

MENTORS DIRECTORY

2009 SUMMER RESEARCH INTERNSHIP AND FELLOWSHIP PROGRAM

Offered by the

**West Virginia IDeA Network of Biomedical Research Excellence
(WV-INBRE)**

to be held at

**The Robert C. Byrd Health Sciences Center
Of West Virginia University**

And

Marshall University

Introduction

The WV-INBRE is pleased to offer summer research internships and fellowships to students and faculty, respectively, from colleges and universities participating in the WV-INBRE program. In 2009 the internship/fellowship period will be from June 1 through July 31, with the Summer Research Symposium to be held on July 30th at Marshall University. Listed in this directory are faculty members at West Virginia University Health Sciences Center and Marshall University who have agreed to participate as mentors in the summer internship/fellowship program. Each mentor has submitted a description of the project(s) that is (are) available to interns and fellows in his/her laboratory. Please review these carefully so that you are aware of what is available for summer projects. Some descriptions are more comprehensive than others; therefore, you may want to contact certain mentors for more detail or to ask for clarifications about the opportunities in their labs. In any case, it is a good idea to speak with potential mentors to be sure you understand what will be expected if you work in his/her lab for the summer.

A listing of mentors with a description of their research and the general area of research is presented on pages 3-5. Mentors and project descriptions begin on page 6. Listed for each mentor is an e-mail address, phone number and, where available, a home-page address. The home-page addresses will allow you to learn about the mentors and their research programs.

Application forms are available on the WV-INBRE web site (<http://www.wv-inbre.net>) at a link under **2009 Summer Program. Applications must be submitted by mail or e-mail; direct electronic submission is not available.**

For general questions about the summer internship and fellowship program, or if you have difficulty reaching a mentor, please contact one of the following individuals who are serving summer research program coordinators.

Dr. Mark J. Reasor
Robert C. Byrd Health Sciences Center of West Virginia University
(304) 293-2418
mreasor@hsc.wvu.edu

Dr. Elsa Mangiarua
Joan C. Edwards School of Medicine
Marshall University
(304) 696-6211
mangiaru@marshall.edu

WV-INBRE website: <http://www.wv-inbre.net>

Directory of Mentors – Mentors are listed by their location; the first list contains mentors at the West Virginia University Health Sciences Center and the second list contains mentors at Marshall University

Mentors at the West Virginia University Health Sciences Center

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Dr. James O'Donnell	Behavioral effects of antidepressant drugs	23
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Dr. Peter Perrotta	Proteomics in identifying proteins in complex biological samples	25
Dr. Elena Pugacheva	Cancer research; cell cycle studies	25
Dr. Visvanathan Ramamurthy	Neurodegenerative disease research	26
Dr. Yon Rojanasakul	Cancer cell apoptosis and chemotherapy	27
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WVU Mentor Listing According to Area of Research

Cancer Research: Gibson; Guo; Ivanov; Jiang; Liu; Olson; Pugacheva; Rojanasakul

Cardiovascular Research: Brock, Dick, Frisbee; Hollander; Liu; Morissette; Mustafa Nurkiewicz; Wysolmerski; Yu

Cell & Molecular Biology/Genetics: Gunther; Hillgartner; Konat; Lukomski; Perrotta; Salati; Schaller; Wenger; Wonderlin; Yu

Chemistry/Physics: Gannett

Drug Action and Metabolism: Callery

Immunology: Brundage; Cuff; Schafer; Sheil

Infectious Disease: Lukomski; Olson;

Muscle Research: Alway; Morissette

Neuroscience Research: Berrebi; Dey; Hileman; Konat; O'Donnell; Ramamurthy

Obesity Research: Hileman

Pulmonary Research: Dey; Nurkiewicz; Scuri; Wu

Reproductive Research: Hileman; Naz

Toxicology Research: Schafer; Sheil

Mentors at Marshall University

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Dr. Eric Blough	Cardiovascular function with diabetes and aging; Development of nano-scale protein-based Cargo transport system	37
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Dr. Piyali Dasgupta	Cancer research	39
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Dr. Richard Egleton	Diabetes and the choroid plexus; green tea and the blood-brain barrier	41
Dr. Philippe Georgel	Effects of chromatin on nuclear function	42
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Dr. Gary Rankin	Kidney toxicology; Metabolism of metadone	48
Dr. Nalini Santanam	Adipose tissue biology in obesity; Pain in endometriosis	49
Dr. Vincent Sollars	Biology of bone marrow cells	50
Dr. Monica Valentovic	Liver toxicity from herbal medications; Mechanisms to reduce diabetic renal complications	51

Marshall University Mentor Listing According to Area of Research

Cancer Research: Claudio; Dasgupta; Delidow; Hardman; Niles; Park

Cardiovascular Research: Blough; Mangiarua; McCumbee

Diabetes: Blough; Egleton; Mangiarua; Santanam; Valentovic

Drug Action and Metabolism: Valentovic

Immunology: Sollars

Infectious Disease: Yu

Molecular Biology: Georgel; Yu

Nano-scale Research: Blough

Neuroscience Research: Egleton; Grover

Obesity Research: Mangiarua; McCumbee; Sanatanam

Pain Research: Santanam

Toxicology Research: Rankin; Valentovic

MENTORS FOR THE 2009 WV-INBRE SUMMER INTERNSHIP AND FELLOWSHIP PROGRAM

I. At The West Virginia University Health Sciences Center

Dr. Stephen Alway

Professor and Chair of Exercise Physiology

Robert C. Byrd Health Sciences Center of West Virginia University

salway@hsc.wvu.edu

(304) 293-0772

<http://www.hsc.wvu.edu/som/ep/faculty/>

The following project is available in my laboratory:

Nutritional interventions to reduce muscle loss in aging.

Frailty in the healthy elderly has become a widespread problem central to the care of geriatric populations. The major factors contributing to frailty are age-associated loss of muscle mass and function. The overall goal of our research is to define cellular and molecular mechanisms of actions of muscle specific genes in muscle growth in aging that may be responsible for loss of muscle cells in old animals. We have found an increase in atrophy (muscle loss) and in genes that regulate apoptosis (programmed cell suicide) in skeletal muscles of old animals when subjected to reduced muscle use or injury. Furthermore, loading muscles increasing oxidative stress in muscles of old animals and this leads to muscle loss and apoptosis (cell death) in muscle nuclei. Reducing oxidative stress reduces apoptosis and loss of muscle mass with chronic loading. In this project we will use nutritional means (modified diets) to attempt to disrupt the genes that signal muscle loss pathways. Secondly we will expose rodents to muscle disuse followed by muscle loading. We will then determine if the nutritional interventions will help muscle to repair/recover in the period of reloading. The student or faculty member will learn techniques for: 1) working with animals 2) Assisting in tissue preparation for small animal surgeries 3) sectioning muscle tissue, 4) staining proteins in tissue sections (immunocytochemistry), 5) measuring the levels of genes expressed, 6) measuring protein expression levels from skeletal muscles (western blotting) and 6) measuring mitochondrial enzyme levels in muscles and isolated mitochondria. They will also have the opportunity to participate in experiments and learn techniques to evaluate muscle function in rodents.

Dr. Albert Berrebi

Professor of Otolaryngology and Neurobiology & Anatomy
Director, Neuroscience Graduate Program
Robert C. Byrd Health Sciences Center of West Virginia University
aberrebi@hsc.wvu.edu
(304) 293-2357
<http://www.hsc.wvu.edu/wvucn/People/Berrebi/index.html>

I have a project available for a faculty fellow, or perhaps for a very motivated undergraduate (upperclassmen).

Project: Sound-evoked responses of auditory brainstem neurons.

A powerful technique in neuroscience is to perform electrical recordings of neuronal activity (by directly measuring membrane voltage excursions) in response to specific, well defined sensory stimulation.

In this project we will perform *in-vivo* single-unit extracellular recordings of neurons in the auditory brainstem of rodents, and measure spiking activity (action potentials) as the animal hears computer-generated sound stimuli. We are particularly interested in the responses of neurons in the superior paraolivary nucleus (SPON) whose activity appears profoundly modulated by inhibitory synaptic inputs.

The fellow will work with other lab personnel to penetrate the brain, place the recording pipette in the appropriate location and monitor the neuronal activity. These experiments will help us understand the role of neural inhibition in shaping the response properties of auditory circuits, and contribute in the long term to the development of refined auditory prostheses.

Dr. Robert W. Brock

Associate Professor and Wyeth Research Scholar
Department of Physiology & Pharmacology
Center for Cardiovascular & Respiratory Sciences
West Virginia University School of Medicine
rbrock@hsc.wvu.edu
(304) 293-1518
<http://www.hsc.wvu.edu/circs/Investigators/brock.asp>

Title: Microcirculation and Inflammation Research

Currently, my lab is focused on clarifying the pathogenic mechanisms associated with the microvascular and endothelial dysfunction that accompanies systemic inflammatory conditions. Participants in the WV-INBRE program will work alongside graduate students and faculty to provide insight to ongoing microcirculation research projects. This work involves the use of a variety of research instrumentation and techniques depending on skill level and interest.

Projects include evaluating vascular control and flow regulation in the kidney and liver microcirculation to determine the effect of various interventional strategies on microvascular function. Specific projects that we are currently working on are:

- 1) Kidney vascular protection in diabetes
- 2) Remote organ damage and protection.

Dr. Patrick Callery

Professor and Chair

Department of Basic Pharmaceutical Sciences

Robert C. Byrd Health Sciences Center of West Virginia University

pcallery@hsc.wvu.edu

(304) 293-1482

<http://www.hsc.wvu.edu/sop/bps/faculty/pcallery.html>

Title: Drug Metabolism Research (This project is appropriate for faculty and/or students)

Participants in the INBRE program will work side-by-side with graduate students and faculty to contribute to ongoing drug metabolism research. Projects range from studying the active site of the enzymes that catalyze drug metabolism reactions to the measurement of drug metabolites in humans with phenotypes associated with the potential for drug interactions. These studies are of importance to the understanding of the fate of drugs in the body as it relates to the optimal use of drugs to treat disease.

Participants join an active team of drug metabolism researchers to work with a variety of research instrumentation and techniques depending on skill level and interest. On-the-job training will be provided. Instrumentation includes HPLC, molecular modeling software, and mass spectrometry. Techniques can include enzyme incubations, PCR, computational chemistry and structural proof of metabolites.

Dr. Christopher Cuff

Associate Professor of Microbiology, Immunology and Cell Biology

Robert C. Byrd Health Sciences Center of West Virginia University

ccuff@hsc.wvu.edu

(304) 293-4622

<http://www.hsc.wvu.edu/micro/faculty/cuff.htm>

Immunity to mucosal virus infection: Many pathogens and toxins enter the body through the gastrointestinal or respiratory tract. These organ systems are lined with non-keratinized (mucosal) epithelium, and a complex immune system helps protect these mucosal surfaces against infection. To understand how the immune system works to protect mucosal surfaces, we study the induction, expression, and regulation of immune responses to a virus called respiratory enteric orphan virus (reovirus) in infected mice and human cells. We examine various aspects of innate and adaptive immunity including dendritic cell function, antibody and cytokine responses, and cytotoxic T-cell responses. The techniques we use include cell culture, flow cytometry, ELISA, and PCR-based assays.

Dr. Richard Dey

Professor and Chair of Neurobiology and Anatomy

Robert C. Byrd Health Sciences Center of West Virginia University

rdey@hsc.wvu.edu

(304) 293-5979

<http://anatomy.hsc.wvu.edu/people/default.asp?id=dey>

The research in my lab focuses on neuroanatomical organization of airway innervation, examining interconnections between airway neurons and airway structures (smooth muscle, blood vessels, glands, epithelium), and on determining neuronal responses to inhaled irritants. Different types of nerves including sensory, sympathetic, parasympathetic, and nonadrenergic/noncholinergic supply the trachea and bronchi. Released neurotransmitters mediate bronchial and vascular smooth muscle tone, mucous secretion, coughing, and breathing patterns in normal conditions and produce defensive responses after inhalation of irritant substances. Airway nerves may also contribute to lung diseases like asthma, chronic cough, and chronic obstructive pulmonary disease (COPD). Although there is considerable information regarding the actions of neurotransmitters, such as acetylcholine, norepinephrine, vasoactive intestinal peptide, substance P and nitric oxide, the mechanisms through which airway nerves contribute to asthma and other airway diseases is not clear. Combinations of immunocytochemical, molecular biological, neurophysiological and pharmacological approaches are used to investigate pulmonary neural responses to inhaled irritants such as ozone, a photochemical environmental pollutant, and toluene diisocyanate, a catalyst associated with occupational asthma used in manufacturing polymers.

If you work in my lab, you will be a participant and contributor to regular lab meetings to discuss important papers in the field and share data and ideas about ongoing experiments. The projects will include training and data collection using confocal and fluorescence microscopes, laser capture microdissection, and real time PCR.

Examples of specific projects:

1. Evaluating pulmonary function, smooth muscle responsiveness and neuropeptide production in ferrets exposed to ozone. Opportunity to learn about effects of ozone (an air pollutant) on the airways. You would learn and collect data using immunocytochemistry, use research microscopes, and measure breathing in lab animals.
2. Evaluating sensory neurons in adult rats exposed to occupational irritants (toluene diisocyanate). Do neural tracing between sensory ganglia and the lung or nasal cavity, measure neurotransmitter levels in sensory neurons, collect data using fluorescence microscopy.
3. Study the effect of ozone exposure during early life (2-6 day old rat pup) on the responses to airway irritants in adolescents (28 day old). This project uses similar technical approaches described above, but involves a different question: does exposure to airway irritants in early life cause abnormal responses later. This is intended to investigate the possibility that children are more sensitive to the detrimental effect of airway irritants.

