity that occurs during abnormal brain development and following CNS injury or insult.

Two primary projects are currently ongoing in the Risher lab:

1) Astrocytes, the primary glial cell type in the brain, secrete a variety of factors that promote synaptogenesis during development and after injury. One family of synaptogenic proteins, the thrombospondins (TSPs), acts through a neuronal receptor, calcium channel subunit α2δ-1, which is known to be altered in some patients with epilepsy, intellectual disability, and autism. Our recent work using rodent models revealed that the interaction between TSP and α2δ-1 differentially promotes synaptic connectivity between males and females. We are currently investigating the genetic, cellular, and molecular mechanisms that underlie these differences, as well as determining the functional relevance of this disparity between the sexes.

2) Neonatal abstinence syndrome (NAS) is a devastating consequence of the national opioid epidemic that is showing striking incidence rates in West Virginia and Central Appalachia. NAS infants are essentially born with an addiction to opiates, and they enter an intense state of withdrawal after cessation of placental exchange-mediated drug exposure. The babies require constant supervision and, approximately 50% of the time, pharmacological intervention before being able to be discharged from the NICU. The long-term effects of NAS on cognition and behavior are predicted to be numerous, but there is currently not much known about how prenatal opioid exposure affects brain development. We have strong preliminary evidence that astrocyte-mediated synaptogenic signaling is among the developmental processes that are significantly disrupted with prenatal opioid exposure (POE). We are now conducting experiments to try to understand the extent to which prenatal opioids influence the formation and maturation of synaptic circuitry in cell culture and rodent models of NAS.

In the Risher lab, students will be exposed to a variety of cellular, molecular, genetic, and imaging techniques. Commonly used methods include animal handling (mouse/rat), primary cell culture, organotypic brain slice culture, Western blotting, immunohistochemistry, plasmid DNA transformation and transfection, confocal microscopy, electron microscopy, 3D reconstruction-based image analysis, genotyping, viral vector work, and single-cell RNA sequencing. Students will have the opportunity to meet regularly with Dr. Risher as well as in a group setting such as our monthly lab meetings.

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Our laboratory is interested in understanding how adolescent binge drinking influences brain function and contributes to the development of alcohol use disorder. Using a rodent model of adolescent binge drinking, our laboratory and others have demonstrated that there are acute and long-term changes to neuronal structure, function, and behavior across multiple cognitive domains.

Over the last few decades, it has become apparent that non-neuronal cells called astrocytes which outnumber neurons and ensheathe many neuronal connections, play an important role in synapse formation, synapse maintenance across the life-span, and synaptic recovery following injury. However, how astrocytes contribute to neuronal and synaptic remodeling following ethanol exposure is not fully understood. Understanding how astrocytes contribute to the long-term effects of adolescent binge drinking in a rodent model is crucial for understanding the impact that underage alcohol exposure can have on the adult brain and how early onset drinking may contribute to the development of alcohol dependence later in life.

We have three ongoing projects:

1. Investigating the acute and long-term effects of binge drinking on astrocyte function.

2. Investigating the role of astrocytes in the development of addiction.

3. Investigating how changes in astrocyte function following adolescent binge drinking influence recovery from secondary injury later in life, e.g., following traumatic brain injury. Techniques used to answer these questions include: intracranial survival surgery for injection of adenoassociated viruses and insertion of optic fibers for optosensors to evaluate calcium and neuro/gliotransmitter release, immunohistochemistry, Western blot, qPCR, neuronal-astrocyte primary co-culture, confocal microscopy, 3D morphometric analysis of astrocytes, and a battery of behavioral paradigms including conditioned place preference, fear conditioning, open field, social interaction, and plus maze.

**Boyd Rorabaugh, Ph.D.**

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**Impact of Methamphetamine use during pregnancy on the cardiovascular function of adult offspring**

My laboratory studies the impact of methamphetamine use during pregnancy on cardiovascular outcomes in adult offspring. We have found that prenatal exposure to methamphetamine leads to myocardial hypersensitivity to ischemic injury, induces long-lasting changes in cardiac gene expression, and alters vascular function in adult offspring. Importantly, some of these effects are sex-dependent. Our data suggest that individuals that were prenatally exposed to methamphetamine may be at increased risk of developing cardiovascular diseases. We are currently trying to understand the mechanisms by which prenatal exposure to methamphetamine induces these cardiovascular changes.

