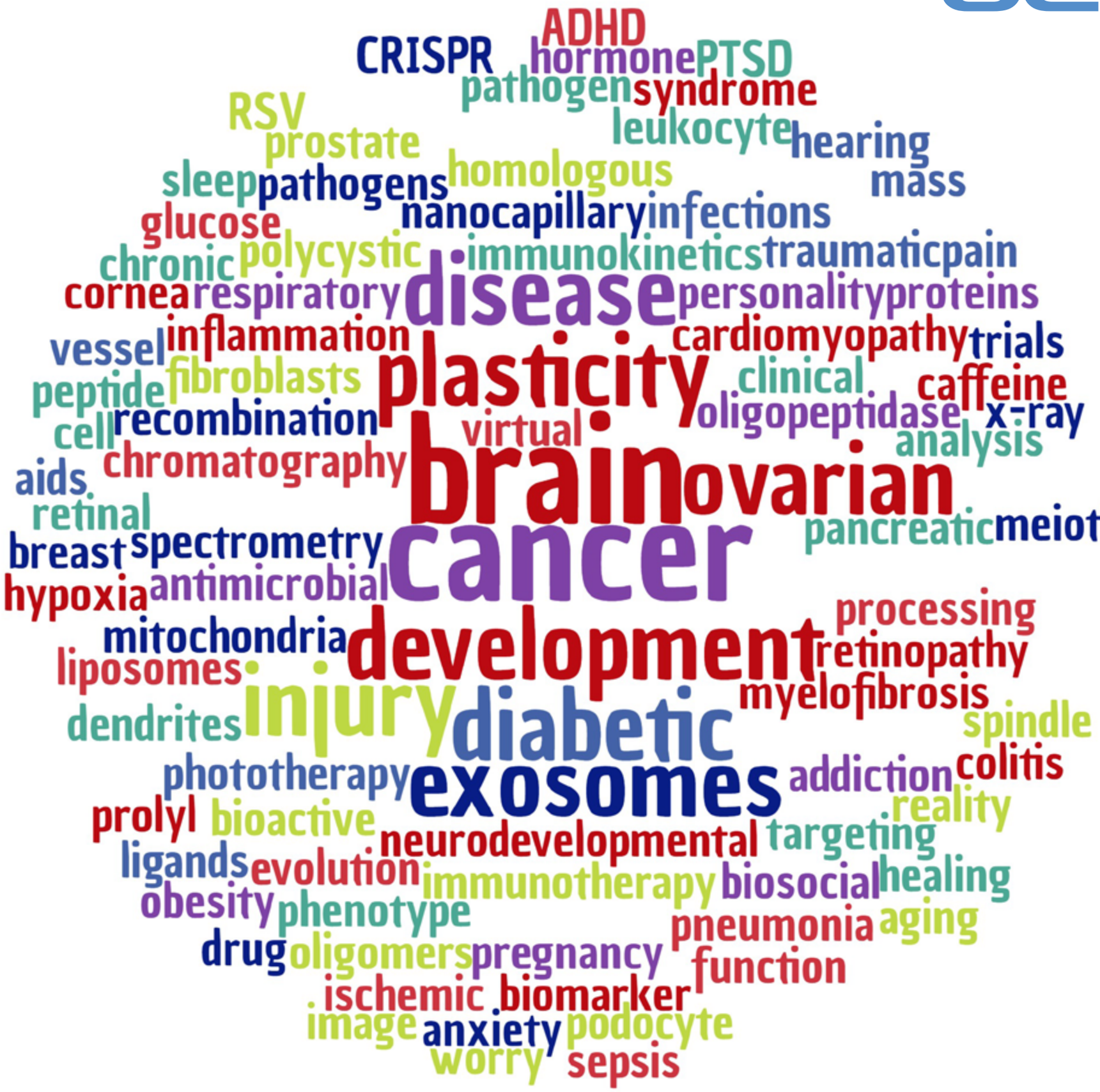
ADHD
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respiratory
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personality
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inflammation
cardiomyopathy
trials
vessel
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ischemic
biomarker
image
anxiety
podocyte
worry
sepsis

2019 Health Research Conference

October 16, 2019
Samis Education Center at OU Children's Hospital

Level 2 Auditorium

2:30 - 2:45 p.m.

Registration

2:45 - 4:25 p.m.

Welcome and Introductions
Michael Carolina, OCAST Executive Director

Bioscience Commercialization
Carol Curtis, Ph.D., Vice President and Director of Investments, i2E

Keynote
Daniel Clark, B.S.I.E., President, Linear Health Technologies

Linear Health Sciences is a medical device company creating products for various types of medical tubing based on its proprietary, breakaway safety-valve technology. This platform technology is designed to improve the healthcare experience for patients, caregivers, and healthcare facilities. Dan's responsibilities as President encompass marketing and commercial efforts, regulatory and technology strategy deployment, as well as overall company vision.

Closing Remarks
Mary Beth Humphrey, MD, Ph.D., Associate Dean for Research, College of Medicine, University of Oklahoma Health Sciences Center, Health Research Committee Chair

Level 4 Rooftop Garden

4:30 - 6:00 p.m.

Networking Reception
Join the speakers, health researchers, and other attendees for hors d'oeuvres and networking in the beautiful Samis Center rooftop garden immediately following the conference.

Special thanks to the Samis Education Center and University of Oklahoma Health Sciences Center for their gracious hospitality in providing space for this event.

FY2020 Funding Opportunity Announcements/Solicitations for all OCAST programs will run:

Open - January 17, 2020

Close - March 2, 2020

[Sign up here for notifications to receive reminders!]

or

[Get more information here.]

Applied Research
Health Research
Intern Partnerships
Small Business Research Assistance
Manufacturing
New Product Development
Inventors Assistance
Seed Capital
Technology Commercialization

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Pioszak: Using powerful x-rays to visualize cardiovascular hormones and guide the development of new drugs for migraine headache and heart attack

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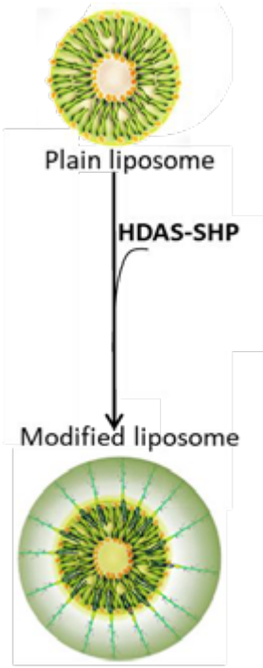
Liposome surface modification: Effectiveness of a new lipo-polymer HDAS-SHP to suppress immune reactivity of injected liposomes

Immunokinetics of superhydrophilic polymer-modified liposome encapsulated hemoglobin

PI: V. Awasthi, University of OK Hlth Sci Ctr **Project: HR17-054** **Research Area: Bio Med Eng**

Project Narrative

- An artificial substitute for red blood cells can be liposomes containing hemoglobin; these liposomes should avoid macrophage uptake and circulate in the body for a long time to mimic red blood cells.
- The overall objective of this project is to modify liposome surface with a novel lipo-polymer HDAS-SHP and evaluate these liposomes for persistence in circulation (See **Fig. 1**).
- The aim during this reporting period was to assess stability of HDAS-SHP on liposome surface and test macrophage uptake.
- A safe and effective blood substitute is capable of addressing shortages of blood in life-threatening trauma, gun-shot/blast injuries, spontaneous bleeding, and surgery.

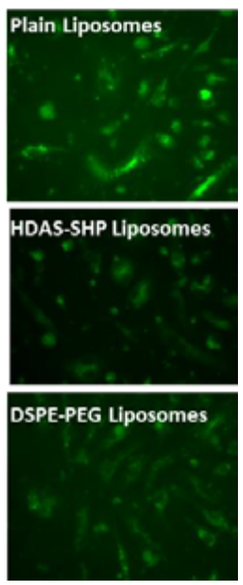


Recent Accomplishments

- HDAS-SHP is stably inserted in liposomes; anchoring stability is comparable to standard lipo-polymers. We have published a comprehensive article on immune properties of liposomes modified with HDAS-SHP.

  Mare, R.; Da, H.; Fresta, M.; Cosco, D.; Awasthi, V. Anchoring Property of a Novel Hydrophilic Lipopolymer, HDAS-SHP, Post-Inserted in Preformed Liposomes. Nanomaterials ***2019****, 9, 1185*

- Compared to standard DSPE-PEG liposomes and plain liposomes, HDAS-SHP liposomes showed reduced phagocytosis by macrophages (Green color in **Fig. 2**).



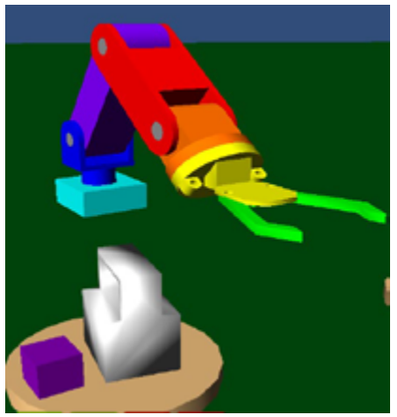
Virtual Learning Environments to support STEM learning for children with autism

J. Cecil, Oklahoma State University HR18-077 STEM Learning for children with autism

Project Narrative

In this OCAST project, our objective is to study the impact of using Virtual Learning Environments (VLE) to help children with autism learn science and engineering.