Dr. Gregory Dick

Assistant Professor

Department of Exercise Physiology
Center for Cardiovascular & Respiratory Sciences
Robert C. Byrd Health Sciences Center of West Virginia University
gdick@hsc.wvu.edu
(304) 293-2542

Title: Ion Channel Research

Participants will work with the sponsor on projects related to ion channels and cellular electrophysiology. Ion channels are transmembrane proteins that open and close in response to various stimuli and permit charged molecules to cross the cell membrane. Projects are aimed at identifying ion channels expressed in vascular smooth muscle cells. These studies are relevant to the treatment of hypertension, as ion channels are the targets of therapeutic agents such as K⁺ channel openers and Ca²⁺ channel blockers. Participants join a laboratory using a variety of techniques to address the research questions. No previous experience is needed – only a desire to learn. Experiments include measuring ion channel function with patch clamp techniques (real-time measurement of current crossing the membrane), cell culture and interfering RNA methods, and assessing ion channel genes and proteins with PCR and Western blots.

Dr. Jefferson C. Frisbee

Associate Professor
Department of Physiology and Pharmacology
Center for Interdisciplinary Research in Cardiovascular Sciences
Robert C. Byrd Health Sciences Center
West Virginia University School of Medicine
jfrisbee@hsc.wvu.edu
(304) 293-6527

Translational Research in Cardiovascular Disease

In a nutshell, “translational research” refers to those investigative efforts that are designed to bridge the gap between traditional laboratory procedures involving animal experimentation or the study of cellular preparations to clinical research involving human subjects. Additionally, a second element of translation spans the gap between the study of the individual human subject to the community or environment in which the individual exists and must interact.

Much of the emphasis in our laboratory is focused on the former of these two definitions, where we study cardiovascular function and dysfunction in rodent models of cardiovascular disease risk factors (e.g., obesity, dyslipidemia, hypertension) and how these conditions lead to a progressive impairment in vascular function. Additionally, we

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are also actively engaged in studies in the clinical setting wherein individual patients recruited to our study provide extensive data with regard to their lifestyle and medical history as well as tissue samples for subsequent analyses. These data are then used for our investigations into the genesis, mechanistic bases and outcomes of vascular dysfunction in human subjects with specific patterns of disease risk factors that are so

common in our society today. Through external collaboration, these data collected from the enrolled subjects are then incorporated into larger databases and models for the study of how the individual interacts with his/her community or environment and how that interaction can predispose an individual to the development of cardiovascular disease.

Our laboratory has opportunities for individual students to explore their interests in any of following two areas, as they relate to the development of cardiovascular disease:

1. basic laboratory animal research
2. community and population research

If any or all of these areas are interesting to you and you wish to discuss them further, please do not hesitate to contact us through the WV-INBRE program. We look forward to hearing from you.

Dr. Laura F. Gibson

Professor and Vice Chair for Research

Department of Pediatrics

Robert C. Byrd Health Sciences Center of West Virginia University

lgibson@hsc.wvu.edu

Phone: (304) 293-1547

<http://www.hsc.wvu.edu/som/micro/faculty/gibson.asp>

Chemotherapy induced cell signaling

While significant progress has been made in the treatment of various types of leukemia, there remains disease that is resistant to standard chemotherapy. Leukemic cells not successfully eradicated by treatment often remain viable in the bone marrow, and later begin to grow and contribute to relapse of disease after cessation of treatment.

Relapsed leukemia is often more challenging to treat than that presented initially, and has a much less promising prognosis. This project will include using a model system of bone marrow and leukemic cell co-culture to investigate the protective effects of the marrow on leukemic cells, and investigate strategies to attempt to make the cancer cells more vulnerable to treatment. Students will learn to do tissue culture, Western blot analysis of proteins, flow cytometry, and confocal microscopy during this investigation.

Chemotherapy effects on bone marrow stromal cells

The bone marrow provides a unique setting for development of blood cell formation, with the regulatory components of the marrow that direct production referred to collectively as the "microenvironment". While the microenvironment is not the intended target of chemotherapy, it is exposed to various drugs during treatment, and can suffer damage from them. We are investigating changes in the microenvironment that result from chemotherapeutic insult, and how these changes may negatively impact patient recovery. This work is focused on alteration of SDF-1, VCAM-1 and disruption of the extracellular matrix subsequent to dose escalated chemotherapy. Students involved in this project will learn to do chemotaxis assays, to culture bone marrow stromal cells *in vitro*, and to do a variety of protein analyses including Western blots and ELISA.

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Infiltration of the CNS by leukemic cells

One of the sites in which tumor cells are particularly problematic to treat is the central nervous system. Using a model of the CNS that includes human brain derived endothelial cells and human derived leukemic cells we are investigating the survival

signals that are important in that anatomical site that may diminish the effectiveness of chemotherapy. This project involves tissue culture of a variety of cell types, protein analysis by Western blot and confocal microscopy, and a variety of gene analysis assays. In addition, the student will learn to do cell cycle analysis.

Dr. Mike Gunther

Associate Professor of Biochemistry and Molecular Pharmacology
Robert C. Byrd Health Sciences Center of West Virginia University
mgunther@hsc.wvu.edu

(304) 293-0714

<http://www.hsc.wvu.edu/som/bmp/gunther.htm>

Superoxide dismutase, mitochondria, and Lou Gehrig's Disease.

Work in my laboratory has focused on discovering the molecular mechanism behind development of Amyotrophic lateral sclerosis (ALS) (also known as Lou Gehrig's disease). About 3% of all cases of ALS result from the inheritance of a mutant form of the gene encoding the enzyme copper-zinc superoxide dismutase (SOD). Several lines of experimental evidence support a role for mitochondrial dysfunction in ALS pathogenesis. We have found that expression of the mutant SOD protein (but not the wild type or no SOD protein) in yeast (*Saccharomyces cerevisiae*) results in decreased mitochondrial electron transport activity that is accompanied by decreases in the content of essential heme cofactors (Gunther et al., 2004, Arch. Biochem. Biophys. 431:207-214). Several currently active projects in my laboratory will follow up on those results.

We are currently attempting to determine the mechanism of the decreased mitochondrial electron transport activity in the strains of *S. cerevisiae* that express the mutant human SOD proteins. Those experiments will involve the isolation of mitochondria from the yeast and assaying the isolated mitochondria for correct assembly of the electron transport complexes using 2-dimensional polyacrylamide gel electrophoresis, Western blotting, and physical biochemical techniques. Because we have observed decreased concentrations of the essential heme cofactors in mitochondria of yeast expressing the mutant human SOD proteins, we predict that assembly of the most affected electron transport complexes has been compromised. These experiments will also be continued in recently developed strains of another aerobic yeast that expresses the mutant human SOD1 proteins. We are also in the process of assaying the enzymatic activities of other mitochondrial proteins to determine whether the electron transport chain is the primary target of the mutant human SOD1 proteins.

Dr. Nancy Lan Guo

Assistant Professor

Department of Community Medicine/Mary Babb Randolph Cancer Center

Robert C. Byrd Health Sciences Center of West Virginia University

lquo@hsc.wvu.edu

Phone: (304) 293-6455

<http://www.hsc.wvu.edu/mbrcc/fs/GuoLab/>

Research Areas in My Lab

Expression-based prognosis of Non-small Cell Lung Cancer

Lung cancer is one of the most aggressive cancer types and is the leading cause of cancer-related deaths in industrialized countries. Non-small cell lung cancer counts for about 80% lung cancer incidences. About 25% to 30% of patients with non-small cell lung cancer have stage I disease and receive surgical intervention alone. However, 35% to 50% of patients with stage I non-small cell lung cancers will relapse within 5 years. It is currently impossible to select specific patients at high risk for cancer recurrence for receiving adjuvant chemotherapy. Our previous research identified tumor metastasis-associated genes from genome-wide transcriptional profiles. Students involved in this project will learn to verify expression patterns of the identified candidate biomarkers using real-time RT-PCR, Western blots, and dot-blot assays of patient tumor resections, blood/serum samples, and lung cancer cell lines.

Population-based prognosis of breast cancer recurrence

Most of breast cancer patients with advance disease stage receive chemotherapy. Nevertheless, only about 50% of patients benefit from it. It remains a critical problem to identify patients who need more aggressive treatment. Based on our previous population-based transcriptional study of over 2,000 breast cancer patients, a 28-gene recurrence signature was identified to stratify patients into prognostic groups with distinct disease outcome. This project seeks to identify potential regulators of this gene expression signature. Students involved in this project will validate the gene expression and protein expression patterns of the identified biomarkers using techniques including real-time RT-PCR, Western blots, dot-blots, and immunohistochemistry.

Chemosensitivity and chemoresistance in human cancer cell lines

Accurate assessment of a patient's predisposition to an anti-cancer drug is an important prerequisite in personalized therapy. Currently, there is no reliable clinical test to predict a patient's response to a particular chemotherapeutic agent. In our previous research, genetic markers and protein products were identified to predict cell line chemosensitivity and chemoresistance for a panel of 118 anti-cancer drugs. This panel includes the FDA approved anti-cancer drugs or drugs that are in late development stage or clinical trials. This project will explore molecular changes involved in drug-treated human cancer cell lines. Potential drug delivery vehicles using nanoparticles will be used in the experiments. Students interested in this project will learn cell culture and lysis assays, RT-PCR, Western blots, and techniques using nanoparticles.

Dr. Stan Hileman

Associate Professor of Physiology & Pharmacology

Robert C. Byrd Health Sciences Center of West Virginia University

shileman@hsc.wvu.edu

(304) 293-1502

<http://www.hsc.wvu.edu/wvucn/People/Hileman/index.html>

Our laboratory has two major interests. One is uncovering brain pathways whereby nutrition influences reproduction. Nutrition is a, if not the, major factor regulating reproduction in mammals. Clearly, the effects of inadequate nutrition are exerted primarily through inhibiting gonadotropin releasing-hormone (GnRH) secretion, a hypothalamic decapeptide necessary for reproduction. However, the mechanism(s) whereby nutrition alters GnRH release are not completely known. The goal of this work is to define the neurobiological pathways controlling food intake and how those pathways are integrated into a system whereby nutrition influences GnRH release and fertility. To accomplish this goal, several surgical, endocrine and molecular biology techniques are employed, including assays, in situ hybridization, immunocytochemistry, neuroanatomical tract tracing and RT-PCR, with both rodents and sheep being used as models. In particular, current experiments focus on neural mechanisms whereby certain circulating metabolic signals, such as leptin, may mediate nutrition-induced changes in reproduction as well as examining potential sex-dependent differences in these systems.

A second major interest is testing the hypothesis that control of food intake is sexually differentiated. Obesity is a major health problem in the U.S. and particularly in West Virginia. Since obesity is a contributing factor to such diseases as diabetes and hypertension, a great deal of interest has arisen in defining the neural mechanisms controlling food intake. However, the vast majority of work has been done using male rodents as a model. Our laboratory has shown that brain neuropeptides and circulating hormones differ between males and female mice in response to fasting or a high-fat diet. This strongly suggests that control of food intake is sexually differentiated and may impact the route of pharmaceutical intervention. Current work focuses on determining whether sex steroids play a role and whether sensitivity to the adipose-derived hormone leptin differs according to sex. This involves using techniques such as radioimmunoassay and polymerase-chain reaction measurement of brain neuropeptide levels.

Other current projects in the lab include:

Examining food intake and body weight later in response to stress in adult rats that have been prenatally stressed. The hypothesis for this study is that prenatally stressed rats will be more susceptible to stress-induced alterations in food intake and body weight during adult life. This is a collaborative effort with Dr. Adrienne Salm of the Department of Neurobiology and Anatomy.

Examining the role of obesity/leptin in renal function and cancer cell growth. Our goal is to determine if elevated levels of leptin play a role in cancer cell growth rate. These studies use in vitro cell culture and polymerase chain reaction to measure mRNA levels of desired endpoints. This is a collaborative effort with Dr. Linda Vona-Davis in the Department of Surgery.

Examining the role of cerebrospinal fluid GnRH on sexual behavior. Our goal is to determine if GnRH secreted into the cerebrospinal fluid of the third cerebral ventricle influences sexual behavior of the sheep. This is a collaborative effort with Dr. Heather Billings in the Department of Physiology and Pharmacology.

Dr. Brad Hillgartner

Professor of Biochemistry

Robert C. Byrd Health Sciences Center of West Virginia University

fbhillgartner@hsc.wvu.edu

(304) 293-7751

<http://www.hsc.wvu.edu/som/bmp/hillgartner.asp>

Work in my laboratory focuses on the regulation of genes involved in the development of obesity, diabetes and atherosclerosis. We are currently investigating how nutritional and hormonal factors regulate the expression of genes controlling fatty acid synthesis and fatty acid oxidation. A student intern or fellow participating in these studies would gain experience in a variety of cell and molecular biological techniques, including cell culture, transfection, DNA and RNA analyses, and Western analysis.

Dr. John Hollander

Assistant Professor

Division of Exercise Physiology

Center for Interdisciplinary Research in Cardiovascular Sciences

Robert C. Byrd Health Sciences Center of West Virginia University

jhollander@hsc.wvu.edu

(304) 293-3683

http://www.hsc.wvu.edu/som/ep/faculty/j_hollander.asp

Title: Cardiovascular Research (This project is appropriate for faculty and/or students)

INBRE program participants will work in conjunction with laboratory personnel on projects examining cardiac diseases. Projects in the laboratory focus specifically on understanding the role played by proteins thought to be protective against cardiac ischemia, diabetes, and aging. The goal of these studies is to provide insight into the mechanism of action of these proteins, with the goal of designing therapeutics to treat cardiac disease state.