INBRE participants will have the opportunity to participate in the identification of methamphetamine-induced changes in cardiac gene expression (analysis of RNA sequencing data, real time PCR, western blotting), conduct experiments with isolated tissues (hearts and blood vessels), interact with other investigators in the department (faculty, graduate students, postdoctoral fellows, and undergraduate students), assess scientific literature, and learn to present their data to a scientific audience.

**Dr. Travis Salisbury**

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Obesity increases the risk for 10 different cancers including breast cancer. We have shown that adipose tissue from the breast tumor microenvironment releases factors that induce signaling in breast cancer cells that stimulates cancer cell migration and invasion. We are investigating the signaling mechanisms by which obesity associated secreted factors stimulate breast cancer cell migration and invasiveness. We hypothesize that the primary pathway involved is the mTOR pathway. Students in my lab would have the opportunity to study these questions in several lines of human breast cancer cells. Our methods are largely molecular biology based; therefore, students would have the opportunity to use real time PCR machines, electrophoresis equipment, and laminar flow tissue culture hoods. Students will also have a choice as to what technique they would like to learn during their internship. Techniques in lab will include, but are not limited to, real-time PCR, western blot, chromatin immunoprecipitation analysis, interfering RNA approaches to gene knockdown and proliferation assays.

**Nalini Santanam, Ph.D., M.P.H., F.A.H.A.**

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The following projects are ongoing in my laboratory:

**Project 1: Vaping and exercise:** Vaping is highly rampant among young individuals. This studywill test the effects of vaping on the heart and other organs. This study will also test if exercise can help these individuals from some of the harmful effects of vaping.

**Project 2: New pain medications:** There are several million individuals who suffer from chronic pain. The current treatmentsthat they are provided are not effective. Our lab is researching alternatives to the current medications for pain.

**Project 3: Heart fat and health:** Obesity is very high in West Virginia. There are several fats in the body including the one that is in or around the heart. We are studying heart fat to understand its role in human physiology.

**Dr. Yevgeniy Shakirov**

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Our research interests focus on telomeres, the evolutionarily conserved protein-DNA complexes that cap linear eukaryotic chromosomes, promote genome maintenance and regulate cellular lifespan. Telomere length shortens with each somatic cell division and is often viewed as the most accurate cellular marker of biological age. Proper maintenance of telomere length has important implications for aging, stem cell-related diseases and cancer. Although considerable variation in mean telomere length exists in yeast, plants and humans, mechanisms underlying telomere length homeostasis are largely unknown.

**Project 1**. **Genetic and epigenetic architecture of natural telomere length variation.**

The main objective of current research in the lab is to elucidate the genetic and epigenetic causes of telomere length variation using the genetically facile plant *Arabidopsis thaliana* as a model. To achieve this goal, we use a plethora of cutting edge natural variation resources available for this organism and a collection of powerful molecular, genomic and epigenetic tools. We recently identified a major effect QTL that explained 48% of telomere length variation in recombinant inbred Arabidopsis populations, with the underlying natural polymorphism mapping to the *NOP2A* gene. Mutations in mammalian *NOP2* orthologs lead to uncontrolled proliferation of cancer cells, and their expression serves as a prognostic marker of tumor development. INBRE program participants will work with laboratory personnel on understanding the role played by NOP2A and other genes in telomere length control. Our studies will have an impact on understanding genetic differences in telomere length between individuals and populations, and may provide novel insight into the molecular basis for different rates of aging and predisposition to telomere-associated stem cell, cancer and age-related diseases.

**Project 2.** **Analysis of the interplay between telomere biology and ribosome biogenesis.**

We have recently identified several components of rRNA maturation machinery, including RPL5, that impact species-specific telomere length set point in plants. Interestingly, human *RPL5* inhibits tumorigenesis, and its inactivation is the most common (11-34%) somatic ribosomal protein defect in multiple tumor types. Indeed, important similarities exist between human diseases known as telomeropathies and ribosomopathies, and our findings argue that components of rRNA maturation machinery may impact species-specific telomere length set point across eukaryotic evolution. IMBRE participants will work with mutants of ribosome biogenesis genes in plants to uncover specific mechanisms linking telomeres with ribosome biogenesis.

