SUMMER 2024 RESEARCH PROJECTS – SURF AND SRF

FACULTY MENTOR:
Salah-uddin Ahmed, Ph.D., Professor of Pharmaceutical Sciences, Executive Director of the Graduate Program, *ASPET member*
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RESEARCH PROJECT:
Identifying novel therapeutic targets in the pathogenesis of rheumatic diseases

TECHNIQUES TO BE LEARNED:

- ELISA
- Western blotting
- In-gel zymography

DESCRIPTION OF PROJECTS AND AIMS:
The Ahmed lab is involved in translational studies to identify the unknown mechanisms that govern the pathogenesis of rheumatic diseases such as rheumatoid arthritis, osteoarthritis, and gout. In particular, our group has been investigating the posttranslational and epigenetic mechanisms that facilitate rheumatic disease propagation. Using synovial cells, tissues, and serum samples from the rheumatoid arthritis patient population, we conduct genomics and metabolomics studies to identify some key mediators that promote chronic inflammation and tissue destruction in rheumatoid arthritis. Aims for the SURF projects in my lab are: 1) Identify the role of proinflammatory cytokine signaling networks in the pathogenesis of rheumatoid arthritis; 2)

Design novel targeted therapies in the treatment of rheumatoid arthritis; and 3) Understand the role of pain receptor pathways in the management of rheumatic diseases.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:
The SURF student is assigned a role and/or participation in an ongoing or new projects where they not only get the chance to learn about the inflammatory signal transduction pathways that promote rheumatic diseases, but also get a chance to sharpen their technical skills in running procedures independently. At the end of the summer, the SURF fellow will be capable of summarizing their progress in the form of a poster presentation. The work will be further expanded and presented at one of the national scientific meetings in which SURF students typically serve as co-authors. These studies have resulted in the publication of manuscripts in the past.

MENTORING PLAN:
The SURF students in the Ahmed lab are assigned a lab mentor (senior graduate student or a postdoctoral fellow) to train the students in the basic skillsets needed to perform research. After a few weeks of rigorous hands-on training, the SURF student is assigned a project of interest in the lab and the lab mentor supervises the student for the remainder of the training. Students are asked to present their findings in the weekly lab meetings, led by Dr. Ahmed, where they share their data in PowerPoint presentations and take on questions and suggestions from all the members of the lab. This opportunity allows students to sharpen their communication skills and engage them in critical analysis of their work.

FACULTY MENTOR:

Zhaokang Cheng, Ph.D., Associate Professor of Pharmaceutical Sciences, Pharmaceutical Sciences
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RESEARCH PROJECT:

Novel role of cell cycle regulators in the heart

TECHNIQUES TO BE LEARNED:

- Cell culture
- BCA protein assay
- Western blot
- Immunofluorescence staining

DESCRIPTION OF PROJECT AND AIMS:

The widely-used anthracycline family anti-cancer drug doxorubicin can cause serious cardiotoxic side effects by inducing apoptotic death of cardiac myocytes. Dr. Cheng's laboratory previously identified cyclin-dependent kinase 2 (CDK2) as a critical mediator of cardiomyocyte apoptosis. These results support a novel function of cell cycle proteins in the regulation of cardiac cell death. The aim of this project is to define the signaling pathways involved in cardiac CDK2 activation. The proposed research could open a new avenue in the treatment of doxorubicin-induced cardiotoxicity.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

Upon joining the lab in early summer, the SURF fellow will work with their lab mentor in Dr. Cheng's group on the proposed project. The SURF fellow will receive expert training in fundamental research skills including cell culture and western blotting. Both the lab mentor and Dr. Cheng will be available to help the SURF fellow with research-related questions, difficulties, and future directions.

MENTORING PLAN:

During the project period, Dr. Cheng will meet with the SURF fellow weekly in a formal fashion to review new data, troubleshoot problems and discuss future plans. In addition, Dr. Cheng will be available to meet with the SURF fellow on a daily basis as questions arise. The Cheng lab has weekly journal club meetings to discuss cutting-edge research. The SURF fellow will have opportunities to participate in journal club discussions to refine their scientific communication and research presentation skills. Through these regular meetings, Dr. Cheng will ensure the successful training of the SURF fellow and completion of the research project.