INBRE participants will interact with graduate students and staff members to answer research questions, using a multidisciplinary approach that includes genetic modification of the heart, cell culture models, and protein analyses. Training will be provided to the participants, which includes molecular cloning, whole heart physiology, RNA, DNA, and protein manipulation, and biochemical analyses.

Dr. Alexey V. Ivanov

Research Assistant Professor

Department of Biochemistry
Mary Babb Randolph Cancer Center
Robert C. Byrd Health Sciences Center of West Virginia University
Email: aivanov@hsc.wvu.edu
Phone: (304) 293-4936
Web: <http://www.hsc.wvu.edu/mbrcc/research/bios/ivanov.asp>

Participants in this INBRE program will join an active and dynamic laboratory at WVU Cancer Center and will work side-by-side with graduate students and faculty to contribute to ongoing research. The laboratory staff is dedicated to providing the best educational experience available.

The focus of cancer research, until the last several years, has largely been on the genetic basis of cancer. However, a mounting body of evidence accumulated for the last ~10 years has indicated that non-mutational (epi-genetic) heritable alterations in gene expression patterns may be as equally important for the evolution of all human cancer types. Specific epigenetic changes, i.e. methylation of DNA and histones in the promoters of many tumor-suppressor genes, which lead to their silencing (shut off of expression), are now well recognized as one of the critical steps in cancer development. However, the identity of specific transcription factors responsible for the initiation of such silencing is currently unknown. The long term objective of our projects is to investigate and characterize KRAB zinc finger transcriptional repressors which are involved in epigenetic silencing in human cancers.

Identification of new epigenetic silencers of KRAB-ZNF family overexpressed in breast cancer.

This project will focus on comprehensive analysis of differential expression of KRAB-ZFP transcriptional repressors, the best candidates for the role of epigenetic silencers, in breast cancer and normal breast cells. Students will learn to do cell culture, immunoprecipitation, Western blot and mass spectrometry analysis of proteins during this investigation.

Role of heterochromatin protein 1 (HP1) in cell differentiation.

This project will investigate specific requirement of HP1 proteins, important epigenetic players, in differentiation of mesenchymal stem cells into chondrocyte, osteoblast, adipocyte and neuronal lineages in *in vitro* cell culture model. Students involved in this project will learn to culture mesenchymal stem cells *in vitro*, to do lentiviral gene transfer and a variety of protein analyses.

Regulation of transcriptional repression by KRAB zinc finger proteins.

This project will focus on identification and studying gene targets for candidate tumor suppressor protein ZNF432, and ZNF263. Students involved in this project will learn to do cell culture, immunoprecipitation, Western blot analysis of proteins and a variety of gene expression analysis assays.

Dr. Bing-Hua Jiang

Associate Professor of Microbiology, Immunology and Cell Biology and
The Mary Babb Randolph Cancer Center
Robert C. Byrd Health Sciences Center of West Virginia University
bhjiang@hsc.wvu.edu
(304) 293-3094

PI 3-kinase and Akt are activated upon the activation of protein tyrosine kinase receptors by growth factors. PI 3-kinase and Akt have been shown to promote cell growth and inhibit cellular apoptosis. The PI 3-kinase signaling pathway has been strongly implicated in human cancer. For instance, the gene coding for the catalytic subunit p110 α of PI 3-kinase is amplified in 50% of human ovarian carcinoma cell lines. The genes coding for Akt1 and Akt2 are amplified in ovarian and breast cancers. As an antagonist of PI 3-kinase, tumor suppressor PTEN is often mutated in various cancers including glioblastoma, endometrial carcinoma, prostate carcinoma, and melanoma. A better understanding of the PI 3-kinase signaling pathway is of great theoretic and therapeutic interests.

Our primary interest is to further investigate the role of PI 3-kinase in cell growth and tumorigenesis as well as to explore potentially new functions of the pathway. Our study demonstrated for the first time PI 3-kinase signaling pathway in angiogenesis. Angiogenesis is required for tumorigenesis and its inhibition represents one of the most promising approaches in cancer therapeutic treatment as well as in certain vascular diseases. We therefore plan to further study the roles of PI 3-kinase and PTEN in angiogenesis. It is known that PI 3-kinase is activated *in vitro* by a variety of growth factors. Several of these growth factors are known to induce angiogenesis. Accordingly, we will determine whether all or only some of the growth factors-mediated angiogenesis requires PI 3-kinase activation *in vivo*. We have also performed representational difference analysis to identify novel Akt targets involved in angiogenesis. We have obtained several new targets that have growth regulatory functions. We will characterize their expression profiles in human cancer, study their regulation patterns by various growth factors and oncogenes, and determine their role in angiogenesis and tumorigenesis. In addition, we have recently found that PI 3-kinase activates expression of hypoxia-inducible factor 1 (HIF-1). HIF-1 plays an important role in normal physiological process as well as in pathological conditions. HIF-1 activity is commonly elevated in human cancer. One project that is available will study how the PI 3-kinase signaling pathway regulates HIF-1 expression, determine whether HIF-1 is regulated by various oncogenes and tumor suppressor genes, and investigate the role of HIF-1 in angiogenesis and carcinogenesis. To study the molecular mechanisms of angiogenesis and tumorigenesis induced by PI 3-kinase signaling, and to develop novel therapeutic agents for many human diseases including cancer.

Depending on the project selected, an intern/fellow will use primary cells and cell lines from animals or humans, to study how PI3K regulates HIF-1 and angiogenesis. He/She will learn cell culture, protein preparation, RNA isolation, Western blot, Northern blot, cell migration and invasion.

Dr. Gregory W. Konat

Professor

Department of Neurobiology and Anatomy

School of Medicine

Robert C. Byrd Health Sciences Center of West Virginia University

gkonat@wvu.edu

(304) 293-0594

Title: Regulation of neuroinflammation

(This project is appropriate for faculty and graduate students)

Inflammation is a deleterious feature of major central nervous system (CNS) pathologies, most notably, stroke, spinal cord or brain injury, and chronic neurodegenerative conditions, such as multiple sclerosis, Alzheimer and Parkinson diseases. Inflammation is mediated by a family of toll-like receptors (TLRs) that recognize a variety of microbial components and substances released from damaged tissues. TLRs are expressed in the CNS by glial cells. The response of glial cells is frequently exaggerated resulting in excessive and prolonged generation of inflammatory substances that damage neurons and other CNS cells. My laboratory seeks to decipher mechanisms that control TLR activity in human glial cells. We have recently shown that the monomeric GTPases of Rho subfamily negatively regulate TLR-mediated expression of inflammatory genes in astrocytes, the major glial cells. Because Rho proteins are activated by signals emanating from many surface receptors, they may act as integrators of various extracellular signals to tailor inflammatory response to actual pathophysiological status of the cells. The elucidation of Rho-mediated feedback mechanisms has immense therapeutic potential to control neuroinflammation through targeted manipulation of TLR signaling pathways.

Participants will work with a variety of research techniques including: culture of human astrocytes; extraction and purification of RNA and proteins; gene expression at the mRNA level by quantitative real-time RT-PCR; gene expression at the protein level by immunoblot and ELISA; immunoprecipitation; enzyme assays.

Dr. Jun Liu

Associate Professor of Physiology and Pharmacology and MBR Cancer Center

Robert C. Byrd Health Sciences Center of West Virginia University

junliu@hsc.wvu.edu

(304) 293-1503

Research interests: My lab focuses on the role of caveolin in tumorigenesis, metastasis and angiogenesis.

1. Tumorigenesis and metastasis: Many features of tumor progression, including increased mitogenic signaling, insensitivity to antigrowth signals, unlimited replication potential, resistant to apoptosis, sustained angiogenesis and elevated invasiveness and motility are influenced by caveolin-1. In the early stages of cancer, caveolin-1 is down-regulated in order to avoid its inhibitory effects on cell growth, whereas its expression is elevated as the cancer advances in order to promote tumor progression. However, the mechanism that regulates caveolin-1 expression during tumor progression remains unclear. My lab has recently identified a novel signaling pathway that governs caveolin-1 up-regulated during epithelial-mesenchymal transition. Furthermore, we have developed animal tumor models and are investigating how caveolin affects the interaction between tumor cells and endothelial cells, a key step for tumor cells invading (intravasation) and exiting (extravasation) blood vessels.

2. Cell signaling, cytoskeleton and cell motility: We have identified a sequence motif that controls caveolin polarity in migrating cells and demonstrated that loss of caveolin polarity impedes cell polarization and directional movement. By using the caveolin depolarization model, we are investigating the role of caveolin in spatial organization of cell signaling, cytoskeleton arrangement and cell migration.

3. Angiogenesis: Angiogenesis, i.e., new blood vessel development, is essential for tumor growth and metastasis. The mechanisms underlying the pathogenesis of neovascularization are not yet fully understood, but involve endothelial cell migration, proliferation and differentiation. Our lab has demonstrated recently that caveolin plays an important role in the regulation of endothelial cell proliferation and directional movement. We hypothesize that caveolin may represent a novel therapeutic target for human cancers.

During training in my lab, students and faculty members will learn the following techniques: gene subcloning, transfection and expression; Western blot analysis; immunofluorescence microscopy; animal tumor models; immunohistochemistry; and cell co-culture system.

Dr. Slawomir Lukomski

Associate Professor

Department of Microbiology, Immunology, and Cell Biology

West Virginia University School of Medicine

slukomski@hsc.wvu.edu

(304) 293-6405

<http://www.hsc.wvu.edu/som/micro/faculty/lukomski.asp>

Streptococcal collagen-like proteins: Structure-Function Relationship

The primary focus of our research is studying the role of the streptococcal collagen-like proteins, Scl, in the pathogenesis of group A *Streptococcus* (GAS). We have characterized Scl structure and domain organization. Recently, we identified several important human molecules that bind Scl in vitro including the complement regulatory protein, factor H (FH) and the low-density lipoprotein (LDL). At present, our research is focused on defining the biological roles of these interactions in terms of host-pathogen interactions. For more information, please, use the link above to access our web site.

Main projects:

1. Scl1-FH mediated immune evasion by rheumatogenic GAS.
2. Involvement of the LDL receptor in the uptake of group A *Streptococcus* by human cells.

Trainee will contribute to one of the ongoing projects depending on the priority. In general, he/she may learn several methodologies used in modern microbiology such as DNA methods (isolation, digestion, gel electrophoresis), PCR amplification, cloning, protein methods (isolation, purification, SDS-PAGE analysis), gene inactivation and immunology techniques.

Dr. Michael Morissette

Assistant Professor

Division of Exercise Physiology

Center for Interdisciplinary Research in Cardiovascular Sciences

Robert C. Byrd Health Sciences Center of West Virginia University

morissettemr@gmail.com

(617) 735-4219

http://www.hsc.wvu.edu/som/ep/faculty/m_morissette.asp

Signal Transduction Pathways in Cardiac Hypertrophy

This WV-INBRE summer research program opportunity would provide an appropriate experience for a student/fellow/intern/or faculty member.

Postnatal heart growth is primarily characterized by an increase in the cell size, or hypertrophy, of cardiomyocytes. Cardiac hypertrophy is thought to be a functionally adaptive response to increased stress or demand on the heart that accompanies pathologic conditions such as hypertension or remodeling after a

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myocardial infarct. Although hypertrophy is initially beneficial, continued growth and remodeling in the heart ultimately leads to heart failure. This contrasts with exercise-induced, or physiological hypertrophy, which does not lead to heart failure.

A student or faculty member would be involved in the elucidation of novel molecular signal transduction pathways that lead to physiologic versus pathologic

cardiac hypertrophy. Depending on the area of interest and expertise of the student or faculty member, the project could include induction of hypertrophy, through exercise training (physiologic) or pressure-overload (pathologic), followed by Western blotting and quantitative real-time PCR to examine changes in proteins, phosphorylation of proteins and transcription. To determine if these changes are causally related to the induction of hypertrophy we will utilize an *in vitro* heart cell-culture model (cardiomyocytes and fibroblasts) along with manipulation of gene expression (via transfection, infection, or siRNA).

The student or faculty member may also be involved in understanding the signal transduction pathways downstream of a well-known inhibitor of skeletal muscle growth, myostatin. We have recently found a role for myostatin in the regulation of heart growth as well. This project would include the techniques mentioned above, as well as the utilization of transgenic models that have enhanced or inhibited myostatin activity specifically in the heart.

Dr. S. Jamal Mustafa

Assistant Dean for Research

Professor of Physiology and Pharmacology
and Basic Pharmaceutical Sciences

Robert C. Byrd Health Sciences Center of West Virginia University

smustafa@hsc.wvu.edu

(304)-293-5830

<http://www.hsc.wvu.edu/circs/investigators/mustafa/>

The following project is available in my laboratory:

Involvement of A_{2B} Adenosine Receptor in Nitric Oxide Production in Coronary Endothelial Cells from A_{2B} Knockout mice.

Adenosine, a purine nucleoside acts through its cell surface receptors namely A₁, A_{2A}, A_{2B}, and A₃ via its coupling to G-proteins. Adenosine causes dilation of the coronary artery mostly through A_{2A} adenosine receptor. However, the involvement of other adenosine receptors in the modulation of coronary artery relaxation is not known. Recently, we have indirectly shown the involvement of A_{2B} adenosine receptor in endothelium-dependent relaxation of porcine coronary artery possibly through nitric oxide (NO). Also, both porcine and human coronary endothelial cells showed an expected PCR product size for A_{2A} and A_{2B} adenosine receptors. This was further confirmed by western blots for and A_{2A} and A_{2B} adenosine receptors. Our recent data using the A_{2A} adenosine receptor knockout mouse support indirectly the involvement of A_{2B} receptor in coronary flow regulation.