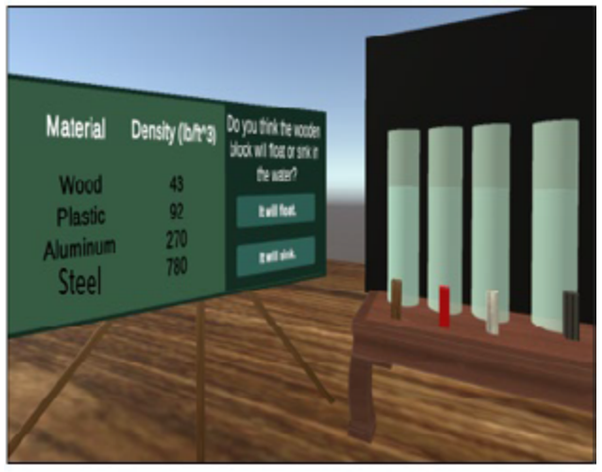
- Initial interactions with school students has started and will continue
- VLEs have been designed and built in interaction with Dr. Mary Sweet-Darter
- We are exploring impact of introducing positive reinforcers (based on Applied Behavioral Analysis ABA principles)



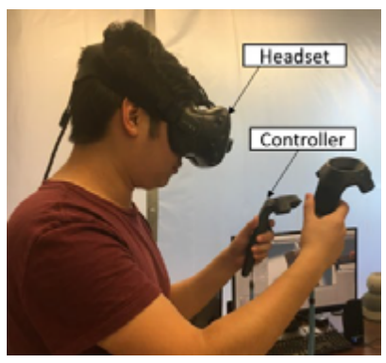
A VLE to learn robotics concepts

Recent Accomplishments

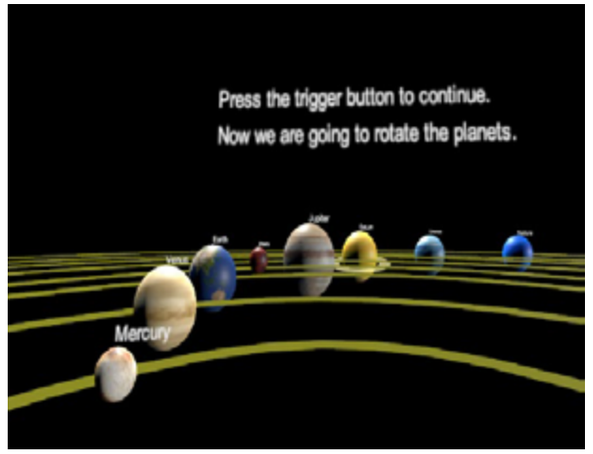
- VLEs include modules to learn basic solar system concepts, density, robotics and other topics
- Assessment plan has been developed to study impact on student learning
- VLEs have been developed using immersive / haptic technology based platforms and the Unity 3D engine



View of a density VLE



An immersive VLE

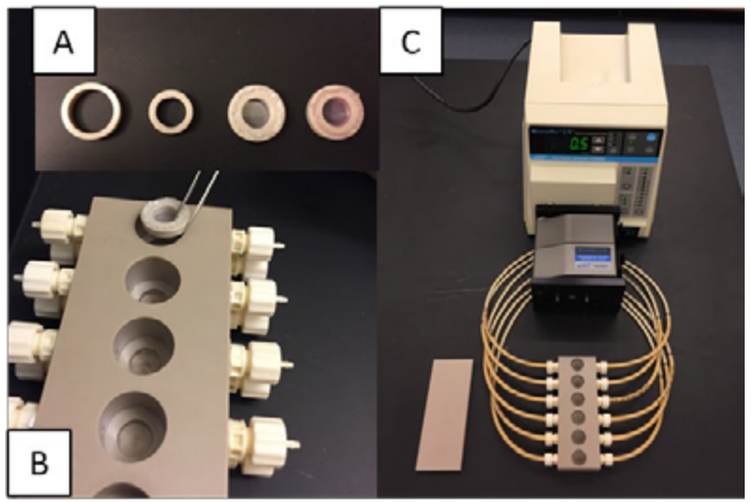


A VLE to learn solar system concepts

Using an advanced tool to generate specialized immune cells for the treatment of a variety of diseases

Ex Vivo Generation of Dendritic Cells from an Advanced Vascular Tissue Construct

PI: Heather Fahlenkamp, Oklahoma State University **OCAST Project: HR16-144** **Research Area: Biomedical Engineering**



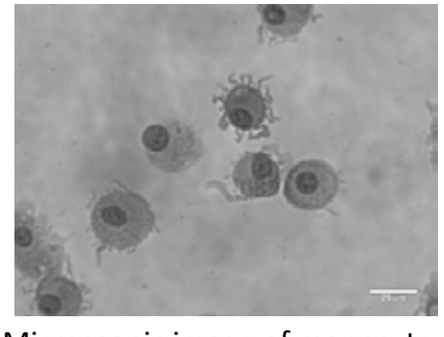
3D tissue-engineered vascular model within the bioreactor plate design. (A) Rings with a thin, porous membrane are coated with collagen and endothelial cells, (B) Rings are placed inside the bioreactor plate, and (C) Monocytes flow through the plate and over the cells and DCs are collected after 2 days.

Recent Accomplishments

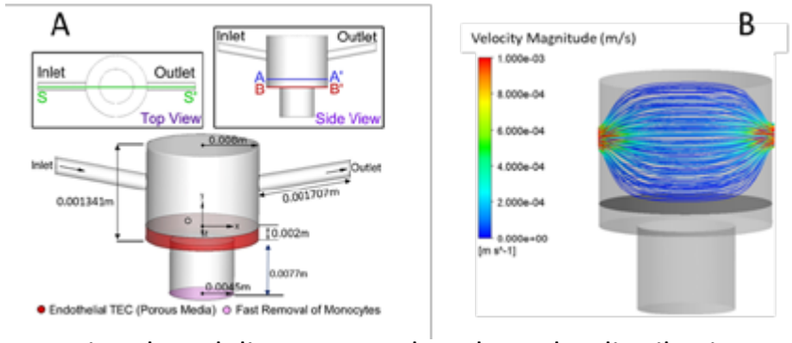
- We have shown that monocytes can differentiate into DCs in the plate bioreactor.
- We have shown that DCs from the plate bioreactor generated a greater immune response to tetanus toxoid compared to DCs generated from a 2D culture method.
- We have modeled the plate bioreactor using a computer program and have determined the flow pattern and DC generation.

Project Narrative

The immune system is linked to various diseases ranging from cancer, viral infections, and autoimmune. Dendritic cells (DCs) are an important class of immune cells, which even in small numbers can stimulate the immune system. However, producing the quantity and/or type of DCs needed for treatment is a major challenge. For this project, we have developed a novel tool to generate DCs from another class of immune cells called monocytes. The tool is described as a 3D tissue-engineered vascular model within a bioreactor. In the body, monocytes migrate through the blood capillaries and differentiate into DCs. We grew a layer of endothelial cells in the bioreactor to mimic the capillaries. Next, we added monocytes to the top of the endothelial cell layer under flow conditions to mimic the blood flow. When required, we mimicked inflammation by treating the endothelial cells with a molecule called TNF-α. To study the migration and differentiation of the monocytes, we collected cells from the endothelial cell layer, as well as above and below the cell layer. We found that monocytes migrated across the endothelial layer, and the number of migrated monocytes increased upon TNF-α treatment, with more cells below the endothelial layer. We also found that a large percentage of monocytes that migrated had differentiated into DCs. The DCs from the bioreactor generated a greater immune response to tetanus toxoid in terms of T cell proliferation compared to DCs from 2D culture methods. We used a computer to model the system in order to understand fluid flow profiles and monocyte migration and differentiation patterns. The results of the computational modeling were used to determine the best settings for the bioreactor. This project is innovative compared to other methods to generate DCs by creating a tool that mimics the natural environment of the body. The long-term goal of this research is to develop DC-based therapeutics to treat a variety of diseases and that can be patient-specific to treat an individual patient.



Microscopic image of monocyte-derived DCs from the plate bioreactor. 400x magnification.



Computational modeling was used to show the distributions and migration of monocytes in the plate bioreactor. (A) Schematic of the plate bioreactor well and (B) Flow pattern and monocyte distribution.

Smart adhesives with antibacterial and bioactive properties

Real-time quantification of cells' viability and 'smart' adhesive resins with antibacterial, bond-promoting and bioactive properties

PI: Fernando Luis Esteban Florez, The University of Oklahoma HSC **OCAST Project: HR16-131.** **Research Area: Biomedical Engineering**

Project Narrative

Secondary (recurrent) caries is the most common cause of failure of dental restorations. The etiology of secondary dental caries is related to the formation of bacterial biofilms at the adhesive interface between teeth and resin composite restorative materials. The problem is exacerbated further because posterior composite restorations tend to accumulate more biofilms when compared to other restorative materials. One approach to solving this problem is the addition of antibacterial agents within dental adhesive resins. Toward that end, we have tested the working hypothesis that addition of nitrogen-doped titanium dioxide nanoparticles in a commercial dental adhesive resin (OptiBond Solo Plus) promotes the attainment of experimental materials with improved biocompatibility properties.