All participants will receive training in molecular cloning, RNA, DNA and protein analysis, aspects of genetic manipulations and bioinformatics.

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**Project 1:**

**The Question:** *“What causes treatment failure in some cancer patients but not others?”* Cancer cells can be viewed as a separate organism within the host that is continually evolving in order to survive. Applying evolutionary theory in this context will help us understand how cancers can reoccur after going into remission or not respond to chemotherapies.The process that cells in our bodies undergo to become cancer cells end up producing a cell that ceases to listen and cooperate with its neighbors, which is necessary for the complex mixture of cell our bodies are. This project will investigate an ability of cells known as phenotypic plasticity, where a cell can change its characteristics in order to adapt to the physiological situation. Our previous investigations have shown that heat shock protein 90 (HSP90) has a critical role in epigenetic gene regulation through histone acetylation and phenotypic plasticity. HSP90 is over-expressed in multiple cancers with poor prognosis. Thus, we propose to investigate the role of HSP90 in the ability of cancer cells to use phenotypic plasticity to adapt to chemotherapy.

**Research Goals:** The approach is to test the effects of inhibition of HSP90 on phenotypic plasticity *in vitro* on a panel of cell lines. We will determine if inhibition of HSP90 can prevent the adaptation to chemotherapies of lung cancer cells newly derived from patient samples, as well as established cell lines. The two adaptive phenotypes we will examine include epithelial to mesenchymal transition (EMT) and expression of drug transporters in a multi-drug resistant (MDR) phenotype. The major tools of investigation will be flow cytometry and functional assays for the phenotypes.

**Specific Project:** Students will induce EMT and MDR in a variety of lung cancer cell lines and study the expression of various protein markers on the surface of the cells using flow cytometry to determine the effects. HSP90 inhibitors will be used to determine how these influence phenotypic plasticity.

**Project 2:**

**The Question:** *“What are the molecular defects that promote age-related dysfunction?”* Our understanding of the processes associated with aging now includes dysfunction associated with epigenetic gene regulation. Understanding the mechanisms that promote evolution of the epigenome and its increasing dysfunction with age in stem cells are critical to therapeutic strategies targeting aging. The results of our investigations in the *Drosophila* and murine model systems indicate a very important gene in epigenetic gene regulation, heat shock protein 90 (HSP90), is well connected to phenotypic plasticity. We propose to investigate if the loss of phenotypic plasticity associated with epigenetic drift and clonal hematopoiesis can be ameliorated by inhibition of HSP90.

**Research Goals:** This project is designed to test the hypothesis that inhibition of HSP90 results in changes in histone acetylation that in turn prevent epigenetic drift and loss of phenotypic plasticity in hematopoietic stem cells. The approach is to test the hypothesis in vitro in a stem cell model of blood cell differentiation.

**Specific Project:** Students will induce epigenetic drift in our stem cell model and study the production of various markers on the surface of cells using flow cytometry. HSP90 inhibition will be performed via small molecule inhibitors to determine if this inhibition can prevent and reverse epigenetic drift of these cells.

**Jiang Tian, PhD**

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**Na/K-ATPase Alpha 1 in Heart Failure and Cardiac Decompensation**

Initial hypertrophic growth of cardiac myocyte is a compensational process in response to reduced cardiac output due to pressure overload or myocardial infarction. Clinical data showed that about 50% of hypertrophic patients eventually become decompensated which led to heart failure and death. Earlier studies found that heart failure patients exhibit significant reduction of Na/K-ATPase in their heart tissue, and their preserved contractile function is correlated with the amount of Na/K-ATPase. Our recent work demonstrated that specifically reducing Na/K-ATPase α1 causes cardiac cell death, reduces cardiac hypertrophy, and decompensates muscle contraction in animal models of cardiomyopathy. The current project is to study the mechanisms of Na/K-ATPase α1 in regulation of cardiac cell survival and metabolic activity through mitochondria-mediated signaling pathways. Specifically, we will investigate the relationship between Na/K-ATPase α1 reduction and mitochondria-mediated cell apoptosis. In addition, we have identified a specific Na/K-ATPase inverse agonist, MB5 (a hydroxyxanthone compound), that can preserve cell membrane Na/K-ATPase by blocking ouabain-induced endocytosis. We will continue to test if MB5 can be used as a potential rescue for Na/K-ATPase in animal models of cardiomyopathy. Students participate in this project could learn basic laboratory techniques including Western blot, RT-qPCR, cell culture, and tissue collection from animal studies. It is also expected for the students to practice experimental design and manuscript writing.