FACULTY MENTOR:

John D. Clarke, Ph.D., Vice Chair and Associate Professor of Pharmaceutical Sciences, *ASPET member*
Email: j.clarke@wsu.edu

RESEARCH PROJECT:

Precision medicine: natural product inhibition of drug transporters

TECHNIQUES TO BE LEARNED:

- Cell culture
- Western blotting

DESCRIPTION OF PROJECT AND AIMS:

Toxicities from drugs and natural products are a significant burden on human health. This precision medicine project investigates inhibition of drug transporters by the natural product kratom. Kratom has stimulant and opioid-like properties and can be purchased at convenience stores, head shops, and many other retailers throughout the United States. The popularity of kratom has increased as people with opioid use disorder seek ways to overcome their addiction. If people take kratom with other prescription drugs, the phytoconstituents in kratom may inhibit drug transporters in the intestine and liver and alter drug exposures and toxicities. We will investigate the effects of kratom constituents on drug transporter function using cell culture and rodent models. Aims of the specific SURF projects in the lab are : 1) identify the inhibitory effects of kratom constituents on transporter function using cell culture, and 2) determine the risk of altered drug disposition after kratom consumption in a mouse model.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

After initial training by laboratory staff, the fellow will be expected to work in an independent manner on all standard laboratory techniques. The fellow will have the opportunity to work with lab staff on non-routine procedures (animal experiments, small molecule analysis, etc.) with the expectation that the fellow will be able to work in a semi-independent manner towards the goals of the project.

MENTORING PLAN:

The fellow will participate in the weekly lab meetings and meet with Dr. Clarke individually as needed, but no less than once every two weeks.

FACULTY MENTOR:
Travis Denton, Ph.D., Assistant Professor of Pharmaceutical Sciences
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RESEARCH PROJECT:
Medicinal approaches to treat neurological diseases

TECHNIQUES TO BE LEARNED:
Depending on the project, trainees will learn some of the following techniques

- Medicinal chemistry
- Cell culture
- Western blotting
- Analytical techniques (e.g., NMR, UPLC-HRMS/MS)
- qPCR
- HRMS- based proteomics
- lentiviral shRNA transduction

DESCRIPTION OF PROJECT AND AIMS:
Projects in the Denton lab focus on small molecule, organic synthesis-based, medicinal chemistry. We also focus on the analytical determination of small molecules in biological matrices (analytical biochemistry). Projects currently ongoing in the lab are multiple, and all focus on trying to make life easier for people who are tormented by devastating neurological diseases.

Project 1: Treatments for ALS, Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and other neurological disorders. In this project, the aim is to develop small molecules to enhance cellular autophagy in an effort to fight neurodegenerative diseases.

Project 2: Treatments for glutamine addicted cancers. In this project, the aim is to develop phosphonic acid bioisosteres of fundamental α-ketoacids and many other small molecules to inhibit newly identified pathways (including the glutaminase II pathway) important for the progression of devastating forms of cancer such as triple-negative breast, castration resistant prostate, non-small cell lung, kidney, and more.

Project 3: Treatment of Alzheimer's disease by targeting the α7 nicotinic acetylcholine receptor (nAChR). In this project, the aim is to synthesize small molecules to target the α7-nAChR, the only nicotinic receptor in the brain of Alzheimer's patients that is not down-regulated. Through this approach, we hope to enhance the cognitive function of people with this disease.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:
For all three projects, the SURF student will synthesize, purify, and characterize new molecules that will be tested for biological activity by our collaborators. The SURF fellow will receive training from the mentor and members of the laboratory so that they can perform procedures independently by the end of the summer.

MENTORING PLAN:
Results will be discussed weekly with the mentor and presented in a poster at the end of the training period.

FACULTY MENTOR:

Darrell A. Jackson, Ph.D., Associate Professor of Pharmaceutical Sciences
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RESEARCH PROJECT:

Sex differences in ischemic/reperfusion-induced expression of calcium permeable GluA2-lacking AMPA receptors

TECHNIQUES TO BE LEARNED:

- Cell culture
- Western blotting
- Maxi-preps
- Transfections
- Fluorescent microscopy
- Hippocampal slice preparation

DESCRIPTION OF PROJECT AND AIMS:

Projects in the Jackson lab focus on the intracellular trafficking of neurotransmitter receptors following neuronal injury. Current research projects that are ongoing in the lab are focused in determining the underlying mechanisms responsible for leading to injury and death of neuronal cells following ischemic stroke injury. The overall goals of these projects are to identify critical signaling pathways that ultimately lead to neuronal death following stroke injury in order to develop more focused therapeutic intervention.