Recent Accomplishments

- Filling of one provisional patent Drs. Florez, Khajotia and Rondinone
- PHF New Investigator Award ($50,000)
- Signing of 2 Material Transfer Agreements
- Submission of one manuscript (Dental Materials Journal)
- 1 Oral and 1 poster presentation at the 2019 IADR conference

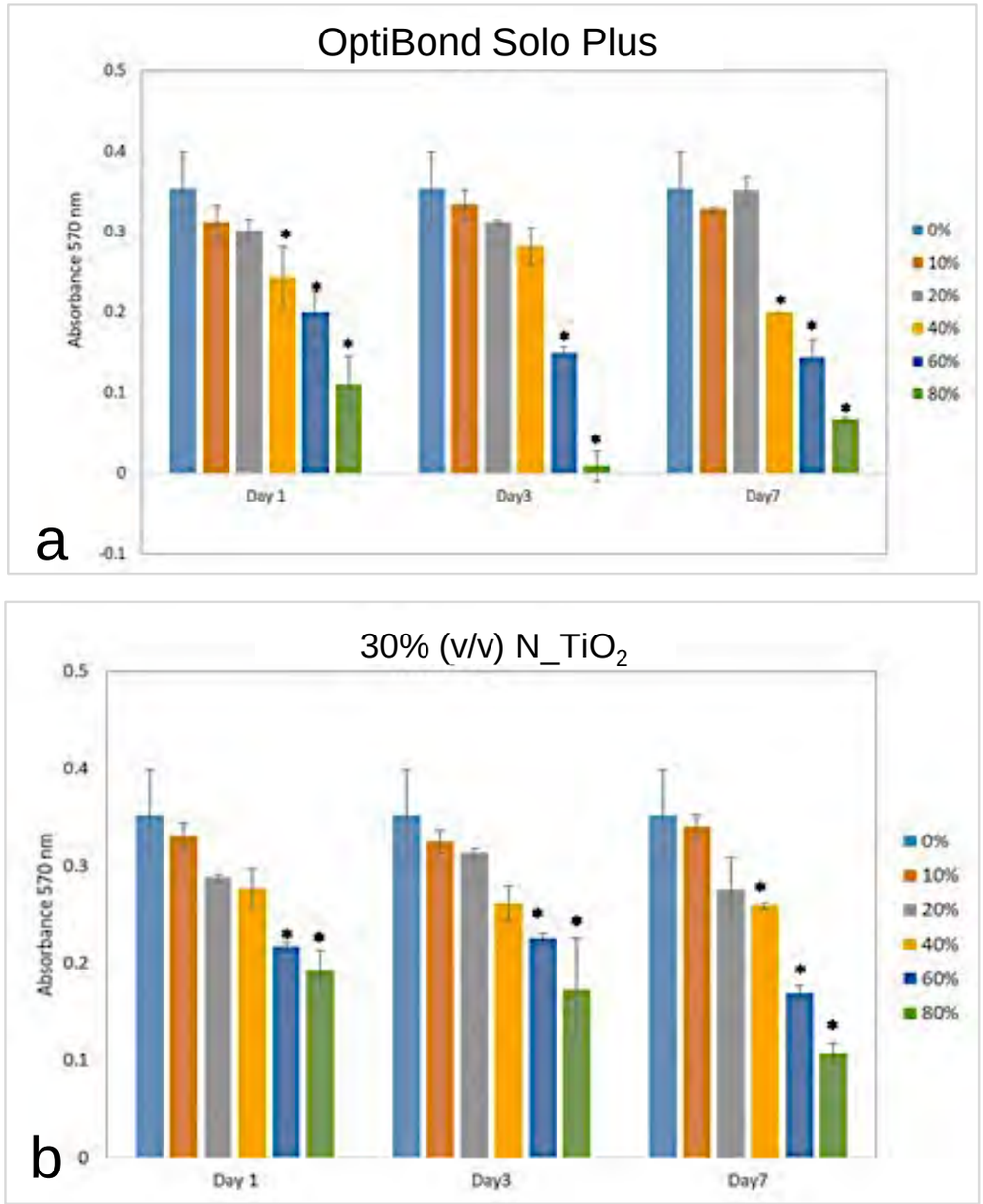


Figure 1. Cytotoxicity of unaltered (a) and experimental dental adhesive resins containing 30% (v/v) of (b) N_TiO_2. Bars with asteristic denote the presence of statistically significant differences ($p<0.05$).

Determining How Diabetic Kidney Disease Starts:

***Computational Modeling of the Onset of Diabetic Kidney Disease*, OCAST Project: HR17-057**

PI: Ashlee N. Ford Versypt, Ph.D., School of Chemical Engineering, Oklahoma State University

Research Areas: Biomedical Engineering, Chemistry, Physiology, & Computational Biology

Overview:

Diabetic kidney disease (DKD) is a severe complications of diabetes and the primary cause for kidney failure. Diabetes and kidney disease are the 6th and 9th leading causes of death in Oklahoma. In DKD, kidney tissue damage is focused in glomeruli, which are bundles of blood vessels within the kidneys where blood is filtered to produce urine. Significant damage to glomeruli occurs before doctors can detect that the kidneys are leaking proteins into urine. Continued damage leads to kidney failure. Because these tissues cannot repair themselves, it is critical to slow DKD before irreversible injury occurs. The connections between the processes that lead to glomerular injury in DKD are not completely understood. Chemical and physical processes in three main glomerular cell types and cross-talk between these cell types have been identified as contributing factors. However, there is currently no way to predict how these factors combine and synchronize to impact the health of glomeruli in DKD. This research project addresses the critical need to compile the many individual factors for DKD into a user-friendly systematic framework. This framework is a computer model that combines the interconnected chemical, physical, and biological factors in the appropriate magnitudes and sequences to make testable predictions. The computational model is expected to clarify complexities of DKD physiology.

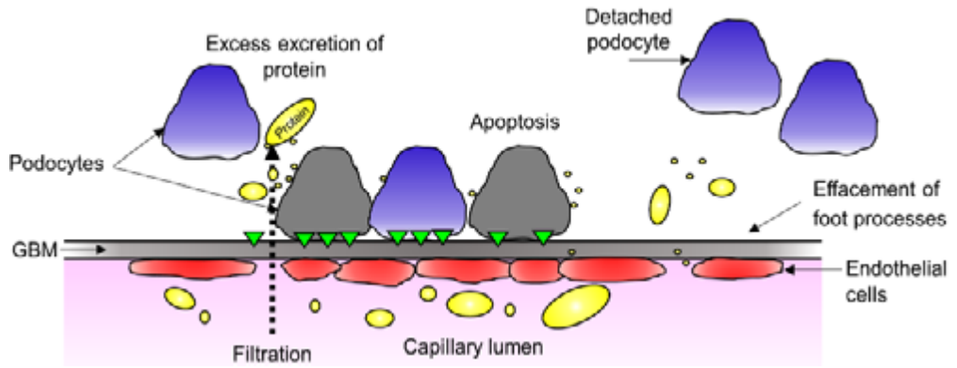


Figure 1: Subset of processes involved in glomerular injury in DKD.

Recent accomplishments:

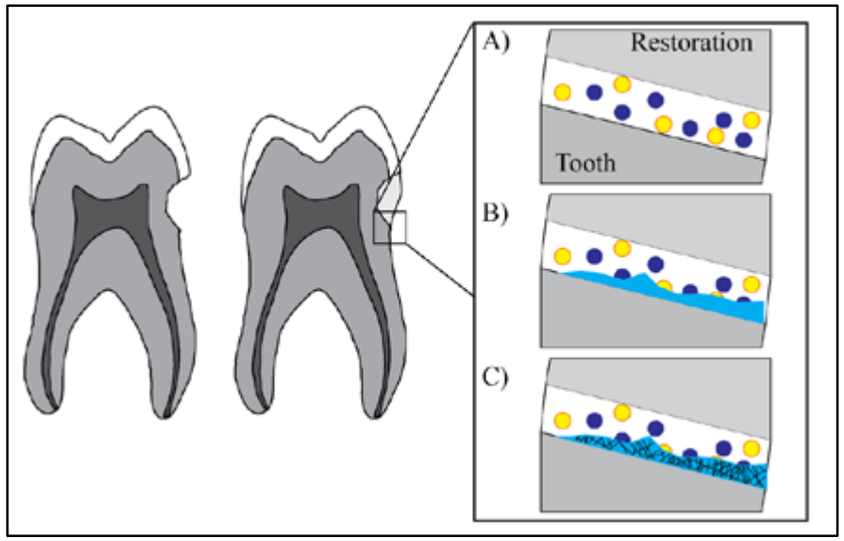
- The team published two computational models for the impacts of glucose on a key hormone's chemical reaction network in the podocyte cells, which are the outer layer of the glomeruli.
- The PI was invited to present the results of this work at an early program dedicated to diabetes at the American Society of Nephrology Kidney Week Conference.
- The results from this work were leveraged as preliminary results in support of the PI's recent NSF CAREER and NIH NIGMS R35 MIRA grants totaling more than $2.3 million in research funding.