This study will directly address the role of endothelial derived mediators including NO from the mouse coronary endothelial cells from the A_{2B} knockout and wild type animals. Recently, we have successfully established a protocol for isolating mouse coronary endothelial cells and maintaining them in culture. Using these cells in culture, we will

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activate the various adenosine receptors using selective agonists to characterize the A_{2B} receptor. NO will be measured as nitrite using the Griess reaction. The expression of various adenosine receptors will also be measured using Real-Time PCR. This will be confirmed by western blot using specific antibodies for adenosine receptors. It is expected that the data generated from this study will directly support the role of endothelial A_{2B} adenosine receptor and its role in coronary flow regulation by adenosine.

Dr. Rajesh K. Naz

Professor of Obstetrics and Gynecology
Professor of Microbiology, Immunology and Cell Biology
Professor of Physiology and Pharmacology
Research Director Reproductive Biology
Robert C. Byrd Health Sciences Center of West Virginia University
Rnaz@hsc.wvu.edu
(304)-293-2554/1570/5631
<http://www.hsc.wvu.edu/som/micro/faculty/naz.asp>

We have two projects for summer for WV-INBRE students.

Project 1: Development of a peptide/DNA vaccine based upon sperm antigens for contraception

Our laboratory is actively involved in the development of a vaccine targeting sperm for contraception for humans. One of the crucial steps in the vaccine development is identifying antigens that are specifically expressed only on sperm and no other somatic tissue, and has a role in fertilization and fertility. We have identified two such antigens namely FA-1 antigen and YLP₁₂ peptide on human sperm that are involved in binding to the human eggs. The genes encoding these molecules have also been cloned from human testes. The vaccines using these antigens cause up to 70% contraceptive effect in female mice, the model used for testing the efficacy of the vaccine. To increase the efficacy of the vaccine, we are continuing our search to identify/clone/sequence additional antigens that are sperm-specific and have a role in fertilization. These projects will be continued during summer 2009. The student will have the opportunity to work/associate with these projects and learn the following techniques: 1) isolation of RNA/DNA, 2) polymerase chain reaction, 3) cloning and sequencing, 4) in-vitro expression of protein, 5) semen analysis, 6) immunization of animals, and 7) sperm functional test using immunocytochemistry and fluorescent microscopy.

Project 2: Human antibody engineering for development of specific diagnostics and non-steroidal immunocontraceptives

Phage display technology has been widely used to obtain a variety of engineered antibodies, including single chain variable fragment (scFv) antibodies against several antigens. ScFv is an antibody fragment that plays a major role in the antigen-binding activity, and it composed of variable heavy (VH) and variable light (VL) chains connected by a peptide linker.

We have synthesized fully functional human scFv using lymphocytes from infertile men and women in vitro that react with human sperm and block its function. These antibodies are being investigated as immunocontraceptives which will not have any side effects. This project will be ongoing during summer 2009. The student will have the opportunity to work/associate with the graduate/post-doctoral fellow doing this project. The student will learn several molecular biology, immunological, and reproductive biology techniques involved in this project.

Dr. Timothy R. Nurkiewicz

Assistant Professor of Physiology and Pharmacology
Center of Interdisciplinary Research in Cardiovascular Science
Robert C. Byrd Health Sciences Center
West Virginia University, School of Medicine
tnurkiewicz@hsc.wvu.edu

(304) 293-7328

<http://www.hsc.wvu.edu/circs/Investigators/nurkiewicz.asp>

Project Title: Airborne Particles and Systemic Microvascular Endothelial Dysfunction

Evidence indicates that acute exposure to airborne pollutants such as particulate matter (PM) increases the risk of pulmonary and cardiovascular morbidity and mortality. This implies that PM affects extra-pulmonary tissues, as evidenced by the occurrence of cardiovascular dysfunction on high pollution days. However, the biological mechanisms by which PM evokes systemic effects remain to be defined. Despite its obvious importance in regulating the delivery of cells and molecules to all tissues, and in the etiology of most cardiovascular diseases, no research has investigated how systemic microvascular function is affected by pulmonary PM exposure. Our preliminary observations in the rat spinotrapezius muscle indicate that endothelium-dependent arteriolar dilation is significantly impaired after pulmonary residual oil fly ash exposure, and this impairment is associated with microvascular oxidative stress. Interestingly, this systemic microvascular effect can occur independent of pulmonary inflammation. My central hypothesis is that acute PM exposure affects peripheral microvascular function, and this effect is achieved by local reactive oxygen species production and/or altered neurogenic input to the systemic microcirculation. A fundamental understanding of these mechanisms is vital in preventing and treating the life-threatening events associated with air pollution. The student or faculty member will have the opportunity to develop surgical and experimental techniques associated with animal studies. These techniques include: rat surgery, intravital microscopy, in vivo measurement of oxidative stress and various micropipette-based techniques.

Dr. James M. O'Donnell

Assistant Dean for Research

Professor of Behavioral Medicine & Psychiatry and
Neurobiology & Anatomy

Robert C. Byrd Health Sciences Center of West Virginia University

jodonnell@hsc.wvu.edu

(304) 293-6232

<http://www.hsc.wvu.edu/wvuicn/wvuicn%5Fdr/jodonnell/>

Molecular Psychopharmacology

The effects of antidepressants as well as drugs used to treat other psychiatric illnesses emerge over time with repeated treatment. Many theories have been proposed to account for this observation, including changes in neurotransmitter receptor sensitivity, altered intracellular signaling mechanisms, and growth of new nerve cells in certain regions of the brain. Recent work in our lab has identified important roles for cyclic nucleotide phosphodiesterase enzymes

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(PDEs) and neuronal monoamine transporters as mediators of the long-term behavioral effects of antidepressant and anti-anxiety drugs. Specific ongoing projects include examination of the role of specific subtypes of PDE4 in mediating neurogenesis and antidepressant effects on behavior, the assessment of PDE2 as a pharmacological target for the development of novel anti-anxiety drugs, and examination of the role of norepinephrine and serotonin transporter

regulation in mediating the long-term behavioral effects of antidepressant drugs. All of these projects involve the use of behavioral, neurochemical, and molecular approaches to identify molecular mechanisms that mediate psychopharmacological effects.

Dr. Joan Olson

Associate Professor of Microbiology, Immunology and Cell Biology
Robert C. Byrd Health Sciences Center of West Virginia University
jolson@hsc.wvu.edu
(304) 293-5843
<http://www.hsc.wvu.edu/som/micro/faculty/olson.asp>

Development of Bacterial Based Anti-tumor Therapy

In exploring novel approaches for treatment of cancers, recent studies have re-directed attention to the potential use of microbes as specific anti-tumor agents. Microbial approaches to anti-tumor therapy have developed from the observation that certain microorganisms display selective or preferential accumulation in a tumor environment and can mediate tumor death. Examples of such microorganisms include: i) facultative or obligate anaerobic organisms, such as *Clostridia spp.*, which can proliferate within a hypoxic tumor environment; and ii) *Salmonella typhimurium*, which has been found to preferentially accumulate in tumors by a mechanism that is suspected to involve macrophage delivery.

We observed during studies of the effects of the bacterium, *Pseudomonas aeruginosa*, on eukaryotic cell function, that cancer-derived cells showed a greater sensitivity to the toxic effects of *Pseudomonas* than normal epithelial cells. The goal of this project is to further examine the cellular mechanism associated with the increased sensitivity of tumor cells to *Pseudomonas*.

The project will involve: 1) culturing bacterial and eukaryotic cells (sterile techniques); 2) Western blot analysis to monitor the effects of *Pseudomonas* on tumor derived cell lines; and 3) drug treatment studies to identify host cell processes targeted by the toxic effects of *Pseudomonas*.

Dr. Peter Perrotta

Associate Professor of Pathology
Director of Proteomic Core Facility
Robert C. Byrd Health Sciences Center
West Virginia University

pperrotta@hsc.wvu.edu
(304) 598-4401
[Dr. Perrotta's Web Page](#)

Title: Proteomics (This project is appropriate for students)

Participants in the INBRE program will work side-by-side with faculty and staff at the Proteomics Core Facility at the WVU Health Sciences Center. The projects involve using sophisticated techniques to identify proteins in complex biological samples. On-the-job training is provided so that the student can actively participate in all of the steps that are used to identify proteins. These steps include protein purification, protein separation (2D gels), and mass spectrometry (MS). If the student has a special interest in bioinformatics, the experience can be tailored so that there is increased exposure to the advanced computer-based techniques used to identify proteins from mass spectrometry data. Exposure to proteomic technology is important because these tools are being used more frequently by medical researchers.

[Proteomic Core Laboratory Web Site](#)

<http://www.hsc.wvu.edu/som/pathology/proteomics.asp>

Dr. Elena N. Pugacheva

Assistant Professor

Department of Biochemistry and Molecular Biology

Robert C. Byrd Health Sciences Center of West Virginia University

epugacheva@hsc.wvu.edu

Phone: (304) 293-5295

<http://www.hsc.wvu.edu/mbrcc/fs/PugachevaLab/>

Molecular mechanisms of lung cancer metastasis

While significant progress has been made in the treatment of lung cancer, there remain substantial problems in metastasis treatment. Tumor cells not successfully eliminated by treatment often remain dormant, and later begin to grow and contribute to relapse of disease after cessation of treatment. Relapsed tumors are often more challenging to treat than that presented initially, and has a much less promising prognosis. This project will include using a model system of cultured lung cancer cells of different origin and tumor stage to investigate the effects of adhesion molecules of Cas family on tumor cells migration, invasion and proliferation properties, and investigate strategies to attempt to make the cancer cells more vulnerable to treatment. Students will learn to do tissue culture, Western blot analysis of proteins, migration and invasion assay, flow cytometry, and confocal microscopy during this investigation. Later stages of the project will include animal imaging and immunohistochemistry.

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Regulation of cell cycle progression by Aurora family kinases

The tumor cells provide a unique setting for analysis of cell cycle malfunctions and identification the new regulatory components that direct constitutive cell proliferation. One of the cell cycle stages called mitosis is the most vulnerable stage of cell division. Inhibition of mitosis is the most widely used chemotherapy target. But most of those

inhibitors are not specific for tumor cells. Thus the most promising targets for cancer treatment will be those molecules which specifically expressed in tumor cells. Aurora kinases are mitosis activators. They dramatically overexpressed in almost all types of cancer, but not in normal cells. New small molecule inhibitors were developed to target Aurora kinases, but they have not been effective in treatment of solid tumors. In our laboratory we are investigating changes in the mitosis of epithelial cells derived from solid tumors that result from Aurora inhibitors application, and how these changes may impact tumor growth. This work is focused on alteration of mitotic signaling cascade and disruption of the actin/tubulin cytoskeleton subsequent to dose increase. Students involved in this project will learn to do cell cycle assays, to culture human normal and tumor cells *in vitro*, and to do a variety of protein analyses including Western blots and Immunoprecipitation.

Cell cycle regulation of primary cilium

Primary cilium is a microtubule based protrusion on the apical surface of the almost every cell of human body. The biology of primary cilia was mostly studied on green algae. Little is known about ciliary dynamics in human cell. Most ciliated cells of human body use it as extracellular antenna to sense the microenvironment. Cilium is a dynamic organelle. Primary cilium gets build up and then disassembles depending upon environmental cues like growth factors, Ca²⁺ concentration, and pressure. Cilium gets disassembled during mitosis and the molecular mechanisms of this event are unknown. Tumor cells do not have primary cilium. We hypothesized that cilium is a negative regulator of cell cycle progression and could be potentially used for future drug target therapy. Using a model of the RPE1 cells (retina pigmentosa epithelial cells) that are well characterized ciliated human cells we are investigating the signals that are important for cilia disassembly in the context of mitosis. This project involves tissue culture of a variety of cell types, protein analysis by Western blot and a lot of confocal microscopy. In addition, the student will learn to do live cell imaging.

Dr. Visvanathan Ramamurthy

Assistant Professor
Department of Ophthalmology and Biochemistry
Center for Neuroscience
West Virginia University
ramamurthyv@wvuh.com
(304) 598-6940
<http://www.hsc.wvu.edu/wvucn/people/ramamurthy/>

Title: Molecular and Biochemical basis behind blinding neurodegenerative diseases

The main focus of our research group is to understand the molecular and biochemical basis behind blinding diseases that affect humans. Participants in the INBRE program will work along with graduate students, postdoctoral fellows and faculty to contribute to this National Eye Institute (NEI) funded research. Projects range from studying the role of genes, in which defects result in severe degeneration of neurons to understanding the basic process that underlie the signaling between neurons that are essential in

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transmission of visual signals to visual cortex in brain. Our studies are of importance in understanding of the role of these genes in visual signal transduction and in developing novel therapeutic regimens such as gene therapy and small molecule inhibitors in treating debilitating diseases.

Participants join an active team of experienced researchers to work with a variety of animal models that mimic the diseases and techniques depending on skill level and interest. On-the-job training will be provided. Techniques include creation of transgenic and knock out mouse models, electrophysiology to measure retinal function, molecular techniques such as PCR, cloning, gene expression in prokaryotes and tissue culture cells, protein purification by FPLC, western blotting and enzyme assays.