Stroke is prevalent in elderly individuals and greater than 70% of all strokes occur above the age of 65. Ischemic stroke, the most prevalent form of stroke, results in delayed neuronal death (DND) in vulnerable brain areas, such as the hippocampus, and is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors lacking the GluA2-subunit. GluA2-lacking AMPARs are Ca^{2+}-permeable and are known to contribute in DND. Most AMPARs expressed on adult hippocampal pyramidal neurons contain the edited form of GluA2 (Q607R) and are thus impermeable to Ca^{2+} entry. These receptors undergo an unusual subunit composition switch in response to ischemic/reperfusion (I/R), changing from a GluA2-containing Ca^{2+}- impermeable AMPAR to a GluA2-lacking Ca^{2+}-permeable AMPAR.

Project 1: The aim is to determine whether activation of estrogen receptors prevent post-ischemic induced internalization and degradation of GluA2-containing AMPARs. We will utilize immortalized neuronal cell line transfected with fluorescence tagged neurotransmitter receptors (AMPAR GluA1 and GluA2 subunit), Rab proteins (use to examine intracellular trafficking of receptors), and estrogen receptors (ER alpha, ER beta, and G-protein coupled estrogen receptor; GPER) to examine whether estrogen signaling pathway can suppress the post-ischemic induced internalization and trafficking of GluA2-containing AMPARs toward degradative pathways.

Project 2: The aim is to determine whether activation of estrogen receptors suppress the oxidative stress signaling pathway responsible for mediating the internalization and degradation of GluA2-containing AMPARs. We will utilize immortalized neuronal cell line transient transfected with fluorescence tagged neurotransmitter receptors (AMPAR GluA1 and GluA2 subunit), Rab proteins (use to examine intracellular trafficking of receptors), and estrogen receptors (ER alpha, ER beta, and G-protein coupled estrogen receptor; GPER). Cell cultures will be treated with an oxidant (hydrogen peroxide) to examine whether estrogen receptor activation suppresses oxidative stress mediated internalization and trafficking of GluA2-containing AMPARs toward degradative pathways.

MENTORING PLAN:

The SURF student will be trained in techniques that include Western blot analysis, cell culture, maxi-preps, transfections, fluorescent microscopy, and hippocampal slice preparation combined with oxygen glucose deprivation/ re-oxygenation by the investigator, Dr. Jackson, and lab technician. The SURF student will meet with Dr. Jackson daily to discuss experimental design and results. Additionally, the SURF student will attend weekly lab meetings to discuss data and related research articles provided by Dr. Jackson.

FACULTY MENTOR

Philip Lazarus, Ph.D., Professor of Pharmaceutical Sciences, *ASPET member*
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RESEARCH PROJECT:

The overlapping metabolism of opioids and cannabis: Potential consequences for toxicity and withdrawal among opioid users.

TECHNIQUES TO BE LEARNED:

- Enzyme activity assays
- hydrocodone metabolite assessment

DESCRIPTION OF PROJECT AND AIMS:

As the U.S. population struggles with the ever-increasing burden of the ballooning opioid crisis, the interaction of cannabis and opioids is becoming an emerging area of research. Cannabis and opioids are metabolized by many of the same phase I and phase II enzymes in the liver, which could potentially influence the effects of either or both of these compounds. Compounds in both classes undergo rapid hydroxylation or oxidation by hepatic cytochrome P450 (CYP450) enzymes, followed by glucuronidation via the uridine 5'- diphospho-glucuronosyltransferase (UGT) class of enzymes, and finally excretion. The inhibition of these shared enzymes by cannabis and its metabolites has the potential to greatly influence opioid plasma levels in patients currently taking opioids as part of a pain management strategy. Preliminary research in our lab indicates that the cannabinoid (-)-trans-Δ9- tetrahydrocannabinol (THC) and three of its major metabolites (11-OH-THC, THC-COOH and THC-COO-glucuronide) are indeed inhibiting a number of CYP and UGT enzymes. Of particular interest is the inhibition of CYP2D6 and UGT2B7, which act to metabolize codeine, morphine and hydrocodone. The aim of the present proposal will be to examine this inhibition in vivo in up to 30 people co-administered hydrocodone and a marijuana cigarette. Given the public perceptions and misconceptions surrounding the efficacy of cannabis, and its potential uses in pain management, opioid withdrawal, and as a part of many patients' general health practices, a better understanding of how these bioactive compounds are influencing metabolic systems within the human liver are necessary. This knowledge will aid in understanding how cannabis fits into the public health crisis surrounding opioid overdose and opioid use disorder, provide a baseline understanding of how cannabis is metabolized, and how those active metabolites are influencing liver function in patients currently taking opioids.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