Automatic Repair of the Filling/Tooth Interface in Dental Restorations

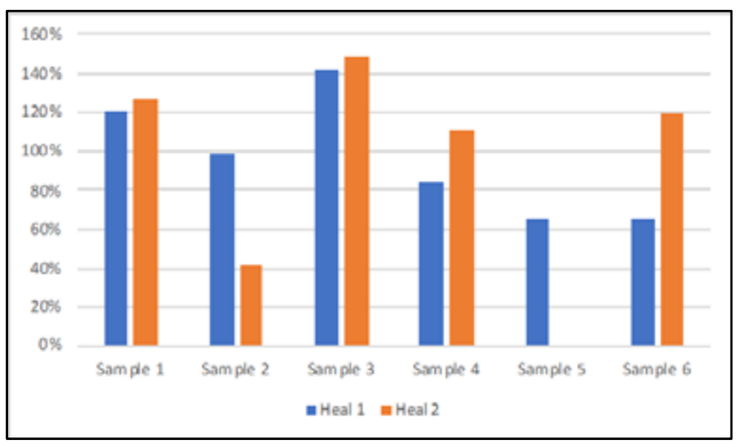
Interfacial Healing in Dental Restoration

PI: Michael W. Keller,Phd, PE The University of Tulsa **OCAST Project: HR16-100** **Research Area: Biomedical Engineering**

Resin-based restorations have become the primary choice of most patients requiring restorative dental work. This preference is based on appearance and a growing concern about the presence of mercury in dental amalgams. While these restorative materials provide benefits, composite resins are prone to failure. The primary cause of restoration failure is damage at the resin-tooth bond leading to the formation of new cavities. A major research area is new strategies for improving material performance and for minimizing the potential of new cavity formation. Material approaches are currently focused on the synthesis of new adhesive resin formulations that are resistant to degradation and attack by microbes. Based on this work, several additives have been suggested by researchers that improve the resistance of the restoration-dentin bond to enzyme attack. These approaches use "passive" materials or processes to improve the durability of the resin-tooth bond. These passive approaches attempt to inhibit degradation processes in order to prevent failure of the interface and eliminate subsequent pathogenic attack on the remaining healthy tooth structure. In this project, we will synthesize and characterize an "active" material that will respond to interface damage by healing and sealing interfacial cracking and failure.



Schematic view of a tooth with caries and subsequent restoration. Inset image shows the damage-healing process for the proposed adhesive resin material. A) shows the pristine, as-placed restoration, B) shows the onset of damage that ruptures capsules, C) shows the release and polymerization of the healing agent repairing and sealing the damage.



Injected healing results where the healing agent is directly pipetted onto the fracture surface. Demonstrates the potential to heal even if refractured.

Recent Accomplishments

Accomplishments

- Synthesized micron and submicron (nanoscale) microcapsules for inclusion at the tooth-restoration interface.
- Developed specimen preparation procedure to enable testing of the new self-healing material.
- Began self healing testing of the restoration.

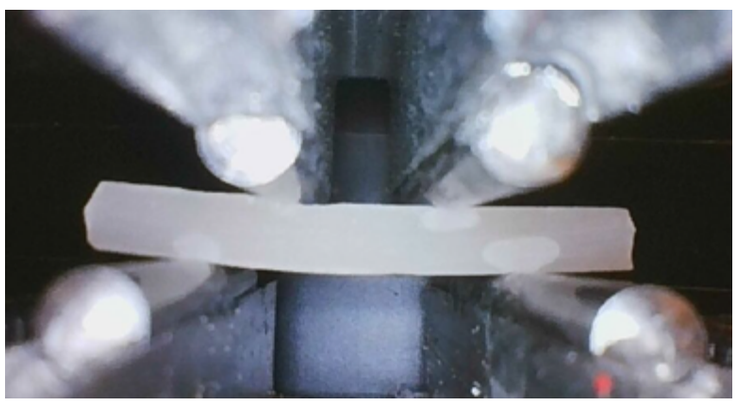


Image of a 4pt bend test specimen

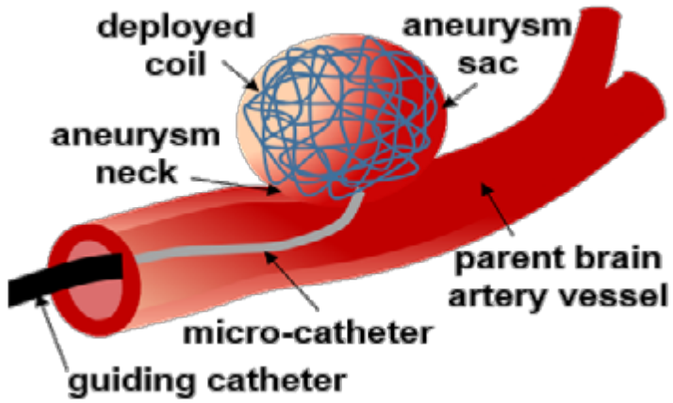
Long-Term Durable, Individualized Solution to Brain Aneurysms

Novel Shape Memory Polymer Devices for Optimal Endovascular Embolization of Intracranial Aneurysms

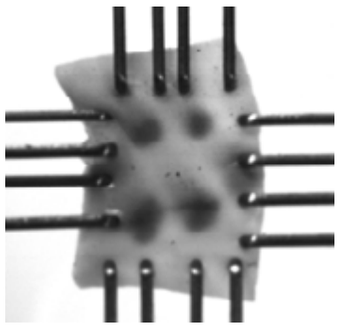
PI: Chung-Hao Lee, The University of Oklahoma **OCAST Project: HR18-002** **Research Area: Biomedical Engineering**

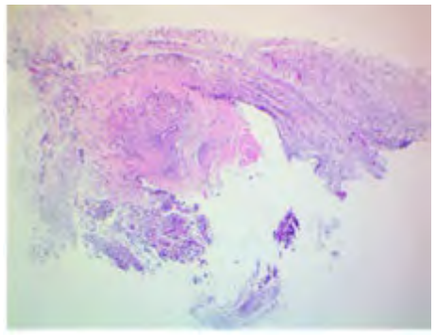
Project Highlights

- Incidental rupture of an intracranial aneurysm (ICA) is associated with 50%-80% of hemorrhagic strokes, leading to a mortality rate reaching as high as 40% within the first week and causing ~15,000 deaths each year in the US.
- Although endovascular coil embolization has been well received by neurosurgeons as a minimally invasive therapy for the elderly patients with ICAs, aneurysm recurrence (41% by 5 years after initial coil therapy, with 26% of those requiring retreatment) remains a clinical issue.
- The goal of this project is to develop shape memory polymer-based embolic devices that account for the complex aneurysm geometry for **patient-specific** endovascular embolization of saccular intracranial aneurysms.
- Optimal aneurysm filling and complete occlusion can then be achieved, for enhancing treatment durability and reducing in-hospital expenditure for managing aneurysm rupture-induced hemorrhagic strokes.

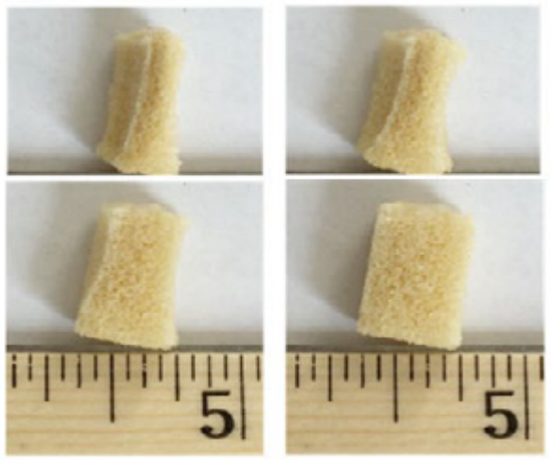


Schematic of a typical dome-shaped saccular brain aneurysm as treated by endovascular coil embolization.





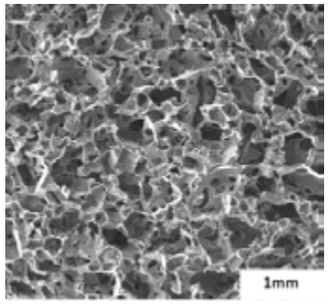
Mechanical & histological characterization of arterial and aneurysm tissues of the proposed *in vivo* rabbit study.



Shape recovery of heating the manufactured SMP foam.

Recent Accomplishments

- An Interdisciplinary Team Has Been Formed: Bioengineering, Materials, Neurosurgery.
- Thermo-Mechanical Characterization Has Been Conducted for Pristine and Porous Shape Memory Polymers (SMPs).
- Research Development Has Led to Three Journal Publications, One Provisional Patent Application, and One Filed Disclosure.



Evaluation of the porous structure of the manufactured SMP foam via scanning electron microscopy (SEM).

Dual-function Nanocoatings with Drug Release Control

Nanocoatings for Controlled Drug Release and Improved Biocompatibility

Yu Mao, Oklahoma State University HR18-005 Biomedical Engineering

Project Narrative

Though drug-eluting stents have been widely used method in treating coronary artery diseases by opening up narrowed blood vessels, syndromes of restenosis and thrombosis after stenting remains problematic. Contemporary stent coatings rely on strategies to address drug efficiency and stent biocompatibility separately. The lack of comprehensive approaches in addressing drug delivery and surface biocompatibility limits the clinical success of stents. The long-term goal is to develop drug-eluting stents that significantly reduce restenosis and thrombotic response while supporting endothelialization after implantation. The overall objective of this application is to identify strategies for simultaneous control of drug release and stent surface biocompatibility.