Dr. Yon Rojanasakul

Professor of Pharmaceutical Sciences

Robert C. Byrd Health Sciences Center of West Virginia University

yrojan@hsc.wvu.edu

(304) 293-1476

<http://www.hsc.wvu.edu/sop/bps/faculty/yrojanasakul.asp>

Cancer Cell Apoptosis and Chemotherapy

Abnormal regulation of apoptosis or programmed cell death is the foundation of neoplastic evolution and cancer development. Most cancer cells have impaired apoptosis regulatory mechanisms and are resistant to chemotherapeutic agents. Therefore, strategies that increase their sensitivity to apoptotic cell death by chemotherapeutic agents would be of great value for cancer treatment. Our laboratory investigates the mechanisms of apoptosis induction by chemotherapeutic and environmental agents with a goal of identifying key molecular targets for drug therapy and disease prevention. The student will learn techniques for 1) growing and transfecting cells in culture, 2) detecting cell apoptosis by microscopic and biochemical methods, and 3) analyzing protein expression by immunoblotting or flow cytometry.

Dr. Lisa Salati

Professor of Biochemistry and Molecular Pharmacology

Robert C. Byrd Health Sciences Center of West Virginia University

lsalati@hsc.wvu.edu

(304) 293-7759

My laboratory studies how gene expression is regulated. My laboratory has discovered a novel way in which this can be regulated: increasing and decreasing the rate of pre-mRNA splicing. To study this interesting step, we use a model gene: glucose-6-phosphate dehydrogenase (G6PD). This enzyme functions in the synthesis of fatty acids in liver and in providing substrates to support growth and protection from oxidative stress in all cell types. Thus, expression of this gene is key to cell growth and development. Our current interests are to determine the cellular signals involved in this regulation. For instance, which of insulin's signaling pathways enhances G6PD gene expression? Do fatty acids inhibit G6PD expression directly or by interfering with the insulin signal transduction pathway? Experiments are currently underway to understand the cellular signals responsible for this regulation. Undergraduate students or faculty working in the laboratory this summer would be involved in these ongoing experiments. This represents an opportunity to study fundamental aspects of how cells function.

Examples of student projects might be:

- subcloning portions of the G6PD gene to provide better DNA constructs for identifying the RNA element
- measuring amounts of intermediates in the insulin signal transduction pathway
- analysis of amounts of G6PD RNA produced in response to various treatments and from various DNA constructs.

These projects would introduce the student to the standard techniques of Cellular and Molecular Biology including restriction enzyme digestion, PCR, transformation, and DNA purification. More advanced techniques would include cell culture, RNA isolation, Western analysis, Northern analysis and probe synthesis.

Faculty members could choose projects that allow them to learn a new technique that would help them in future work. Such projects might include:

- conducting animal studies to understand the role of insulin in regulating G6PD expression.
- maintenance of cell lines in tissue culture and tests of G6PD expression in response to various treatments. These tests would include not only expression of the endogenous gene but also transfected DNA constructs.
- the possibility also exists for faculty members to use protocols already in the laboratory to perform experiments related to their own particular research interests. My laboratory routinely does most standard techniques of Molecular Biology, eukaryotic cell culture including stable and transient transfection, and the specialized techniques of RNA biology.

Dr. Rosana Schafer

Associate Professor of Microbiology, Immunology and Cell Biology
Robert C. Byrd Health Sciences Center of West Virginia University
rschafer@hsc.wvu.edu
(304) 293-3104

A variety of chemicals in the environment have been proposed to impact the immune system via estrogenic or anti-estrogenic effects, commonly referred to as endocrine disrupters. Propanil (3,4-dichloropropionanilide) is a widely used pesticide with immunotoxic effects. Recent studies in our laboratory have demonstrated that propanil enhances the humoral immune response after vaccination of female and male C57Bl/6 mice with heat-killed *Streptococcus pneumoniae* (HKSP). The ovaries are required for this enhancement because ovariectomy completely abolishes it. Interestingly, the enhancement of the immune response by propanil is seen in both intact and castrated male mice. This sexual dimorphism in the response of gonadectomized male and female mice led to our hypothesis that the immune system undergoes sexual differentiation. The preliminary data further demonstrate that propanil requires normal gonadotropic support of the ovaries to stimulate the antibody response in females. Surprisingly, however, the two major ovarian steroids, estradiol and progesterone, are NOT required to enhance the immune response. These data suggest that propanil modulates the female immune system through potentially novel interactions with the endocrine system. Although propanil was initially chosen for study in our laboratory because of its widespread use as a pesticide, the study presented here is intended to use propanil as a model chemical that will contribute to our understanding of interactions between the immune system and the endocrine system. Therefore, in this proposal, propanil will be used as a model environmental agent to investigate a novel estrogen-independent mechanism of endocrine-mediated regulation of the immune system. In addition, based on the differential requirement for gonads in males and females after exposure to propanil, we will examine the potential role for sexual differentiation of the immune system in adult immune responses.

Central hypothesis

The central hypothesis is that early events in development cause sexual differentiation of the immune system that results in the differential requirement for gonads in adult females and males for propanil to enhance the immune response to *S. pneumoniae*.

Specific Aim 1. Contrast the gene expression profiles in the ovaries with those in the testes after exposure to propanil and vaccination with heat-killed *S. pneumoniae*.

Specific Aim 2. Test the hypothesis that sexual differentiation of the immune system influences the effect of propanil on the adult immune response to vaccination with heat-killed *S. pneumoniae*.

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This unique propanil-HKSP vaccination model system will allow us to investigate a novel effect of the ovary on immune function, an important area that is not well understood. In addition, we will test the hypothesis that sexual differentiation of the immune system contributes to the differential requirement for gonads in the

immune response in adult animals after exposure to propanil. The expected outcome of the first specific aim is the identification of genes that are differentially expressed in the ovary and the testes when the animal is exposed to propanil at the time of vaccination with HKSP versus gene expression after vaccination alone. This will establish a basis for future experiments to determine the mechanism by which chemical exposure enhances the immune response to the vaccine and the role of the ovary in the response. In addition, it will provide information on ovarian mediators other than estradiol or progesterone that alter immune responses, which in turn could impact pregnancy and immune-mediated infertility. The expected outcome of the second specific aim is that early events in development affect the sexually dimorphic immune response demonstrated in adults. Overall, the results will form the basis for future research in the development of new diagnostic or therapeutic targets to predict and treat those at risk of developing ovarian disease, immune-mediated infertility, and autoimmune diseases.

Dr. Michael Schaller

Professor and Chair
Department of Biochemistry
West Virginia University School of Medicine
mschaller@hsc.wvu.edu
Phone: (304)293-9514

FAK is an important enzyme that plays a key role in regulating axonal guidance and angiogenesis during embryogenesis and has been implicated in the development of human cancer. Major efforts in the lab are dedicated to determining molecular mechanisms regulating FAK activity and function.

Molecular basis of ligand binding to FAK

We have identified phospholipids as ligands for FAK that lead to its activation. One important goal is to determine how different phospholipids bind to FAK and a mutational approach will be used. Students will learn site-directed mutagenesis, protein expression in bacteria and mammalian cells, Western blotting and fluorometric techniques to measure protein/ligand interactions.

Role of phospholipids in regulating FAK in response to endothelial shear stress

Shear stress is an important factor in the development of atherosclerosis and FAK is activated in response to this mechanical stimulation. Pharmacological, siRNA, dominant negative and overexpression approaches will be used to evaluate the role of several regulators of phospholipids in controlling FAK activation in response to shear stress. Students will learn tissue culture, protein expression in mammalian cells, transfection and Western blotting.

Dr. Mario Scuri

Assistant Professor of Medicine
Department of Pediatrics
West Virginia University School of Medicine
Robert C. Byrd Health Sciences Center

mscuri@hsc.wvu.edu

(304) 293-0511

Cell (304) 276-3159

Project Title: Combined effects of nanoparticles and Respiratory Syncytial Virus on lung development and neurogenic inflammation

Our ongoing research in the field of childhood asthma include projects ranging from the study of the effects of viruses on the plasticity of nerves in the airways to the expression of several mediators of neurogenic inflammation in a rodent model of childhood asthma as well as in human samples. Understanding the basic mechanisms underlying airway diseases in infancy and early childhood is of great importance in the effort to identify therapeutic targets for novel treatments which may prevent or lessen the severity of these pathological conditions in young individuals.

Participants will work with pediatric residents, graduate students and faculty members and will be involved in a number of research techniques that may include RT-PCR, immunohistochemistry, cell culture and animal surgical procedures. Training will also include participation in lab meeting, journal clubs and data management.

Dr. James Sheil

Associate Professor of Microbiology, Immunology and Cell Biology

Robert C. Byrd Health Sciences Center of West Virginia University

jsheil@hsc.wvu.edu

(304) 293-7416

<http://www.hsc.wvu.edu/micro/faculty/sheil.htm>

The major research focus of our laboratory group for faculty and students involved in summer research with the WV-INBRE program is as follows:

A major concern with diagnosing and treating environmental exposure effects due to pesticides is the lack of a definable disease relationship following exposure. We are particularly interested in better understanding the harmful effects of pesticides to the body's immune system and establishing such a disease relationship. Specifically, our research concerns the pesticide, propanil, and its reported adverse effects on cell-mediated immunity. Since exposure to many immunotoxic pesticides (including propanil) results in a variety of ill-defined pathologic effects, it is difficult to correlate pesticide exposure with a particular disease consequence.

Recent studies from our laboratory demonstrate that propanil exposure has a delayed but clearly inhibitory effect on the function of cytotoxic T cells, which are the key effector cells of cell-mediated immunity. An important observation is that the early effects of propanil exposure on cell-mediated immune function are minimal, but the long-term effects after a single initial exposure result in a complete loss of functional reactivity for these cytotoxic T cells. These effects bear a striking resemblance to those seen in a variety of specific disease states – including HIV

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infection and AIDS, cancer, and various chronic virus diseases – which share a common disease mechanism, involving what is known as the PD-1/PD-L1 pathway. This project will examine whether this same disease mechanism is responsible for the observed effects following propanil exposure. ***This study could be especially***

important in terms of its implications for the diagnosis of disease and treatment following exposure to environmental contaminants in general.

Techniques that will be used in these projects include cell culture, flow cytometry, computer modeling, T-cell proliferation assays, mixed lymphocyte response assays, ⁵¹Cr-release assays, and protein purification methods. This research project will be conducted in the context of a larger, interactive laboratory group including 3 faculty collaborators.

Dr. John Thomas

Director, International Tri-University Biofilm Research Consortium¹

Professor, Departments of Pathology and Periodontics

West Virginia University Schools of Medicine and Dentistry

Robert C, Byrd Health Sciences Center-North, Room 2115

JThomas@hsc.wvu.edu

phone: 304.293.3204 fax: 304.293.1627

<http://www.hsc.wvu.edu/som/pathology/thomas/>

BIOFILMS (Anti-Koch/Anti-Pasteur)

Microorganisms exist as free living phenotypes (planktonic) and attached phenotypes (biofilms), never mutually exclusive. However, given the opportunity, 99.9% of microbes prefer the biofilm phenotype, given its legacy of survival, emphasizing microbial diversity, heterogeneity and co-aggregation. Biofilms emphasize cooperation (the “We” concept (Metchnikoff) vs. the “I” concept (Pasteur)).

Recently, a growing list of human diseases, both oral and medical, have been linked to biofilms and hastened further understanding of their mechanisms of communication (Quorum Sensing), 3-D structures and methods of avoidance of the immune system; means of treatment is still lacking.

Students will learn extensive molecular and non-molecular (culture) techniques, advanced imaging and CT-radiography to unmask this unique community via four divergent, but connected areas of translational research (listed below), working simultaneously with investigators and students from Cardiff University, Wales, UK and the National University of Singapore, Singapore. Given the translational nature of our research, students may spend time with physicians in the ICU

1. Hospital Associated Pneumonia / Ventilator Associated Pneumonia (VAP)

The most costly Nosocomial infection in the ICU is VAP, adding upwards of 15 days and \$150,000 per patient while utilizing 50% of the ICU resources. Our investigations focus on the endotrach as a “biofilm engine”, emphasizing poor oral hygiene. In addition to evaluating impact of a silver coated endotrach using a unique VEL Model (Ventilator-Endotrach-Lung), (<http://www.hsc.wvu.edu/biofilms/velModel/index.asp>) we are currently very interested in defining the relative rate of genetic exchange (antibiotic resistance) under the growth conditions of an endotracheal biofilm during Mechanical Ventilation for a patient with COPD or ARDS.

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¹Cardiff University, School of Dental Medicine, Cardiff, Wales, UK.
National University of Singapore, School of Dentistry, Singapore.

2. Triphasic Chronic Wound Model

Growing evidence links biofilms to chronic wounds and the lack of tissue regeneration and wound closure. Using methods refined with the VEL Model (above) and Cardiff University, Department of Tissue Engineering and Regenerative Science, we have developed a triphasic wound model, using both procaryotic (bacteria) and eucaryotic (human) cells, which are layered to mimic a wound environment, to study the interface between the two, and the potential use of “replacement therapy” (probiotics) to initiate closure. Students will learn histologic techniques, cell culture, biomarkers and biofilm 3-D imaging with CLSM (Confocal Laser Scanning Microscopy).

Dr. Sharon Wenger

Professor of Pathology

Robert C. Byrd Health Sciences Center of West Virginia University

swenger@hsc.wvu.edu

(304) 293-0446

<http://www.hsc.wvu.edu/som/pathology/Facultybio/wenger.asp>

Two projects are available for summer interns.