The fellow will be trained in the enzyme activity assays and analytical techniques needed to accomplish the experiments described above. The trainee will work directly with a graduate student trainee on the lab techniques for hydrocodone metabolite assessment, and a research nurse on the clinical aspects of the study.

MENTORING PLAN:

The SURF trainee will have bi-weekly meetings with the mentor as well as attending bi-weekly laboratory meetings. Once trained, the student will be able to work fairly independently, and will report on progress at meetings independently.

FACULTY MENTOR:

Kathryn E. Meier, Ph.D., Interim Chair and Professor of Pharmaceutical Sciences; Associate Dean of Undergraduate Programs; *ASPET member*
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RESEARCH PROJECT:

Role of CCN proteins in lysophosphatidic acid signaling

TECHNIQUES TO BE LEARNED:

- Cell culture
- Western blotting
- siRNA-mediated knockdown
- Cell adhesion assays

DESCRIPTION OF PROJECT AND AIMS:

This laboratory studies the effects of lipid mediators on signal transduction in cancer cells. In particular, Dr. Meier's group has been investigating the roles of CCN matricellular proteins in the action of lyophosphatidic acid (LPA) in prostate cancer cells. LPA is a lipid mediator that activates GPCRs, thereby inducing synthesis of CCN1 and CCN2. The roles of the CCNs in LPA action are being investigated using knockdown approaches in conjunction with proteomic analyses. The aim of the project is to determine whether CCNs mediate pro-oncogenic signal transduction events that occur several hours after addition of LPA. If so, then CCNs present a target for therapeutic intervention in prostate cancer.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

At the beginning the summer, the fellow will work closely with Dr. Meier's staff to learn the techniques mentioned above. After the training, the fellow will work semi-independently on their project. The trainee will be assigned a new aspect of the project to pursue as their own.

MENTORING PLAN:

There will be weekly lab meetings to discuss progress and future directions. Dr. Meier will meet individually with the trainee, as needed, to discuss results and to plan for oral and poster presentations.

FACULTY MENTOR:

Senthil Natesan, Ph.D., Associate Professor of Pharmaceutical Sciences
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RESEARCH PROJECT:

Membrane-facilitated allosteric modulation of GPCRs and ion channels

TECHNIQUES TO BE LEARNED:

Basic to intermediate level computational techniques, such as:

- Building 3D structures of small molecules
- Geometry optimization
- Charge calculations
- Preparation of protein-ligand complex structures
- MD simulations using AMBER and GROMACS
- Calculating binding free energy

DESCRIPTION OF PROJECT AND AIMS:

Recent advances in the structural biology of GPCRs and ion channels, along with biophysical and computational studies, suggest that amphiphilic and lipophilic molecules may gain access to binding sites of these receptors by first partitioning into the membrane and then reaching the sites via lateral diffusion through the lipid bilayer. The highly ordered structure of the lipid bilayer may guide a lipophilic or amphiphilic drug molecule to a specific depth within the bilayer, affecting its local concentration near the binding site, and influencing its binding kinetics. Additionally, the constraints of the lipid bilayer including its composition and biophysical properties might play a critical role in "pre-organizing" ligand molecules in appropriate orientation and conformation to facilitate receptor binding. Despite its conspicuous involvement in molecular recognition processes, the critical role of membrane in ligand-binding mechanism to lipid-exposed transmembrane binding sites remains poorly understood. The aim of this project is to gain mechanistic understanding of the structure-membrane interactions relationship (SMIR) of several approved drugs. Knowledge of SMIR will not only provide useful insights to receptor binding kinetics, but also enhance our ability to take advantage of the apparent membrane contributions in rational design of drugs targeting transmembrane proteins with improved efficacy and safety.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

The SURF fellow will learn basic to intermediate-level computational techniques, including building 3D structures of small molecules, geometry optimization, charge calculations, preparation of protein-ligand complex structures, performing MD simulations using AMBER and GROMACS, calculating binding free energy, etc. As these techniques require extensive initial training, the fellow will work very closely with Dr. Natesan and his postdoc and graduate students in the beginning and will become semi-independent toward the completion of the project.