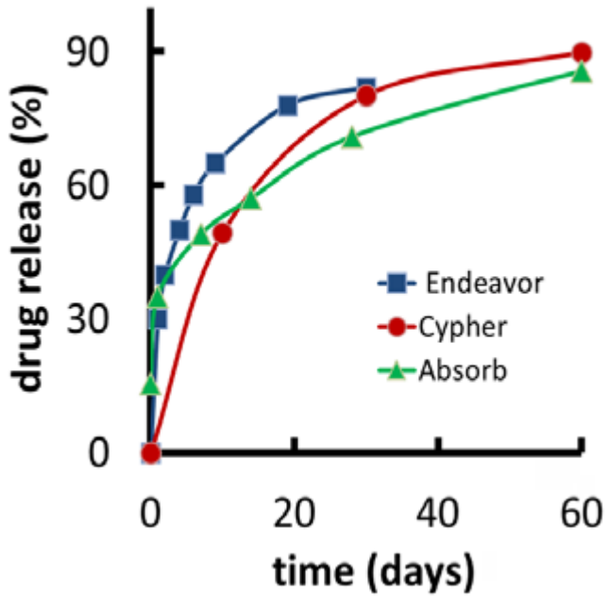


Figure 1. Drug release profile of commercial drug-eluting stents.

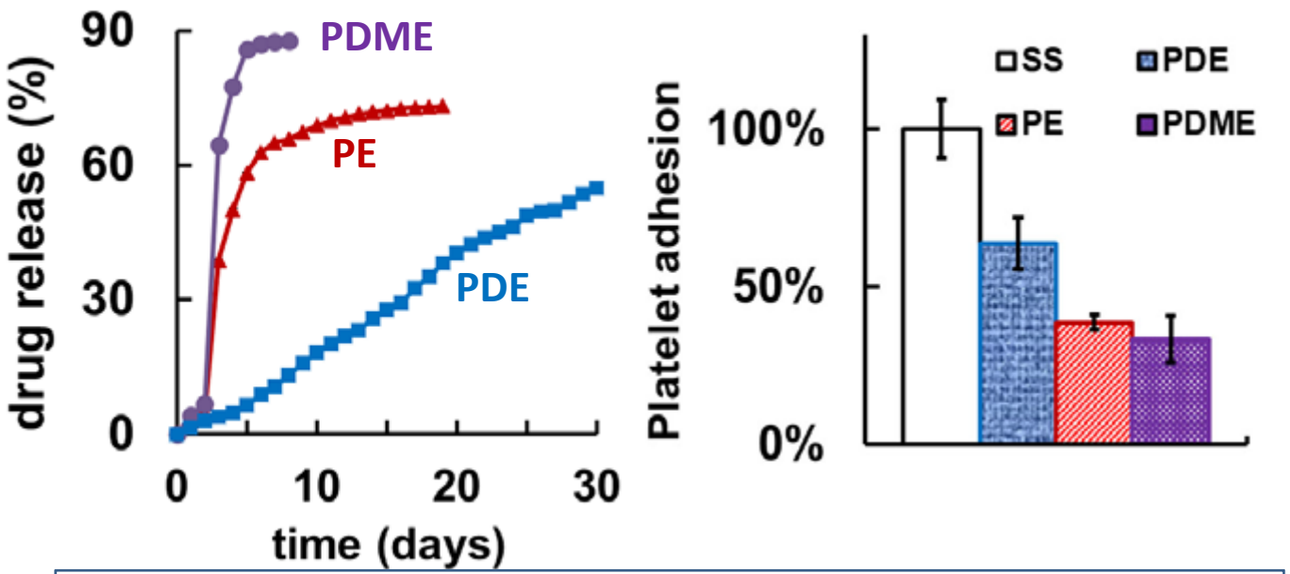


Figure 2. A) Close-to-linear drug release via PDE nanocoatings compared with the burst release through PMDE and PE nanocoatings. B) Platelet adhesion on nanocoated surface with different compositions.

Recent Accomplishments

- ❑ Regulation of drug release kinetics by adjusting nanocoating composition and thickness.
- ❑ Modeling of the zero-order drug release for mechanism understanding.
- ❑ Modulation of drug release rate using dose density.

Artificial intelligence in medicine: Developing a computer aided high accuracy treatment evaluation tool for ovarian cancer patients

Quantitative image analysis for predicting early response of ovarian cancer patients to chemotherapy

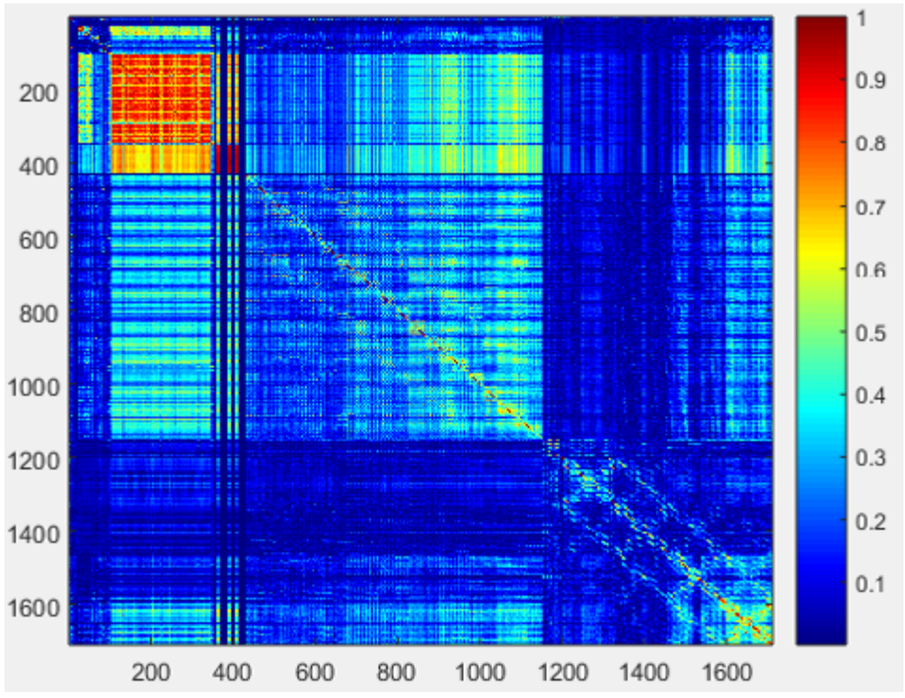
PI: Dr. Yuchen Qiu, The University of Oklahoma **OCAST Project: HR15-016** **Research Area: Biomedical Engineering**

Project Summary

Ovarian cancer is the second most common gynecologic malignancy with the highest mortality rate. In 2019, ovarian cancer may result in 22,530 newly diagnosed cases and 13,980 deaths within United States. As a highly aggressive disease, most of the ovarian cancer patients are diagnosed with metastatic tumors which are scattered at different organs. Among different types of the treatments, chemotherapy is the most effective therapy to control these metastatic tumors. However, the therapy responses vary largely among different individual patients, and physicians can not accurately predict the response at the early stage of treatment.

In order to address this challenge, we developed a novel method to quantify the characteristics of metastatic tumors on patients' CT images for therapy response evaluation. Specifically, this method first segments the target tumors from background, and then computes a large amount of features to estimate different tumor properties including geometry, density, texture, and boundary shapes. After that, we utilize the artificial intelligence (AI) technology to analyze these features to predict tumor response to ongoing therapy. In the last four years, we have accomplished the following tasks:

- Establish a patient database for developing and assessing our novel predicting model
- Develop a compute aided diagnosis (CAD) scheme to accomplish the tumor segmentation, feature computation and predicting score generation
- Use the database to evaluate the model performance; the initial results indicate that our new model outperforms the conventional methods.



Heatmap (Upper) and histogram (Lower) of 1712 features, demonstrating the feature correlation

Delivery of toxic protein to aggressive prostate cancer tumors that have quit responding to conventional treatments

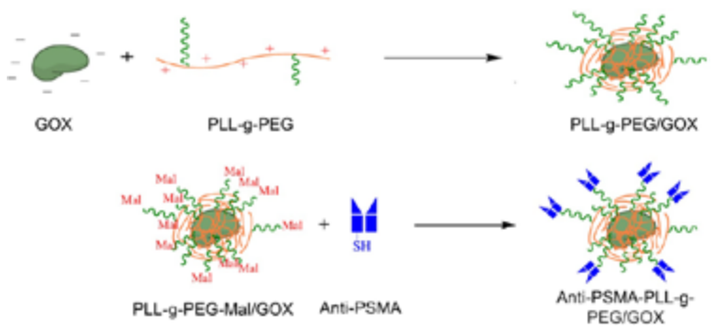
Targeted Delivery of a Reactive Oxygen Species Generator for Treatment of Hormone Refractory Prostate Cancer

PI: Joshua D. Ramsey, Oklahoma State University **OCAST Project: HR19-104** **Research Area: Biomedical Engineering**

Project Highlights

Prostate cancer is the most common type of non-skin cancer among men in the United States. Glucose oxidase (GOX) and other reactive oxygen species (ROS) forming enzymes are of significant interest as anticancer agents due to their potent cytotoxicity. Our overall objective is to use a nanoparticle delivery system to target delivery of GOX to prostate cancer cells.