One project in the cytogenetics research laboratory is to identify the underlying cause of pregnancy loss. About half of these losses are due to chromosomal abnormalities, however it is not always possible to obtain viable tissue. The proposed study will examine tissue that has been saved from pregnancy losses that could not be cultured for metaphase cells due to non-viability. The tissue will be analyzed using fluorescence in situ hybridization (FISH) DNA probes, a technique that targets specific areas of chromosomes. Various FISH probes for different chromosomes commonly involved in pregnancy losses will be used on saved tissue to help identify the presence of chromosome abnormalities. In addition, previous records in the laboratory will be tabulated in order to identify recurrent chromosomal abnormalities.

The second project is the study of chromosomally normal and abnormal cells in an individual, known as mosaicism. As an individual with mosaicism ages, the abnormal cell line disappears from the blood. The purpose of the project is to determine if the telomere length differs between the two cell lines, providing an explanation for loss of the abnormal cell line (shorter telomeres). Telomeres cap the ends of all chromosomes and shorten each time a cell divides. At some point, the telomeres become very short, at which the cell stops dividing and dies. Telomere length will be measured by size of the fluorescent signal at the ends of chromosomes using a computer image capturing system.

Protocols that may be used for these projects include sterile culture technique, harvesting cells for chromosomes, preparing slides, hybridization of probes to chromosome preparations. Equipment that will be used include centrifuge, automatic pipeters, water bath, slide warmer, inverted, light and fluorescent microscopes, digital imaging system, and computer program for visualization and measurement of fluorescent signals. Collection of data will also be necessary.

Dr. William Wonderlin

Associate Professor of Biochemistry and Molecular Pharmacology

Robert C. Byrd Health Sciences Center of West Virginia University
wwonderlin@hsc.wvu.edu
(304) 293-3159
<http://www.hsc.wvu.edu/som/bmp/>

The following project is available in my laboratory:

Measuring the permeability of the endoplasmic reticulum to small molecules.

The endoplasmic reticulum (ER) is an intracellular compartment that plays an essential role in signal transduction, the synthesis of secretory and integral membrane proteins, and cellular homeostasis. Recent studies from our laboratory have indicated that small molecules can move between the cytosol and lumen of the rough ER via the same pathway used by newly-synthesized proteins—the pore of a ribosome-bound translocon complex. Our long-term goal is to understand how changes in the molecular traffic through a ribosome-bound translocon can be altered during disease, potentially leading to cellular stress and cell death. We have preliminary data indicating that these ribosome-bound translocons are stimulated by aggregates of proteins formed during the development of neurodegenerative diseases such as Parkinson's Disease, and we hypothesize that the increased "leakiness" of the endoplasmic reticulum might contribute to neuronal death. The summer project will focus on identifying the mechanisms whereby protein aggregates can stimulate the opening of ribosome-bound translocons and how that stimulation affects the endoplasmic reticulum. This project will provide opportunities to learn techniques in cell biology, protein biochemistry, fluorescence assays, and cell culture.

Dr. Zhongxin Wu

Assistant Professor
Neurobiology and Anatomy
Robert C. Byrd Health Sciences Center of West Virginia University
zwu@hsc.wvu.edu
(304) 293-7222

There are abundant epidemiological studies linking embryonic and early postnatal environmental tobacco smoke (ETS) exposure with childhood asthma and wheezing, but the underlying mechanisms that occurs in utero and early postnatal periods to explain this link remains unknown. Human lung development consists of five stages: embryonic phase, pseudoglandular phase, canalicular phase, terminal sac phase and alveolar phase. During these periods, growth of epithelial and connective tissues, blood vessels and nerves is highly coordinated in order to maintain the normal structural and functional relationships of the respiratory system. Neurotrophic factors, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are essential in promoting and maintaining differentiation, growth, and survival of the central and peripheral nervous systems, including innervation of the airways. Disruption of normal synthesis and release of these neurotrophic factors and resulting changes in airway innervation after inhalation of toxic material is well documented in adult lungs and leads to disease-related abnormalities in respiratory system. The goal of this projects to study effects of prenatal, neonatal and early postnatal ETS exposure on lung function, neurotrophin expression and the resulting altered control of airway innervation.

Dr. Robert Wysolmerski

Professor of Neurobiology and Anatomy
West Virginia University Health Sciences Center
rwysolmerski@hsc.wvu.edu
(304) 293-2213

The overall aim of the laboratory is to define the events that initiate and regulate endothelial cell contraction in an effort to elucidate the basis of increased vascular permeability. The primary hypothesis underlying the proposed work dictates that exposure to inflammatory mediators activates the endothelial cell actomyosin based contractile system. Calcium (Ca^{2+}) dependent and/or Ca^{2+} -independent stimulated phosphorylation of the myosin II regulatory light chain results in myosin II filament formation, an obligatory step leading to endothelial cell motility and contraction. In nonmuscle cells, myosin II is a major cytoskeletal protein which has the ability to convert the chemical energy of ATP into the mechanical work of cell contraction through its interaction with actin. Myosin light chain kinase (MLCK) -catalyzed phosphorylation results in mono- and diphosphorylation of myosin II regulatory light chains at site Ser19 or sites Ser19 and Thr18, respectively. In endothelial cells, we have shown that a small GTPase dependent enzyme, p-21 activated kinase (PAK2), catalyzes Ca^{2+} independent activation of nonmuscle myosin II by phosphorylation of the regulatory light chain that is restricted to site Ser19. This PAK2-mediated monophosphorylation results in a less forceful contractile response than when the regulatory light chain is diphosphorylated by MLCK. More recent studies have shown that PAK2 also phosphorylates unactivated MLCK which results in inhibition of MLCK activation by Ca^{2+} .

The goal of our studies is to biochemically and morphologically characterize myosin II activation by Ca^{2+} -dependent and Ca^{2+} -independent signaling pathways in endothelial cells. The working hypothesis is that a specific pools of myosin II, determined by the heavy chain isoform specificity (myosin IIA vs IIB vs IIC), are activated by enzyme specific modifications. Functionally, regulated differential phosphorylation of myosin II would allow the endothelial cell to react to a variety of physiological signals with graded contractile responses.

Projects:

- 1) Characterize myosin II heavy chain isoform function. Studies will establish the localization and states of phosphorylation of myosin IIA and IIB in endothelial cell spreading, migration and in agonist stimulated confluent monolayers.
- 2) Investigate the role of Rho-GTPases in endothelial cell contraction. Experiments will be carried out to assess the role of Rho-kinase vs MLCK in basal and agonist stimulated isometric force development.

Student researchers will be exposed to the following techniques in the laboratory: 1) casting 3-D tissue constructs, 2) measuring nonmuscle isometric force production, 3) confocal microscopy, 4) cell motility measurements 5) tissue culture methods for establishing and maintaining endothelial cells, 6) western blots, and 7) transient transfection of endothelial cells.

Dr. Han-Gang Yu
Associate Professor of Physiology & Pharmacology

Robert C. Byrd Health Sciences Center of West Virginia University
hyu@hsc.wvu.edu
(304) 293-2324

Project-1: Cardiac Pacemaker Ion Channels

The human heart beats continuously close to 3 billion times in a normal lifetime (70 beats/min, 80 years life expectancy). This never-stop rhythm is generated due to the concerted interactions among a number of specialized proteins called ion channels that sit across the cell membrane. Dysfunction of ion channels (Na^+ , K^+ , Ca^{2+} , etc.) that generate electrical activities in cardiac muscle is the primary cause of many forms of cardiac arrhythmias.

The central interests in my lab lie in (1) how those ion channels work, (2) why the channels behave abnormally in pathological conditions, and (3) normalize the arrhythmias by correcting the dysfunctional ion channel behavior using gene therapy strategies.

Project-2: Novel Mechanisms in Controlling Calcium Influx

Calcium entry into the cells is the primary triggering signal for nearly all the physiological responses. Calcium influx is thus tightly controlled by a number of membrane and cytosolic proteins. Disruption of the fine modulation of calcium influx is the primary cause for diseases such as atrial fibrillation and deteriorated muscle contraction in heart failure.

We have recently discovered a novel mechanism that controls the Ca^{2+} entry into the cell. We are currently focusing on how this new mechanism can render an additional protection to the cardiac performance under physiological and pathological conditions.

Students participating in the projects will have the opportunities to learn a variety of experimental techniques including single-cell patch-clamping, cell culturing, viral-based DNA recombination, cardiac myocytes isolation and viral infection, DNA analysis (restriction enzyme digest, electrophoresis, sequence analysis, PCR mutagenesis, RT-PCR), protein chemistry (Western blotting, co-immunoprecipitation), cell biology (immunohistochemistry, fluorescence imaging), and RNA Interference.

Given the background, interested students will be assigned a specific small project such as (1) ion channel trafficking to plasma membrane, or apoptosis in cultured mammalian cells caused (2) altered protein tyrosine phosphorylation state or (3) ion channel mutants.

II. Mentors at Marshall University

Dr. Eric Blough

Associate Professor

Department of Biological Sciences

Marshall University

blough@marshall.edu

(304) 696-2708

Website: <http://www.science.marshall.edu/blough/>

Participants in the INBRE program will join an active and dynamic laboratory and will work side-by-side with graduate students, post doctoral fellows and faculty to contribute to ongoing physiological research. The laboratory staff is dedicated to providing the best possible educational experience available. Two projects are available. Each project is appropriate for faculty and/or students. On-the-job training will be provided.

Title: The effects of gender on cardiovascular function with diabetes and aging. The long term objective of this project is to investigate how gender influences cardiovascular (vascular, cardiac) structure, intracellular signaling and function. These questions are addressed using a variety of different models (animal, cell culture, ex-vivo tissue preparations) along with molecular (RT-PCR, immunoblotting, protein isolation), morphological and physiological (Echo, EKG, muscle physiology) tools. It is anticipated that the data gleaned from these studies will provide important knowledge regarding the etiology and treatment of cardiovascular dysfunction.

Title: Development of a nano-scale protein based cargo transport system. The long term objective of this project is to investigate the potential of using electrical and / or magnetic fields to build a bio-molecular transporter (using the muscle proteins actin and myosin filaments) that could be used to deliver molecular cargo to addressable coordinates on a micro-patterned surface (e.g. a molecular conveyor system).

Dr. Pier Paolo Claudio

Associate Professor
Department of Biochemistry and Microbiology
& Dept. of Surgery
Marshall University,
Huntington,
West Virginia

claudiop@marshall.edu

Phone: (304) 696-3516

http://www.bms.marshall.edu/research_groups/cancer_biology/claudioresearchsummary.aspx

The focus of our laboratory is to understand the molecular mechanisms governing malignant transformation in order to tailor novel therapeutic strategies. Toward this end, we have carried out in the past 15 years studies to understand the crosstalk between those factors that contribute to cancer progression versus those that protect from it.

Gene therapy offers great potential for combating and curing a wide range of pathologic lesions. One of the major limiting factors in gene therapy has been the

Imaging guided drug delivery

The emphasis of our most recent research efforts is on imaging guided drug delivery. The recent emergence of "molecular imaging" has set the stage for an evolutionary jump in diagnostic imaging and therapy. The ability to incorporate drugs or genes into detectable site-targeted nanosystems represents a new paradigm in therapeutics that will usher in an era of image-based drug delivery.

We have developed a novel gene therapy system based on the use of commercially available ultrasound contrast agents and adenoviruses that enhance the specificity of gene transfer *in vitro* as well *in vivo*. Ultrasound-mediated microbubble destruction improves the efficacy and reduces the non-specific expression of gene therapy vectors providing a useful tool for manipulating gene expression in the living animal. We are currently working on further developing this useful targeting gene therapy tool to help closing the gap that still exist between laboratory bench and bedside application.

Students involved in this project will experience working with animals and with human cancer cells. Students will learn novel techniques aimed at eradicating growth of cancer cells *in vivo*. Students will also participate in the analysis of the molecular mechanisms underlying tumor growth and progression.

Dr. Piyali Dasgupta

Assistant Professor

Department of Pharmacology, Physiology & Toxicology

Joan C Edwards School of Medicine

Marshall University

dasgupta@marshall.edu

(304) 696-7321

The following projects are available in my laboratory:

1. **Role of nicotine in lung cancer progression:** Smoking bears a strong correlation to the development of lung cancer. In our laboratory we study the signaling pathways underlying the proliferative effects of nicotine. We determine the effect of nicotine the growth of lung cancer cells, their angiogenesis and invasive behavior. Students working on this project will learn (i) to measure the effects of nicotine on the growth of lung cancer cells (ii) the potential of certain compounds to inhibit the effects of nicotine and thereby suppress the growth of human lung cancers
2. **Anticancer Activity of capsaicin:** Capsaicin is the major active ingredient of chili peppers. Preliminary data in our laboratory shows that capsaicin manifests anti-cancer activity in human small cell lung cancer. We plan to extend these studies and determine if capsaicin can attenuate the growth of human brain tumors. Gliomas are a highly aggressive type of human brain cancer. We are interested in investigating whether capsaicin can attenuate the growth of human glioma cells. If you are interested in this project, you will learn (i) to perform specific assays to determine whether capsaicin can inhibit the growth of glioma cells (ii) to examine which molecular pathways contribute to this process

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

Dr. Beverly Delidow

Associate Professor of Biochemistry and Molecular Biology

Joan C. Edwards School of Medicine

Marshall University

delidow@marshall.edu

(304) 696-7266

http://bms.marshall.edu/research_groups/cancer_biology/beverly_delidow.aspx

1. Role of β -catenin in melanoma. The incidence of melanoma has increased to an alarming degree in recent years. While early melanoma is both preventable and treatable, later stages of this disease are more difficult to manage. β -catenin is a signaling protein well-known to play a central role in several cancers, however comprehensive study of the role of β -catenin in melanoma is lacking. We are examining the location and action of β -catenin in mouse and human melanoma cell lines, as well as its response to the anti-tumor agent, retinoic acid. Our data indicate that retinoic acid reduces the tumor-promoting activity of β -catenin by several mechanisms within melanoma cells. The summer researcher would be invited to participate in experiments to examine the biological activity of β -catenin in melanocytes and melanoma cells by a number of means. The likely techniques would include subcellular fractionation and western blotting, fluorescent immunocytochemistry, RNA isolation, real-time PCR, transfection and reporter gene assays.

