# MENTORS DIRECTORY

## 2023 AMERICAN HEART ASSOCIATION UNDERGRADUATE SUMMER INTERNSHIP RESEARCH PROGRAM

to be held at

**The Joan C. Edwards School of Medicine**

**at Marshall University**

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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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**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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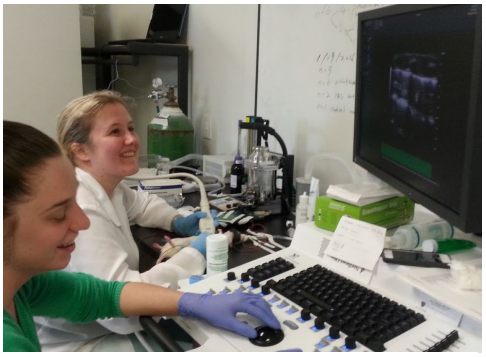

*Echocardiographic assessment of rodent cardiac function by Dr. P. Marck and undergraduate fellow A. Bryant.*

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.

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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.

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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 study will 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: Heart fat and health:** Obesity is very high in West Virginia. There are several fats in the body including the ones that is in or around the heart. We are studying heart fat to understand its role in heart disease.