**Dr. Monica Valentovic**

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Our laboratory is focused on exploring new interventions that will reduce the adverse effects of drugs. We have recently focused on examining ways to reduce the toxicities of cancer chemotherapy agents, antiviral agents, and radiocontrast agents. Projects available in my lab:

**Project #1. Reducing serious cancer chemotherapy side effects.** This is an ongoing project that has been funded by a federal grant from NIH. Our laboratory is evaluating new compounds that may reduce the adverse effects experienced by individuals treated with cancer chemotherapy drugs. All cancer chemotherapy agents induce side effects and reducing these side effects will allow a better quality of life for the individual and potentially improve the success of the cancer chemotherapy agent. A long-term goal is to develop methods to improve the effectiveness of the cancer chemotherapeutic agents while lessening the side effects. This project has clear clinical relevance and is translational. The drugs we are exploring are used in controlling breast, lung, ovarian cancer and leukemia. An individual involved in this project will investigate cellular changes in toxicity, specifically we want to explore changes in the mitochondria as well as post-translational modifications of proteins caused by exposure to cancer chemotherapy drugs including doxorubicin, cisplatin camptothecin or irinotecan.

**Project #2. Potential role of e-vape flavoring agents in renal impairment.** E-cigarettes and e-vaping have a complex series of flavoring aldehydes. Recent studies have shown alterations in genetic expression in the lung, kidney, brain and liver following vaping in rodent models. We will examine changes mediated by flavoring aldehydes on human renal proximal tubules. This project will examine the impact of flavoring aldehydes on mitochondrial proteins critical in generation of ATP.

**Project #3. Examination of the mechanism of renal damage by an antiviral agent used in in treating HIV and hepatitis B patients**.Patients with HIV or hepatitis B must take antiviral agents to slow the progression of their disease. These drugs are taken for very long period of time even years. Side effects often occur after someone takes an antiviral agent for over 1 year. We are examining the mechanism of damage to the kidney by a commonly used antiviral agent. We are using a normal human proximal tubular epithelial cell culture model for this study. We have preliminary results to suggest certain agents can reduce the side effects of the antiviral agents but would not impact the pharmacologic activity.

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1. **Determining how obesity contributes to initiation and progression of myelodysplastic syndromes (MDS), which are blood and bone marrow cancers.** MDS are blood and bone marrow cancers that are often caused in part by overactive inflammation in hematopoietic stem and progenitor cells. Obesity has been linked to MDS and acute myeloid leukemia (AML) but has not been studied in our double knockout (Tifab and miR-146a KO) mouse model of MDS/AML. We will perform studies such that MDS susceptible mice are subjected to control and Western diet to determine mechanisms by which obesity contributes to initiation and progression of disease. We anticipate mechanisms to involve diet/obesity-driven changes in hematopoietic stem and progenitor cell differentiation. We will also plan to determine if this can exist as an epigenetic effect (Do offspring of parents on poor diet have increased susceptibility to MDS initiation and/or progression?)

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1. **Defining the mechanisms by which obese individuals are more susceptible to infection and have lowered vaccine efficacy.** We hypothesize that this occurs through dietary and obesity-driven effects on hematopoietic stem cells, which we have found to be important in providing immune protection against pathogenic threats. We are investigating multiple vaccines in this study. We will also determine if this can exist as an epigenetic effect (Do offspring of parents on poor diet have decreased vaccine-induced immune protection against infectious disease?).

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Vascular disease is one of the major complications of diabetes and hypertension in the United States. Exosome, a major type of extracellular vesicle, is emerging as a novel mechanism of intercellular communication. Increasing evidence suggests that exosomes could convey proteins/genetic materials to recipient cells/tissue/organs in both physiological and pathological conditions. The Wang Lab at Marshall University majorly focuses on understanding the pathophysiological roles of exosomes and their potential therapeutical applications in diabetes/hypertension-associated vascular diseases including ischemic stroke and vascular dementia. Our long-term goal is to establish an exosome-based therapy for treating vascular diseases.