MENTORING PLAN:

Students will share their progress every week during the lab meeting. However, the mentor is available to meet at any time (walk-in) for discussion or to answer any questions.

FACULTY MENTOR:
Mary Paine, R.Ph., Ph.D., Professor of Pharmaceutical Sciences, *ASPET member*
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RESEARCH PROJECT:
Natural product-drug interactions: elucidating mechanisms and causative ingredients using translational research methods

TECHNIQUES TO BE LEARNED:
- *In vitro* assays to recover kinetic and binding parameters and dissolution profiles
- *In vitro-in vivo* predictions
- Pharmacokinetic analysis
- Clinical research procedures

DESCRIPTION OF PROJECT AND AIMS:
Patients often supplement their prescribed drug regimens with botanical and other natural products, which are readily available and often perceived as safe. In parallel, patients may not inform their health care providers, and health care providers may not query their patients, about their use of natural products. These practices raise concern for potentially adverse interactions between natural products and pharmaceutical drugs. Mechanisms underlying these understudied natural product-drug interactions can be pharmacokinetic (what the body does to the drug or natural product), pharmacodynamic (what the drug or natural product does to the body), or both. Common pharmacokinetic mechanisms include inhibition of drug metabolizing enzymes and transport proteins by the natural product, leading to enhanced or reduced systemic concentrations of the object drug and potentially, harmful effects or reduced efficacy of the drug. The aims of this translational research project are to (1) determine key kinetic or binding parameters or dissolution profiles of constituents in a given natural product using human-derived *in vitro* systems, (2) apply the metrics recovered in Aim 1 to mechanistic static models to make *in vivo* predictions, (3) conduct clinical natural product-drug interaction studies involving human participants to evaluate the predictions made in Aim 2, and (4) determine the human pharmacokinetics of natural product constituents and object drugs using plasma and urine obtained from Aim 3.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:
Dr. Paine will work with the fellow to develop a project related to one or more of the four research aims based on the fellow's interests and skillset. An experienced member of the research team (graduate student, post-doc, or clinical research assistant) will work with the fellow directly. The fellow is expected to become semi- or fully independent by the end of the summer.

MENTORING PLAN:
The fellow will meet with Dr. Paine regularly to monitor progress. In addition to participating in various SURF activities, the fellow will interact daily with group members and participate in group meetings.

FACULTY MENTOR:
Bhagwat Prasad, Ph.D., Associate Professor of Pharmaceutical Sciences, *ASPET member*
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RESEARCH PROJECT:
Interindividual differences in drug disposition and response

TECHNIQUES TO BE LEARNED:
- Mass spectrometry-based proteomics and metabolomics
- Bioanalysis of small molecules in biological samples
- Cell culture
- In vitro drug metabolism and transport
- In silico pharmacokinetic modeling

DESCRIPTION OF PROJECT AND AIMS:
The research in the laboratory of Bhagwat Prasad focuses on the prediction of inter-individual differences in drug disposition and response. The Prasad lab utilizes quantitative mass spectrometry (targeted and untargeted proteomics and metabolomics) to quantify drug metabolizing enzymes, transporters and endogenous metabolites in human tissues and biofluids. These data along with genomics information and in vitro data are integrated into physiologically-based pharmacokinetic (PBPK) models to predict variability in drug and endobiotic disposition. The integrated genomics-proteomics-metabolomics approach is further applied to discover specific biomarkers of drug metabolizing enzymes and transporters for predicting disposition and response of xeno- and endo-biotics in special populations such as pediatrics where clinical data are not generally available. The SURF project will involve research on the following aims:
1. Analyze effect of selected drugs on proteomics levels of drug metabolizing enzymes and transporters in human intestinal and hepatic cell lines such as LS180 and HepG2 cells.
2. Perform drug metabolism and drug-drug interaction studies on selected drugs in human intestinal and hepatic cell lines such as LS180 and HepG2 cells.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:
The SURF fellow will work under the mentorship of Dr. Prasad and a senior postdoc in Prasad lab on the proposed project. The SURF fellow will receive training on mass spectrometry and in vitro and in silico drug metabolism. The fellow will attend Prasad lab meetings weekly and present his/her research work twice in the lab meetings. The fellow will be encouraged to present the research findings as a poster presentation in scientific meetings.