2. Regulation of pituitary cell function by the multifunctional protein, β -catenin. β -catenin performs functions as both a cell adhesion molecule and as a transcriptional regulator. We have evidence that pituitary tumor cells require β -catenin to maintain high levels of prolactin production. This is of interest for two reasons. 1. Hyperprolactinemia results in reproductive dysfunction and is one of the presenting symptoms of prolactin-secreting tumors. These tumors are currently usually controlled by drugs that have significant side effects. Finding an alternative means of treatment would be an advantage. 2. The pituitary tumor cells use prolactin as an autocrine growth factor and will grow continuously. Treatment to control prolactin levels usually controls tumor growth, but a small percentage of tumors escape drug sensitivity. Controlling that growth is critical to treatment of pituitary tumors because of their location next to the optic nerve and brain vasculature. We are currently exploring the mechanism of the link between β -catenin and prolactin gene expression, using a variety of cellular and molecular techniques. A summer researcher joining this project would learn cell culture, transfection and reporter gene assays, subcellular fractionation, western blotting and RT-PCR.

Dr. Richard D. Egleton

Assistant Professor of Pharmacology, Physiology and Toxicology

Joan C. Edwards School of Medicine

Marshall University

egleton@marshall.edu

(304) 696-3523

<http://regleton.googlepages.com/>

The following projects are available in my laboratory:

Project #1: Diabetes and the Choroid plexus.

The choroid plexus is a region of the brain that produces most of the brains fluids. During diabetes there is an increased risk of hydrocephalus (water on the brain). In this project we will investigate the molecular changes in the choroid plexus induced in an animal model of diabetes. This project will involve some animal handling, tissue sampling, Western blot analysis, immunofluorescence microscopy, and real-time PCR, as well as using various pieces of equipment to monitor blood glucose and ion concentrations.

Project #2: Green tea and the blood brain barrier.

The blood brain barrier (BBB) protects the brain from various toxins, and promotes optimal conditions for neuronal function. Green tea and its constituents have been promoted as a potential co-therapy in a number of diseases including cancer. There is evidence that EGCG a major constituent of green tea can alter the metabolism of several drugs by regulating the expression of transporters and metabolizing enzymes. The BBB expresses a number of these transporters and enzymes, a change in BBB expression could lead to significant changes in brain delivery of drugs. This project will investigate the effects of EGCG on BBB expression of proteins involved in metabolism and excretion of drugs. This project will involve tissue culture, Western blot analysis, immunofluorescence microscopy, real-time PCR, transport studies and some HPLC.

Instrumentation:

These projects may involve using fluorescent and UV plate readers, real-time PCR, microscopy, blood gas analyzers, glucometers, gel rigs, HPLC, centrifuges, balances and other standard lab equipment.

Dr. Philippe Georgel

Marshall University
Department of Biological Sciences and Department of Biochemistry
Marshall University
304-696-3965
georgel@marshall.edu
<http://mupfc.marshall.edu/~georgel>

Research in my laboratory is centered on the effects of chromatin on nuclear functions, with an emphasis on transcription regulation linked to epigenetic modifications. Epigenomic research pertains to studies investigating changes in the regulation of gene Expression that reflect altered states of DNA organization rather than direct changes in DNA sequence. Human DNA is packaged into repeated units of nucleoproteins (DNA plus histones) referred to as chromatin. It has long been established that both chromatin remodeling and the equilibrium between chromatin folding and unfolding act as regulating mechanisms of gene activation or repression. We recently designed a method that allows us to make physical measurements of defined chromatin fragments directly cleaved from the genome. Our results strongly suggested that the textbook dogma linking chromatin condensation with gene repression and unfolding with transcription activation was not necessarily true for all genes, and may need to be revised. Our most current research project is focused on studying the effects of Sulforaphane (SFN), a substance derived from Broccoli on Prostate Cancer (PCa). We are investigating SFN effect using PCa cell lines as a model system. Initial experiments indicated that SFN can affect epigenetic modifications. We have identified a link between PCa-specific histone post-translational modifications and sulforaphane treatment. We are also investigating the mechanism of action of various chromatin-associated proteins, such as MeCP2 and Sir3, on chromatin compaction and transcription regulation in various *in vitro* and *in vivo* systems. Finally, we have recently started to look at the connection between chromatin remodeling complexes and cell differentiation, using mouse salivary glands as model systems. This project is also linking epigenetic to cellular functions.

Interns will have the opportunity to learn certain of the following techniques.

Molecular Biology: cloning and sub-cloning. Protein over-expression.

Biochemistry: Protein purification (conventional chromatography, affinity chromatography)

Southern, Northern and Western blotting.

In vitro chromatin reconstitution

Electrophoresis mobility shift assay (in agarose or acrylamide matrix).

Immuno depletion assay.

RT-PCR

Site-directed mutagenesis.

Chemical protein cross-linking.

HPLC and FPLC.

Biophysics: Hydrodynamic analysis (utilizing the analytical ultra-centrifuge XLA and model-E from Beckman).

Analytical agarose "Multi-gel" system or Quantitative Agarose Gel Electrophoresis (QAGE).

Cell Biology: Basic cell culture (fibroblasts, Drosophila cells and mouse primary cell culture).

Dr. Lawrence M. Grover

Professor of Pharmacology, Physiology, and Toxicology
Marshall University Joan C. Edwards School of Medicine

Three projects are available in my laboratory for INBRE participants to join:

1. Growth hormone mechanisms regulating hippocampal synaptic function. Growth hormone is a powerful regulator of biological function. A major surge of growth hormone release occurs during sleep, and interruption of normal sleep substantially reduces growth hormone availability. Growth hormone has a special role in maintaining brain function, including the function of the hippocampus, a brain area essential for learning and memory. Our objectives in this study are to learn the biological processes through which growth hormone regulates the synaptic functions of the hippocampus, and to learn how sleep deprivation alters the normal biological processes through which growth hormone acts. By learning how growth hormone regulates hippocampal synaptic function, and how this regulation is affected by sleep deprivation, we will achieve improved understanding of, and more effective treatment for, the consequences of long-term, chronic sleep deprivation.

2. Models of signaling mechanisms in LTP. In this project we study synaptic strengthening (potentiation) in order to understand the brain mechanisms for memory. We focus on the hippocampus, which is the major brain structure involved in memory formation. This project combines experimental and computation approaches in order to model the molecular processes which occur during synaptic potentiation. The computational portion of the project is done at Ohio University in the laboratory of Dr. Bill Holmes, and the experimental portion of the project is done at Marshall University in my laboratory. By determining the brain mechanisms used for normal memory function we will improve our understanding of how memory is adversely affected by neurological disorders and diseases.

3. Role of brain-derived neurotrophic factor (BDNF) in depression and response to anti-depressant medications. Despite extensive study over many decades, the underlying causes of depressive disorders, and the biological basis of commonly used treatments, are still poorly understood. In this project, we focus on the potential ability of anti-depressant medications to stimulate production of brain-derived neurotrophic factor (BDNF) and alter synaptic function in two brain areas, the hippocampus and the medial prefrontal cortex. By increasing our understanding of the roles BDNF plays in depression and the response to anti-depressant medications, we will contribute to improved therapies for depression.

Methods and instruments used: Much of our experimental work uses isolated thin tissue slices of live rat brain. Brain tissue slices are prepared by dissection and sectioning on a vibrating microtome. We use electrophysiological techniques to record, analyze and measure the electrical activity of neurons in brain slices. Brain slices are viewed under a low power microscope, and micro-manipulators are used to place stimulating and recording electrodes into brain slices. Brain electrical signals are amplified, then digitized and store on a personal computer. The computer is used for measurement of recorded signals. We also measure expression and alteration of specific protein molecules using anti-body based techniques (western blotting, immunohistochemistry, and ELISA). We primarily study neurotransmitter receptor proteins, and extra- and intracellular signaling proteins.

Dr. Elaine Hardman
Associate Professor

Dept of Biochemistry and Microbiology
Joan C. Edwards School of Medicine
Marshall University
hardmanw@marshall.edu
(304) 696-7339

Projects available in my laboratory would relate to the following work:

Role of omega 3 fatty acids for suppression of cancer

We have several funded projects in this laboratory. All the projects relate to the suppression of risk for cancer by changing the fat consumed in the diet. Omega 3 fats are usually obtained by consumption of fish or fish oils, canola oil and some vegetables whereas omega 6 fats are especially high in corn and soybean oil and in the meat of animals fed corn or soybeans. One projects is to assess the ability of *in utero* exposure to various types of omega 3 fatty acids in the diet of the mother mouse to prevent or delay the development of breast or prostate cancer in the offspring. Breast and prostate tissues contains many fat cells. The fat cells produce signaling molecules that influence the growth of the potentially cancerous epithelial cells. We assess the change in cancer growth after a dietary change. Some individual projects might be to identify changes in protein expression or changes in cell signaling molecules in the glands. We also have a human clinical trial in progress. Laboratory assays related to this trial include changes in sensitivity to chemotherapy and changes in activity of the transcription factor, NF κ B, in samples from patients. Another potential project would be to determine whether providing omega 3 fat to leukemia cells would slow growth and increase chemosensitivity. After orientation to the laboratory, the participant would contribute to outlining a project that is of personal interest and that would benefit the overall effort in the laboratory. Participants who choose to work in my laboratory might learn: mouse handling, dissection, mouse anatomy, immunohistochemistry for identification of protein expression in tissues, cell culture, gas chromatography, enzyme linked immunoassays, protein assays, polymerase chain reaction, genotyping, microscopy, flow cytometry, diet preparation, protein blotting, basic statistical analysis of data and data presentation graphics.

Dr. Elsa Mangiarua

Professor
Department of Pharmacology, Physiology and Toxicology
Marshall University School of Medicine

mangiaru@marshall.edu

(304)696-6211

<http://musom.marshall.edu/phys/elsa.htm>

Obesity-associated hypertension

Obesity has been associated with many health problems including hypertension. We are trying to find out why obese individuals have a greater risk of becoming hypertensive than lean individuals. Obesity is associated with changes in the vascular wall that may lead to hypertension. Previous research in our laboratory has shown that genetically obese rats fed a high fat diet and given saline to drink develop hypertension. The high-fat diet contains an abundance of fatty acids, specifically oleic acid. It has been previously reported that dietary fatty acids can modulate markers of inflammation. We propose that an inflammatory process may mediate the vascular response to oleic acid. We are currently analyzing the expression of nitric oxide synthase as a marker of inflammation in vascular smooth muscle cells in culture in response to oleic acid treatment. Students working in my lab will have the opportunity to work with rats, learn cell culture techniques, western blot analysis, immunohistochemistry, and ELISA analysis.

Dr. Will McCumbee

Professor of Physiology

Marshall University School of Medicine

Mccumbee@marshall.edu

(304) 696-7366

<http://musom.marshall.edu/phys/mccumb.htm>

Project Title: Obesity Associated Diseases

The principal objective of this research project is to determine how obesity leads to the development of heart disease, high blood pressure and type 2 diabetes. We are particularly interested in the interplay between diet and obesity in the development of these diseases. We have observed, for example, that the expression of certain genes is altered when obese rats are fed a high fat diet. Recently we have acquired a strain of genetically obese rats that develop both high blood pressure and diabetes. This further increases our research options. Depending on their interests, INBRE participants may choose to collaborate with researchers who are working to determine whether these changes in gene expression result in corresponding changes in messenger RNA production and protein synthesis. In other phases of the study, the participants will have the opportunity to work directly with live animals. They can learn how to conduct dietary studies, perform minor surgical procedures, give injections, measure blood pressure, and determine the concentration of specific hormones and metabolites in the blood and other body fluids. Also available, are studies designed to assess the functional response of specific organs such as the heart, kidney, and blood vessels to dietary manipulations. For example, participants can use isolated blood vessels from lean and obese rats subjected to different dietary regimens to compare their responses to contractile agents. The functions of the kidney, an organ that plays a major role in blood pressure regulation, can be assessed by measuring the activity of specific enzymes and the concentration of various metabolites in the urine, whereas functional changes in the heart can be monitored using echocardiographic measurements.

Dr. Richard M. Niles

Professor and Chairman of Biochemistry and Microbiology

Joan C. Edwards School of Medicine

Marshall University

niles@marshall.edu

(304) 696-7323

<http://musom.marshall.edu/bio/niles.htm>

Projects in my laboratory are:

Vitamin A and Melanoma: The incidence of melanoma is rapidly growing in the US population. In WV, it is the cancer with the highest incidence in the 20-35 year old population. We are studying how the biologically active form of vitamin A, retinoic acid affects the properties of human melanocytes and how this is altered in human melanoma cells, making them resistant to cancer prevention effects of this vitamin. Subprojects include: Characterization of the nuclear receptors for retinoic acid in human melanocytes and melanoma; investigating the metabolism of vitamin A in normal human melanocytes and melanoma.