We expect the project will result in a targeted nanoparticle for delivery of GOX for treating hormone-refractory and non-refractory prostate cancer, the first which has a median survival time of approximately one year from its onset with little, if any, improvement with chemotherapy. Ultimately, the project will lead to improved treatments for prostate cancer, which will benefit the lives of Oklahomans suffering from this disease.

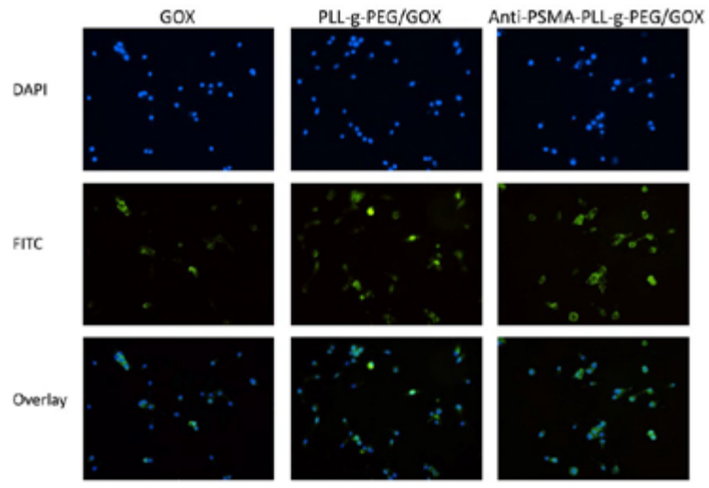


Schematic showing how GOX is encapsulated into a nanoparticle and targeted to prostate cancer cells.

Nicholas Flynn, a scientist from our lab, and Mary Parnell, an undergraduate student, inspect cancer cells under the microscope.

Recent Accomplishments

- We have demonstrated that GOX can be encapsulated within a nanoparticle without severely disrupting its activity.
- We have shown also that these nanoparticles can be targeted toward cancer cells, and that they are more toxic than the free protein alone.
- Our preliminary work was recently published in Macromolecular Bioscience.
- We have begun synthesizing a library of polymers to improve the nanoparticles before moving into animal studies.



Confocal microscopy image showing how the cellular uptake of free enzyme (GOX) compares to untargeted and targeted nanoparticles encapsulating GOX. The cell nuclei are stained blue and the GOX protein is stained green.

A novel wearable vibration therapy device for treating upper limb functional impairment in stoke

Development and evaluation of vibration-based wearable upper-limb rehabilitation device

PI: Hongwu Wang, University of Oklahoma (HSC) **OCAST Project: HR18-034** **Research Area: Biomedical Engineering**

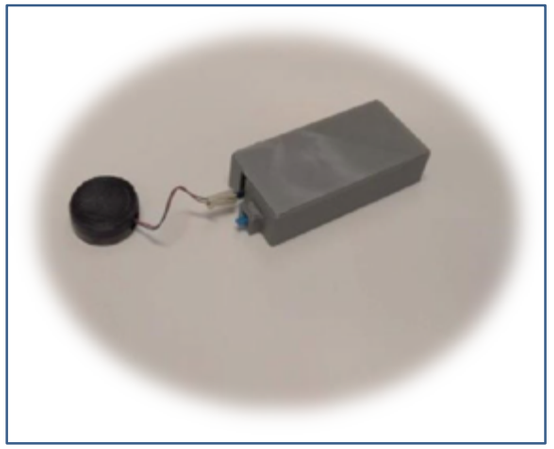
Project Highlights

Neurological disorders and injuries account for the 6.3% of the global burden of disease.

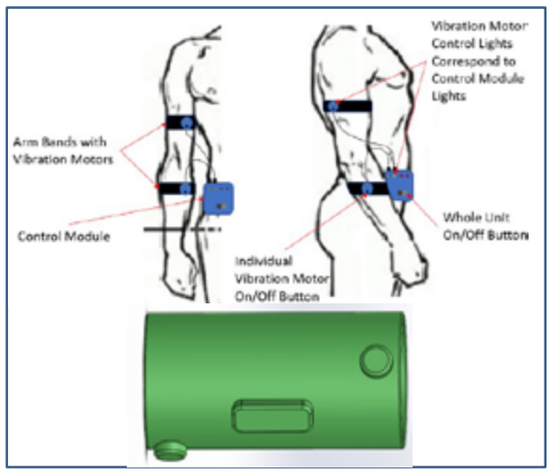
Increasing incidence of neurological disorders and the severity of the disability that they result in, are urgently seeking solutions to prevent or stop the progression of damage and to optimize residual capacity. Functional recovery only lead to 20% of patients' fully resumption of their social life and job activities mainly due to **underdoes**.

Focal vibration (FV) therapy, a non-pharmacological, non-invasive treatment, has had satisfactory outcomes as a useful tool in neurorehabilitation.

We are developing and evaluating a wearable vibration therapy device that delivers **individualized** and **precise** vibration to target muscles. The device will increase and coordinate muscle recruitment and build muscle strength and endurance. The device provides patients opportunity to apply the prescribed vibratory stimuli in-home and/or at community settings to **sustain the dosage** needed. It also allows therapists to monitor usage and compliance and to adjust the doses based on progression.



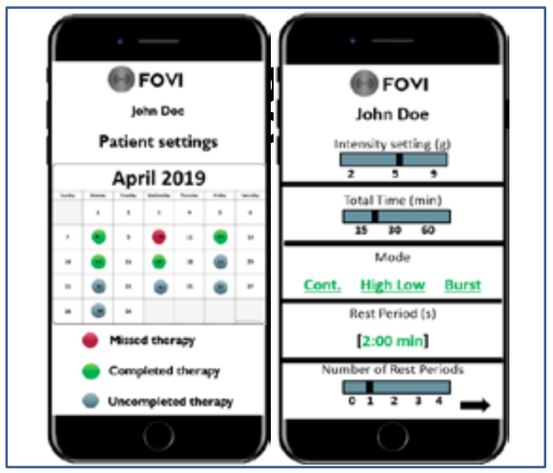
The current prototype with the control hub and a single pod



Device concept with the wearable sleeve to attach the hub and pod

Recent Accomplishments

- We utilized a user participatory design approach, and formed the design matrix and criteria.
- We designed and developed the second generation of a functional prototype with an app.
- We started design and fabricate the sleeve to fit over the upper limb.
- We had a provisional patent pending.



The app interface for the wearable device allows for customization

Identify Bio-Tissue Properties by Advanced Simulation-Based Measurements

Non-contact, in vivo Measurement of Hyper-Elastic Response of Bio-membranes for Predicting Traumatic Injuries

PI: Shuodao Wang, Oklahoma State University **OCAST Project: HR18-085** **Research Area: Bio-Medical Engineering**

Project Highlights

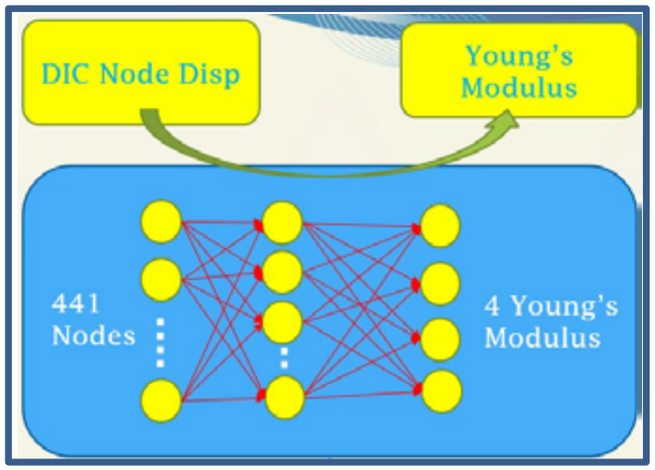
Developing advanced simulation and analysis techniques of the human anatomy is of great importance. Building these models requires experimental data of the physical properties of various bio-tissues. However, characterizations of bio-tissues are extremely difficult due to:

(1) traditional in vitro measurements cannot obtain the authentic properties of living tissues, since their properties change over time once removed from their bio-contexts.

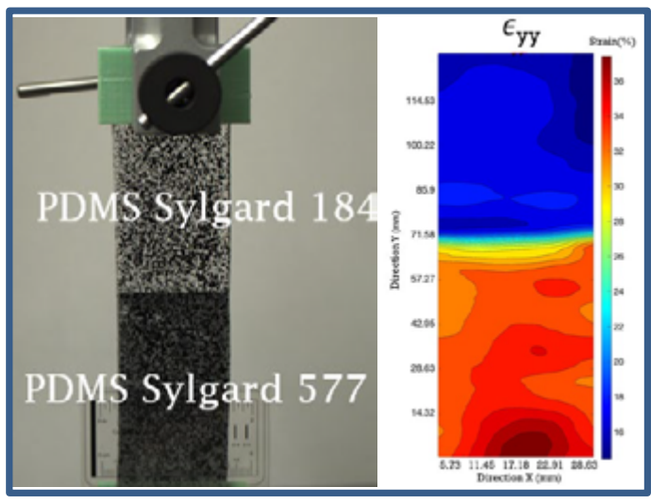
(2) most bio-tissues are soft and thin. E.g. tympanic membranes, meninges, heart valves.

(3) These tissues are made of distinctively different materials distributed spatially.