MENTORING PLAN:
Dr. Prasad meets with all trainees twice weekly in 1-on-1 meetings. In the first meeting, he will review their project goals, weekly research progress, and provide constructive feedback on study design. In the second shorter meeting, trainees are encouraged to ask big picture questions about their projects and review literature. Dr. Prasad encourages his trainees to challenge our hypothesis to encourage critical thinking. All trainees participate in our weekly 2-hr lab meeting that involves presentations by two members and extensive discussions and feedback from everyone. SURF students will be presenting their progress twice during their tenure in the lab meeting. SURF trainees work with a senior PhD student or a postdoc in the lab to get assistance on rigorous planning and execution of the research. All SURF fellows undergo lab-specific safety trainings before beginning any wet-lab research in the lab.

FACULTY MENTOR:

Zhenjia Wang, Ph.D., Associate Professor of Pharmaceutical Sciences
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RESEARCH PROJECT:

Engineering cell-membrane-derived nanovesicles for treating lung diseases

TECHNIQUES TO BE LEARNED:

- Isolation of human neutrophils
- Formulation of neutrophil membrane-derived nanovesicles using nitrogen cavitation techniques
- Characterization of sizes of nanovesicles and drug loading using dynamic light scattering and HPLC
- Utilization of acute lung injury/inflammation mouse models

DESCRIPTION OF PROJECT AND AIMS:

Inflammation is the immune response to eliminate invading pathogens to prevent tissue injury, but excessive vascular inflammation is the pathogenesis of most diseases. Targeting inflammation pathways in vasculature may be an attractive means to manage the host immune responses for prevention of disease development. In this project, we use a mouse lung disease model to examine our hypothesis. Bacterial infection causes acute lung inflammation/injury (ALI) that quickly precipitates acute respiratory distress syndrome (ARDS). Current therapies are lung-protection ventilation and fluid-conservative management. There is no formally recommended pharmacological therapy for ALI/ARDS. Despite advances in care devices and efforts to develop new therapeutics, the mortality is still unacceptably high at 40%. In pathogenesis of ALI/ARDS, vascular inflammation promotes neutrophil adhesion and transmigration into the lungs. Neutrophils, a type of blood circulating leukocytes, bind and adhere to activated lung endothelium via several binding molecules between a neutrophil and an endothelium. Inspired by this unique intercellular interaction, we have created nanovesicles made from the neutrophil membrane. We propose that neutrophil nanovesicles are a new drug delivery platform for delivering drugs to inflamed mouse lungs to control ALI development. Based on our preliminary results uing neutrophil nanovesicle production, intravital microscopy, and LPS- or bacterium-induced acute lung injury mouse models, we have demonstrated the feasibility of this proposal, novel concepts and a great impact in nanomedicine. The aim of this project are as follows:

Aim 1: To determine the properties of human neutrophil membrane formed nanovesicles required for endothelial targeting.

Aim 2: Active loading of NF-κB inhibitors inside nanovesicles for improved therapies of mouse ALI induced by LPS.

Aim 3: Co-delivery of an antibiotic and an anti-inflammatory drug by nanovesicles to enhance bacterial killing and resolve host inflammation in a bacterium-induced ALI mouse model.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

A PhD student will be assigned to work with the SURF fellow daily. The PhD student will teach the summer fellow some basic lab techniques related to this project, so that the fellow can work semi-independently by the end of the summer. The SURF fellow will attend weekly group laboratory meeting, and present literature and results related to his/her/their project.

MENTORING PLAN:

The mentor will set up individual meetings with the SURF fellow weekly to discuss project progress and to troubleshoot. The mentor will advise the SURF trainee to prepare their poster and present their project.