Ongoing projects for students to be involved in:

**Project 1: Role of exercise-intervened exosomes in ischemic stroke**. We have previously demonstrated that exercise intervention could modulate the release of exosomes. Our recent data shows that the exosomal-mediated communications between endothelial progenitor cells (EPCs) and brain cells such as endothelial cells and neurons are compromised in hypertension conditions. Exercise is a well-known nonpharmaceutical approach for cerebrovascular disease and has been shown to modulate the function of EPCs. Given that exosome function varies on cellular status and origin, we speculate that exercise intervention can modulate EPC-derived exosome (EPC-EX)-mediated intercellular communication in the ischemic stroke brain. In this project, we aim to investigate the effects and underlying mechanisms of exercise-regulated exosomes in protecting the brain from ischemic stroke.

**Project 2: Angiotensin-converting enzyme 2 (ACE2)-primed exosomes: potential application in vascular dementia.** Vascular dementia (VaD) is the second most common type of dementia- related disease in the world. Thus far, there is no curative treatment for VaD. Risk factors such as hypertension cause cerebral microvasculature damage and dysfunction which proceeds to neurodegeneration as one of the underlying pathological mechanisms of VaD. Hence, protecting the cerebral microvasculature might provide a new strategy for alleviating neuronal cell loss and improving cognitive function in VaD. Accumulated evidence provided by others and our group has suggested that EPCs and EPC-EXs have a promising therapeutic application for cerebrovascular diseases. Angiotensin-converting enzyme 2 (ACE2), a negative regulator of the renin-angiotensin system, plays a critical role in hypertension-related cerebrovascular diseases. We have recently reported that EPC-EXs can convey ACE2 to protect vascular endothelial cells. The goal of this project is to investigate the potential effects of combining ACE2 and EPC-EXs for treating hypertension-related VaD.

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My research focuses on bacterial biofilms, lung infections and gut microbiota. Four projects are ongoing in the Yu lab.

**Project #1: Cystic Fibrosis Biofilms.** Individuals afflicted with cystic fibrosis (CF) are susceptible to recurrent lung infections with a bacterium called *Pseudomonas aeruginosa*. During the infection in CF, this bacterium forms a capsule (biofilms) by producing a polysaccharide called alginate. Alginate is a virulence factor that allows greater adhesion to lung epithelial cells, as well as protection from antibiotics and the host’s immune system. We study how alginate production is regulated. Elucidation of the alginate pathways will lead to better understand the pathogenesis, and development of novel therapeutics for treatment in CF.

**Project #2: Testing Antimicrobials**. Most of bacterial lung infections starts with the colonization of upper respiratory tract. Aspiration of oropharyngeal secretions containing colonizing bacteria deep into the lung allows for the establishment of lower respiratory tract infections. We are using an inhalation exposure system to introduce bacteria into the distal airways of the mouse lungs, causing the development of pneumonia. This model is being utilized to test the safety and efficacy of novel antimicrobials against the multiple drug-resistant (MDR) lung infection. The goal of this project is to develop novel therapeutics against the MDR Lung infections and pneumonia.

**Project #3: SFB Probiotics.** Gut microbiota, a bacterial community made up of 1,000 different species, are important to human health. Among all the species, there is a morphologically-distinct symbiotic member known as segmented filamentous bacteria (SFB). The SFB belongs to a group of clostridia bacteria, which cannot be grown *in vitro*. However, the SFB play a vital role in the development of the immune system in mice. More specifically, SFB have been shown to attach to the apical epithelium of the small intestine to induce the interleukin-17-producing T helper (TH17) cells. TH17 cells are important for the protection against intestinal pathogens as well as in maintaining gut homeostasis. In this project, we will examine possibilities of how to develop the SFB into a novel probiotic to prevent and control the gastrointestinal diseases in children.

**Project #4: New Biopolymer Development**. Through removal of major pathogenicity genes from genome and validation via the genome resequencing, we created a non-pathogenic strain of *P. aeruginosa* that produces large amounts of alginate. Alginate is a polysaccharide widely used in biomedical applications. It consists of an unbranched linear biopolymer comprised of two sugar monomers, β-D-mannuronate and its C5 epimer α-L-guluronate. Through introduction of changes of genetic codes for the alginate biosynthetic enzymes, we hope that we may be able to use the non-pathogenic strain to produce alginate with custom compositions.