Therefore, it is important to develop an in vivo, non-contact testing method to accurately characterize properties of living tissues. This project focuses on using stereomicroscopy and inverse modeling techniques for measuring the hyper-elastic mechanical properties of soft tissues. The expected results will have profound impacts on studies that are related to mechanical behaviors of soft tissues, enable new simulation models for bio-tissues, and inspire new healthcare devices.



Machine learning for identifying material properties from the measured deformation.



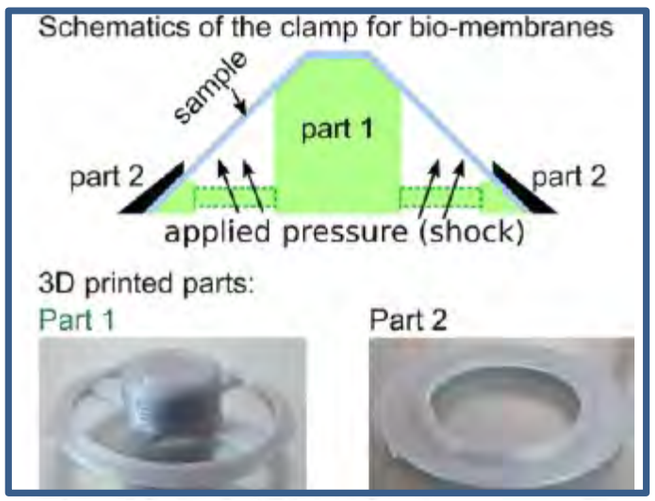
Full-field strain measurement by digital image correlation for material identification.

Recent Accomplishments

(1) Fabricated 3D synthesized membranes and performed preliminary bulge pressure tests.

(2) Conducted full-field digital image correlation experiments and collected data for inverse simulations.

(3) Developed inversed modeling methods by both iterative simulations and machine learning. Adopted "virtual experiments" to expedite the inverse simulation efforts and validate the adopted methodologies.



3D synthesized soft membranes and testing devices for mimicking bio-tissues.

Acquired loss of a crucial protein in cancer cells can lead to the development and progression of deadly childhood cancer, neuroblastoma

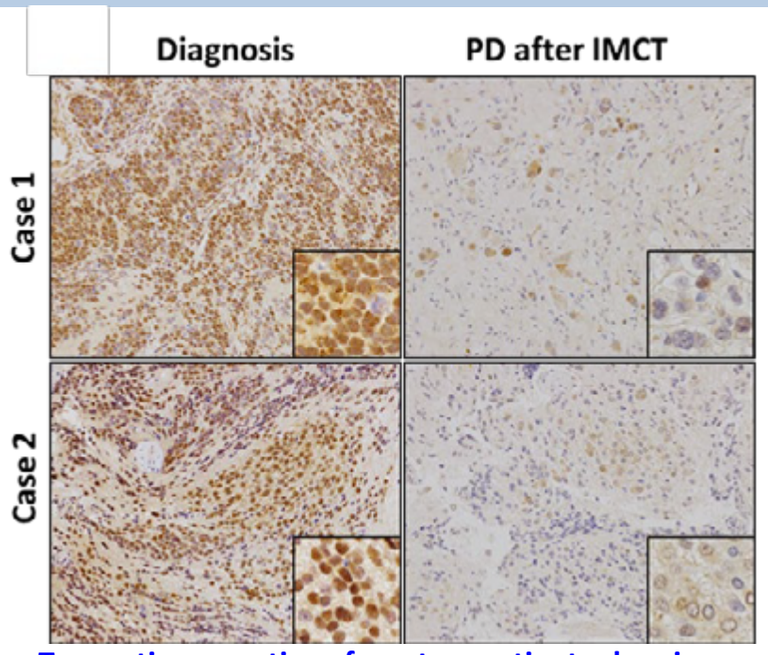
Cre-Conditional RD3-Loss Driven Neuroblastoma Mouse Model: Novel Tool for Preclinical Studies on Disease Evolution

PI: Natarajan Aravindan, OU Health Sciences Center **OCAST Project: HR19-045** **Research Area: Cancer Research/Cancer Biology**

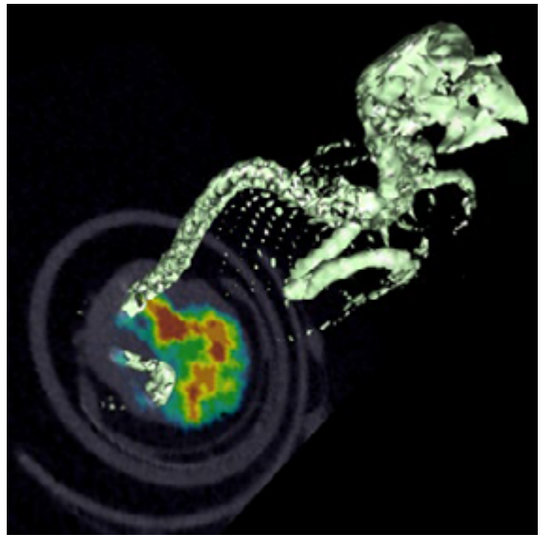
Project Highlights

Neuroblastoma is the most common cancer in infants and young children. Despite four decades of colossal clinical and research efforts to combat this tumor, cure for patients with aggressive disease is critically challenging, contributing to about one-tenth of all childhood cancer deaths. The rapid transition time from curable to incurable disease reflects the continuous acquisition of molecular rearrangements in these cancer cells. Our studies recognized the loss of a protein called Retinal Degeneration Protein 3 (RD3) in aggressive tumors and, also indicated that such loss plays critical role in the tumor progression. The proposed work focus on developing a novel preclinical mouse model by selectively *knocking out RD3* gene in desired (neural crest) cells and, to study whether RD3 loss develops spontaneous neuroblastoma and/or prompt the disease aggression.

In the long run, this research could lead to recognize the mechanism(s) of neuroblastoma initiation and progression and, would allow us to develop more improved therapeutic strategies for better cure of this deadly disease in children.



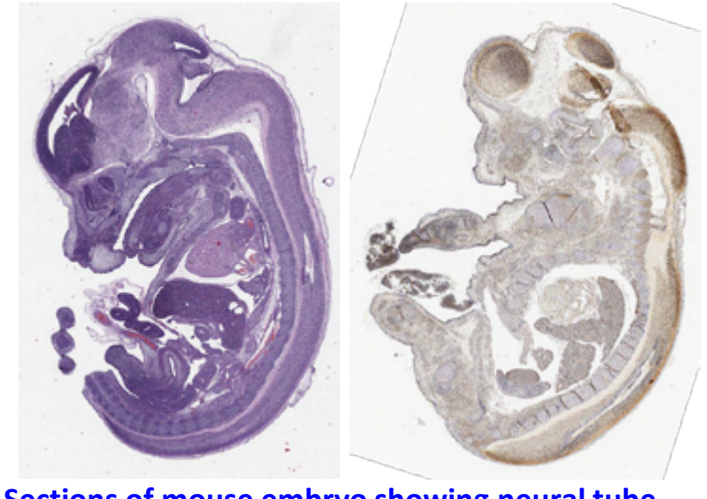
Tumor tissue sections from two patients showing availability of RD3 protein at disease diagnosis and its loss in progressive disease (PD) after intensive clinical therapy



PET image showing a mice bearing NB

Recent Accomplishments

- We identified that RD3 is expressed in human fetal normal tissues
- We determined that RD3 regulates pathogenesis and spreading of tumor in animal models
- We defined that RD3 loss is an acquired processes in cells that survive clinical therapy
- We recently started characterizing the RD3 floxed mice. Floxed mice allows the specific gene to be deleted when crossed with tissue (neural crest) specific enzyme expressing mice.



Sections of mouse embryo showing neural tube and RD3 protein availability

A dual functional small protein identified by phage based biotechnology can smartly home to tumor sites and trigger the antitumor immune responses

Cancer immunotherapy by tumor-homing immune checkpoint-blocking dual-functional peptide

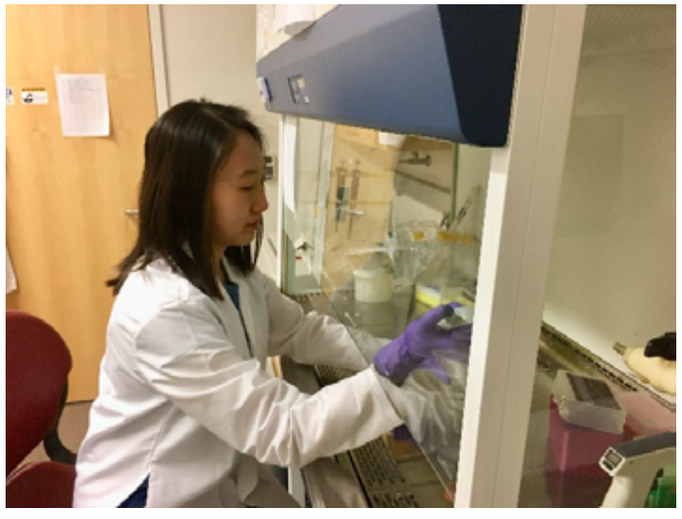
PI: Binrui Cao, University of Oklahoma **OCAST Project: HR17-043** **Research Area: Cancer Research**

Project Narrative

Cancer cells can avoid antitumor immune responses by interacting with the "switches" (called immune checkpoints). Cancer immunotherapy is to block the immune checkpoints and induce antitumor immune responses. However, the current protein blockers may cause serious side effects. This project aims to develop a novel anti-tumor drug that can block immune checkpoints with minimum side effects and low production cost. Our proposed drug is a dual functional peptide (small protein) composed of two components, one for selectively recognizing tumor sites and another for blocking immune checkpoints. These dual functional peptides could selectively move to tumors and induce antitumor immune responses. Moreover, due to their lower production complexity, the production costs of peptides will be relatively low.