FACULTY MENTOR:

Boyang (Jason) Wu, Ph.D., Associate Professor of Pharmaceutical Sciences, *ASPET member*
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RESEARCH PROJECT:

Novel Role of LMO4 in lethal prostate cancer

TECHNIQUES TO BE LEARNED:

- Cell culture
- DNA and RNA extraction
- Western blotting
- RT-qPCR
- ELISA
- MTS cell proliferation assay

DESCRIPTION OF PROJECT AND AIMS:

This laboratory currently centers on prostate cancer research, with particular interests in exploring the molecular mechanisms underlying the development of aggressive behavior, lineage plasticity, and therapy resistance to antiandrogen and chemotherapeutic drugs in advanced prostate cancers. We recently identified aberrant upregulation of transcription cofactor LIM-domain only protein 4 (LMO4) in established enzalutamide (an antiandrogen drug)-resistant prostate cancer cell lines using RNA-seq approach. LMO4 has been originally implicated in regulating embryonic and neural development by interacting with other transcription factors and shown to play roles in certain types of cancers. Using enzalutamide-resistant cancer cell lines, the aim of this project is to elucidate the molecular mechanism by which LMO4 confers the aggressive growth, lineage plasticity and therapy resistance in prostate cancer cells. We will also determine whether disrupting the nuclear interactome of LMO4 could reverse antiandrogen drug resistance and synergize with other therapies for improved treatment efficacy.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

At the beginning of the summer, the fellow will work closely with Dr. Wu's postdocs to learn basic cell and molecular biology techniques necessary to execute the project in the in vitro setting. After obtaining required training for animal studies, the fellow is also welcome to participate in observing animal experiments and assisting other lab members with animal experimental procedures, such as measurement of tumor growth and scoring body conditions of mice. After the training, the fellow will work semi-independently on their project.

MENTORING PLAN:

The fellow will meet with Dr. Wu twice a week or more frequently as needed to discuss the project-related literature and new discoveries in the field, experimental performance and results, and any problems encountered during project execution. In addition, the fellow will be exposed to reading scientific papers assigned by mentor, which are not only related to the project but also in a broad way to allow the fellow to learn the frontiers of research in prostate cancer specifically and the cancer field in general. The fellow will report research progress and give a research summary presentation to the lab at the end of the summer.

FACULTY MENTOR:
Hui Zhang, PhD, Assistant Professor of Pharmaceutical Sciences
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RESEARCH PROJECT:
Double negative T (DN T) cell-related cancer immunology and immunotherapy

TECHNIQUES TO BE LEARNED:
Depending on the project, the trainee will learn some of the following techniques: cell culture, animal handling, immune cell isolation, flow cytometry, ELISA, molecular biology (DNA, RNA, and protein extraction, RT-PCR, qPCR, western blotting, RNA-Seq, etc.), immunohistochemistry, and immunofluorescence.

DESCRIPTION OF PROJECT AND AIMS:
The research in the Zhang Lab is focused on immunology and cancer biology, with a specific emphasis on DN T cell-related cancer immunology and immunotherapy. Cancer immunotherapy, specifically immune checkpoint blockade (ICB)-based immunotherapy, is currently one of the most effective and promising approaches for cancer treatment. However, only about 20% of cancer patients clinically respond to ICB therapy. The major problem is that lymphocytes, specifically CD8+ T cells, cannot effectively infiltrate the tumor. CD8 T cell infiltration in the tumor relies on type I conventional dendritic cells (cDC1). NK cells play a critical role in cDC1 migration and maturation in tumors. Therefore, finding the factors controlling NK cell infiltration and activation in tumors is the key to improving ICB-based immunotherapy. We recently found that DN T cells are crucial in initiating antitumor immune response. DN T-NK axis controls tumor growth and shapes tumor microenvironment. Our current project aims to dissect the cellular and molecular mechanism of how DN T cells modulate NK and myeloid cells in the tumor to shape tumor microenvironment and antitumor immunity. To this end, we use mouse models of melanoma and breast cancer to study tumor progression in adoptive cell transfer, gene expression in the tumor, and immune cell function of tumor-infiltrating lymphocytes and myeloid cells.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:
At the beginning of the summer, the fellow will work closely with Dr. Zhang and his lab technician to learn some basic and specific procedures, such as animal handling and molecular and cellular techniques. After the training, the fellow will work semi-independently on his/her project.

MENTORING PLAN:
For the first two to three weeks, Dr. Zhang will work with the SURF student daily to teach the student basic lab skills and procedures. Once the student is familiar with and comfortable doing the proposed experiments, the student will work on the research project more independently. However, Dr. Zhang is available anytime if any help is needed. There will be a lab meeting weekly to discuss progress and future directions. From week 6, Dr. Zhang will work with the student to analyze data and prepare a poster/oral presentation.