Techniques: “real-time” RT-PCR, cell culture, HPLC, enzyme assays

Expression and Function of Hypoxia-Inducible Factor-1alpha (HIF-1alpha) in Human Melanoma

Some cells in a tumor encounter a hypoxic (low oxygen) environment. In order to ensure their survival, these cells increase the amount of HIF-1alpha protein which turns-on genes that stimulate new blood vessel formation and genes that shift the fuel metabolism of the cell so energy can be obtained in the absence of oxygen. We have found that more advanced melanoma cells have high amounts of HIF-1alpha under normal oxygen tension. We are studying the mechanism by which these melanomas increase their expression of this factor and what genes are the target of this factor under normoxic conditions.

Techniques: cell culture, Western blotting, gene transfections, animal models, DNA microarrays

Restoration of Silenced Genes in Melanoma by Phytochemicals

Studies on clinical specimens of melanoma show that the expression of a number of genes is silenced through epigenetic mechanisms. Epigenetic changes occur without mutation/deletion to the DNA, such as methylation of CpG nucleotides, modification of histones affecting chromatin structure or other factors such as microRNAs that ultimately result in decreased or absent level of the target protein. We are elucidating the mechanism for the frequent silencing of the retinoic acid receptor beta gene in melanoma and the ability of phytochemicals such as genistein found in soy and EGCG found in green tea to reactivate the expression of this gene. This is a team project with Dr. Philippe Georgel from Biological Sciences and Dr. Vincent Sollars from Biochemistry and Microbiology.

Techniques: cell culture, DNA methylation analysis, chromatin immunoprecipitation, PCR

Dr. Maiyon Park

Assistant Professor of Biochemistry and Microbiology

John C. Edwards School of Medicine, Marshall University

parkm@marshall.edu

(304) 696-3680

http://bms.marshall.edu/research_groups/neuro_and_development/faculty_pages/park.aspx

My lab explores the mechanisms underlying **pancreatic cancer** development using human tissue, and tissue culture cells as model system. We also are planning to use mice as a model system using **Chmp1A** conditional knockout mice. We are specifically interested in understanding how **Chmp1A** regulates **P53** and/or **Rb** (Retinoblastoma) mediated tumor suppressor pathway in the suppression of the progression of human pancreatic cancer.

Summer Student Projects:

Investigating the regulation of P53 and/or Rb pathway by Chmp1A in the suppression of human pancreatic tumor

A. Construction of deletion mutation of Chmp1A and use these constructs for

- (1). Growth analysis of human pancreatic tumor cells using deletion mutations of Chmp1A. Full-length Chmp1A will be used as a positive control in these growth assays.
- (2). Regulation of P53 by Chmp1A using immunohistochemistry and immunocytochemistry in combination with tissue culture.
- (3). Investigate the effect of Chmp1A over-expression or knockdown on the up-stream or downstream components of P53 signaling pathway. Transfection, and Western blot analysis will be used.

B. Construction of mutation of Chmp1A and use these constructs for

- (1). Growth analysis of human pancreatic tumor cells using deletion mutations of Chmp1A. Full-length Chmp1A will be used as a positive control in these growth assays.
- (2). Regulation of Rb by Chmp1A using immunohistochemistry and immunocytochemistry in combination with tissue culture.
- (3). Investigate the effect of Chmp1A over-expression or knockdown on the up-stream or downstream components of Rb signaling pathway. Transfection, and Western blot analysis will be used.

The methods students will use during the course of summer research:

1. DNA purification
2. General cell culture assays such as splitting, transfecting, and counting cells
3. Western blot analysis
4. GST-pulldown assay to test binding of proteins
5. Immunocytochemical analysis to determine sub-cellular localization of proteins
6. Scanning, densitometric analysis followed by statistical analysis using excel program
7. Make figures using Photoshop

Dr. Gary O. Rankin

Professor and Chair of Pharmacology, Physiology and Toxicology
Joan C. Edwards School of Medicine

Marshall University
rankin@marshall.edu
(304) 696-7319
<http://musom.marshall.edu/pharm/rankin.htm>

The following projects are available in my laboratory:

Project #1: Succinimide-induced nephrotoxicity: The succinimide ring is incorporated into hundreds of chemicals used as drugs, agricultural fungicides, and industrial agents. Toxicity to the kidney (nephrotoxicity) has been associated with exposure to succinimide antiepileptic agents and some agricultural fungicides. Recent work has determined that succinimide metabolites are responsible for inducing the kidney damage, females are more sensitive than males to succinimide-induced nephrotoxicity, and the stereochemistry of the metabolites contributes to nephrotoxic potential. This project seeks to determine the exact nature of the toxic metabolites and sub-cellular renal targets of the metabolites, how metabolites gain entry into the kidney and the toxicogenomics of succinimide-induced nephrotoxicity.

Project #2: 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 nephrotoxicity occurs.

Project #3: Propanil is an herbicide used in agriculture to decrease grasses in rice field. Recent work in our laboratory has demonstrated that propanil is toxic to the kidney. However, the mechanism by which propanil induces nephrotoxicity is unclear. In this project the role of bioactivation of propanil to toxic metabolites is being explored along with possible mechanisms by which propanil or its metabolites induce toxicity to the kidney

Project #4: Methadone is a drug used to reduce the dependence of heroin addicts on heroin. However, some methadone users die unexpectedly when using normal doses of methadone. Preliminary studies have suggested that there may be a defect in the inactivation of methadone in the liver in these individuals who die unexpectedly. The purpose of this study is to determine if genetic polymorphisms are responsible for these deaths.

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. Toxicogenomic studies involve isolation techniques for obtaining genetic material from treated and control rat kidneys. Additional techniques may involve Western blotting, quantifying urinary contents (protein, glucose), measuring blood urea nitrogen and glucose levels, and real time PCR techniques. Instrumentation will primarily involve the use of balances, centrifuges and UV-visible spectrophotometers. High pressure liquid chromatography and thermocycler use is also possible.

Dr. Nalini Santanam
Associate Professor
Department of Pharmacology, Physiology & Toxicology

Joan C Edwards School of Medicine
Marshall University
santanam@marshall.edu
Tel: (304) 696-7321

The following projects are available in my laboratory:

1. **Adipose tissue biology in obesity:** Obesity is a major risk factor for cardiovascular disease and diabetes. Changes in adipose tissue (the fat storing endocrine organ) composition and function during obesity contribute to the increased risk. There are several projects in my laboratory related to this area. For example,
 - (i) **Identification and isolation of adipose specific stem cells:** There are reports in the literature that the composition of the adipose tissue changes during obesity. This change might be due to changes in the progenitor stem cells. We are interested in demonstrating these changes in adipose specific stem cells in some of our ongoing obesity related studies. If you are interested in this project, you will learn (i) to isolate adipose specific stem cells from the adipose tissue collected from various ongoing studies, (ii) using specific antibodies detect and identify adipose specific stem cells using either flow cytometry or immunohistochemistry.
 - (ii) **Adipose specific miRNA isolation and detection:** miRNA are approximately 17-24 nucleotide single-stranded RNA that post-transcriptionally regulate gene expression. Recently adipose specific miRNA has been identified and their role in obesity associated diseases has been proposed. If you are interested in this project, you will learn how to isolate and detect miRNA using miRNA isolation and detection techniques.
2. **Pain in Endometriosis:** Endometriosis is a disease that affects 10% of younger women. The major symptom of this clinical condition is pain. In our laboratory we are conducting cell culture, animal and human studies to understand the pathophysiology of pain in this disease.

TECHNIQUES:

The techniques that are routinely performed in our laboratory:

3. Cell culture techniques
4. Isolation and quantification of RNA (including miRNA) and DNA from cells or tissues
5. Detection of genes using PCR/ Real time PCR
6. Detection of proteins using Western Blotting
7. Detection of reactive oxygen free radicals in cell culture system.
8. Animal studies: Studies on atherosclerosis, obesity and pain

Dr. Vincent Sollars

Assistant Professor of Microbiology, Immunology and Molecular Genetics
Joan C. Edwards School of Medicine

Marshall University
sollars@marshall.edu

(304) 696-7357

http://bms.marshall.edu/research_groups/pathogenesis_and_aging/sollars/default.aspx

About five hundred billion blood cells need to be replaced in the human body each day. This feat is accomplished by the exponential amplification of cells from a pluripotent precursor cell known as the hematopoietic stem cell (HSC). The HSC does not generate the required 5×10^{11} cells directly on a daily basis, but instead generates highly prolific progenitor cells that produce the nine major hematopoietic cell types. When developmental decisions go wrong in this system leukemias occur due to the uncontrolled amplification of progenitor cells. Defining the regulation of these progenitor cells is important to our understanding of stem cells, leukemias, and other blood disorders. My laboratory investigates these progenitor cells using the mouse as a model system. The following projects are available in my laboratory related to this area of research:

Project #1: Epigenetic regulation of the wntless-integrin (WNT) pathway:

Cells that have identical genomes can respond in markedly different ways to the same stimulus, such as a cytokine, with the outcome being determined largely by the previous developmental history of the cell. This developmental history is recorded as a form of "cellular memory" in an epigenetic format consisting of modifications to the cell's genome, such as DNA methylation. An important biological signaling pathway known as the WNT pathway is regulated by DNA methylation. This signaling pathway has been found to be involved in many cancers including those of the blood, such as leukemias. We are exploring the effect of this regulation on progenitor and stem cell biology by using a mammalian cell culture system and in mice. The student or faculty member will have the opportunity to learn techniques for: a) mammalian cell culture, b) flow cytometry protocols, c) recombinant DNA technologies, and d) mouse dissection techniques.

Project #2: Determination of the frequency of progenitor cells in bone marrow:

I have developed two assay systems for measuring the frequency of progenitor cells in the bone marrow of mice. Comparison of these values in different strains of mice will enable the determination of factors responsible for the regulation of progenitor cells. The hypothesis for this research is that different frequencies of progenitor cells in various mice may be exploited to determine what genes are important in the regulation of progenitor cells. The objective of this project will be to determine the frequencies of progenitor cells in various strains of mice. The student or faculty member will learn techniques for: a) mammalian cell culture and cytokine stimulation of bone marrow, b) mouse dissection techniques, and c) flow cytometry protocols.

Dr. Monica Valentovic

Professor of Pharmacology

Joan C. Edwards School of Medicine, Marshall University

valentov@marshall.edu

(304) 696-7332

<http://musom.marshall.edu/pharm/valentov.htm>

Projects available in my lab:

Projects #1 & 2 The effect of herbal agents (nutraceutical) on susceptibility to toxins: Two ongoing projects are investigating the cellular mechanisms for nutritional agents: 1) to reduce acetaminophen (Tylenol) mediated liver damage and b) to reduce or prevent the adverse effects of cancer chemotherapeutic drugs. These projects have direct clinical relevance. An individual involved in this project will investigate cellular changes in toxicity, examine the involvement of oxygen radicals and will examine protein expression and modification by acetaminophen or cisplatin.

Project #3 Mechanisms to reduce diabetic renal complications: Diabetes mellitus is the major cause of kidney failure in the United States. The long term goal is to examine what makes the diabetic more susceptible to kidney failure. These results may then be applied to develop new treatments for diabetics. Individuals (students or faculty) involved with this project will participate in examining cellular changes that may increase cellular stress in the diabetic kidney.

Dr. Hongwei David Yu

Professor of Biochemistry and Microbiology

Joan C. Edwards School of Medicine, Marshall University

yuh@marshall.edu

(304) 696-7356

http://www.bms.marshall.edu/research_groups/pathogenesis_and_aging/yu.aspx

Dr. Yu's laboratory focuses on biofilm genetics, innate immunity and antibiotic resistance in bacteria. The students will work side-by-side with graduate students and research staffs to tackle issues such as what environmental cues trigger bacteria to form a biofilm, how bacteria invade a host causing the development of pneumonia and how to combat the antibiotic resistance in bacteria.

Project #1: Genetics of Biofilms. Bacteria in nature often grow as aggregating colonies attached to a surface known as a biofilm. Biofilm formation is a leading cause of chronic disease. To grow a biofilm, bacteria need to produce polysaccharides resulting in the formation of slimes. We are studying how bacteria know when to start making slimes. The model microorganism is a ubiquitous biofilm-forming bacterium called *Pseudomonas aeruginosa*. By examining the molecular switch that controls the transition between biofilm and non-biofilm formation, we want to understand how bacteria make biofilms, thus to control biofilms. The faculty or students will be exposed to techniques such as cloning, gene knockout, transposon mutagenesis, and protein overexpression and purification, and Western blot.

(continued on next page)

Project #2: Resistance to Pneumonia. At any given moment, we breathe in bacteria into our lungs. However, few of us will develop pneumonia. This is because we have a robust defense system to fight off the invading bacteria. The main defenses in the lungs

include resident macrophages (big eaters), white blood cells and small proteins with potent antimicrobial activities. We have developed various models in the laboratory to study how these cells eliminate the bacteria, how the host realizes the incoming bacteria by producing a battery of small molecules in order to recruit the white blood cells to the lungs, how to evaluate and boost the activities of novel antimicrobials in pneumonia mouse model. The techniques used for this project include cell culture, plate counts, lung infection mouse models, lung pathology, cytokines measurement and analysis, PCR, real-time PCR, immunohistochemistry, and image analysis using bioluminescent and fluorescent markers.

Project #3: Combating antibiotic resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) and tobramycin-resistant *P. aeruginosa* (TRPA) are two infectious agents that cause a significant morbidity and mortality in immuno-compromised individuals. There is an urgent need to develop new antibiotics to combat these pathogens. To develop such a therapeutic, we are testing a series of rationally designed peptides with potent activity against bacterial pathogens. We also screen for more of these antimicrobial peptides (AMPs) for candidates with a broad spectrum of anti-MRSA and -TRPA activities. We hope to identify the novel AMPs with increased efficacy and reduced toxicity. Students working for this project will learn basic methods and technologies used in the clinical microbiology laboratory.