3rd Round	Frequency	4th Round	Frequency
ARSLEPAPSRHS	1	ARSLEPAPSRHS	11
TLNVPPAKRSLS	1	TLNVPPAKRSLS	8
TLGLRPVPVATT	1	TLGLRPVPVATT	4
MKAHHSQLYPRH	4	MKAHHSQLYPRH	3
SYPSNALSLHKY	2	SYPSNALSLHKY	3
GSAARTISPSLL	3	TPHGYQPMQGKT	3
NYLPHQSSSPCR	2	GSAARTISPSLA	3
		DNHAKXPLRGSS	3
		AHIESTFARXTH	2
		HDRMTKSSFSPP	2
		TLSLPGFTFVPT	2
		DHISRSQRAPLG	2
		WGVTKPIRTSTL	2
		SNKNLDTRILTK	2
		SNIGALQFLPPP	2
		TLGLRPVPVATT	2

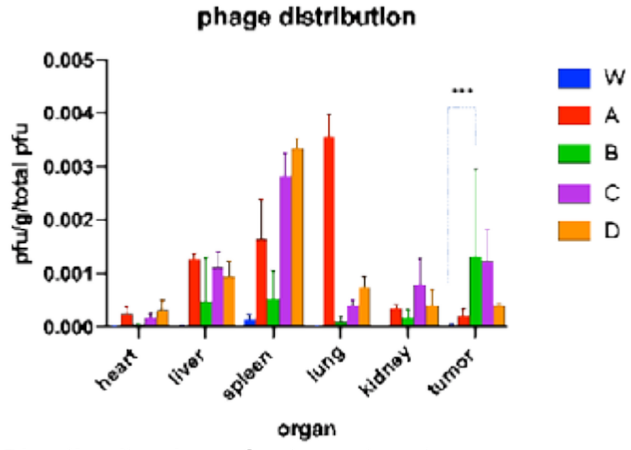
The selected melanoma tumor-homing peptides and their frequencies. After four rounds of selection, a total of 68 colonies were sent for sequencing (14 clones from Round 3 output and 54 clones from Round 4 output)



Liwei Zhang, a graduate student from our lab, performed *in vivo* phage biopanning to identify melanoma tumor homing peptide.

Recent Accomplishments

- We have identified two melanoma homing peptides, SYPSNALSLHKY and TLGLRPVPVATT, using the *in vivo* phage display technique.
- We have demonstrated the melanoma tumor-homing capability of the selected peptides by biodistribution study .
- We will inject the dual functional peptides into the melanoma tumor models to evaluate its selectively inhibiting tumor growth by blocking immune checkpoints.



Bio-distribution of selected melanoma tumor-homing phages in the melanoma tumor-bearing mice. (A-D) Phage displaying peptide A (ARSLEPAPSRHS); peptide B (SYPSNALSLHKY); peptide C (TLGLRPVPVATT); peptide D (TLNVPPAKRSLS); W: wild type phage

Nanotechnology-based photoimmunotherapy for metastatic cancers

Phototherapy for metastatic breast cancer using immunologically modified nanoparticles

PI: Wei R. Chen, University of Central Oklahoma **OCAST Project: HR16-085** **Research Area: Cancer Research/Cancer Biology**

Project Highlights

Combining laser and nanomaterials, we are developing a nanotechnology-based photoimmunotherapy for treatment of metastatic breast cancer. Specifically, we designed a upconversion nanoparticle (UCNP)-based nanoplatform, loaded with rose bengal (RB) for photodynamic therapy, indocyanine (ICG) for photothermal therapy, and maleimide (mal) for capturing tumor antigen. When this unique nanoplatform, UCNP/ICG/RB-mal, is administered to the target tissue, under laser irradiation, photothermal, photodynamic, and photoimmunological interactions can be synergized for cancer treatment. The results of this project may lead to an effective cancer therapy.

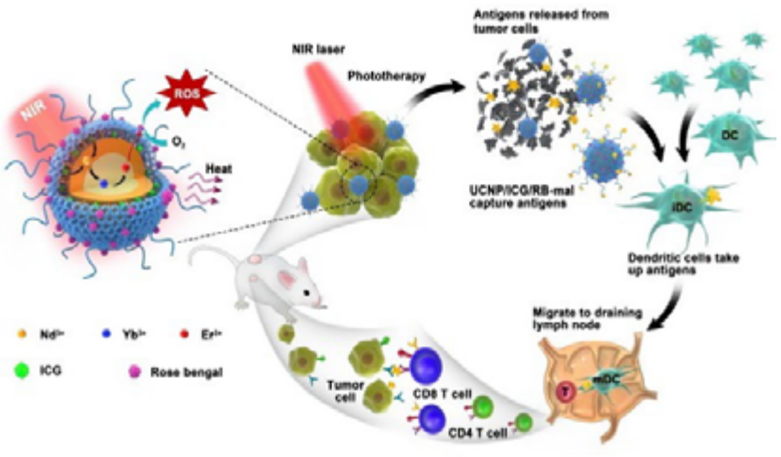


Fig 1. Schematic: synthesis & functions of UCNP/ICG/RB-mal
Upconversion nanoparticle (UCNP) – base
Rose bengal (RB) – photosensitizer
Indocyanine green (ICG) – photothermal and PDT agent
Maleimide (mal) – tumor antigens capturing agent

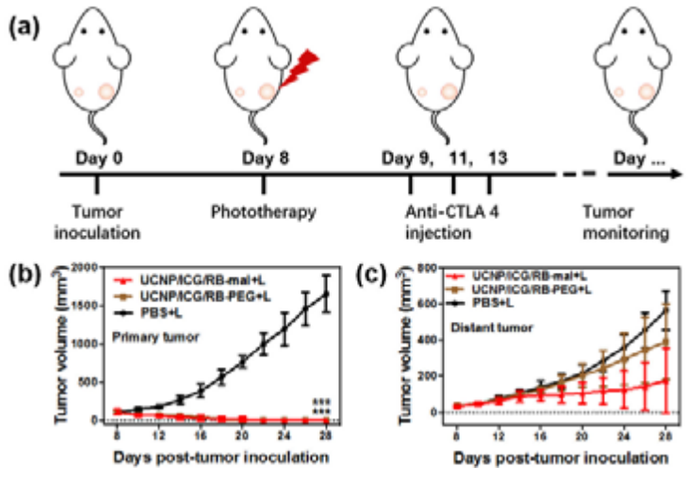


Fig 3. Abscopal effect of laser + UCNP/ICG/RB-mal in treating 4T1 mammary tumors in mice. (a) Schematic; (b) Treated primary tumor size; (c) Untreated secondary tumor size.

Recent Accomplishments

- We synthesized the immunologically modified nanoplatform UCNP/ICG/RB-mal and proposed its mechanism in cancer treatment (Figure 1).
- We treated a highly metastatic mammary tumor (4T1) in mice using laser + UCNP/ICG/RB-mal and achieved long-term survival (Figure 2a). The combination use of a checkpoint inhibitor, anti-CTLA-4, further enhanced the therapeutic effect (Figure 2b).
- Laser + UCNP/ICG/RB-mal induced immune response (increased tumor infiltration of immune cells).
- The induced immune response provided abscopal effect to control untreated tumors (Figure 3).

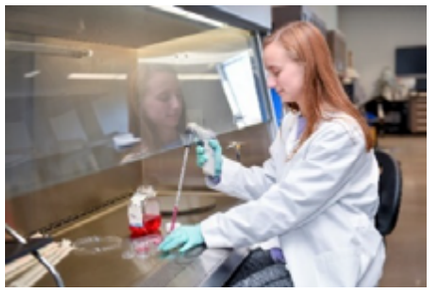


Fig 4. Sara Zukerman, a student in my lab, working on tumor and immune cells.

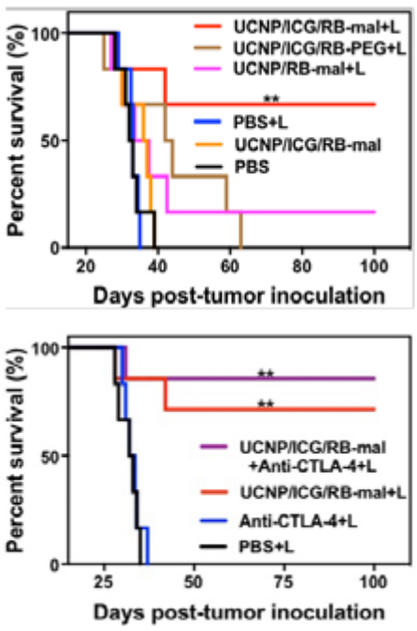


Fig 2. (a) Survival rates of tumor-bearing mice treated by Laser + UCNP/ICG/RB-mal; (b) Survival rates of mice treated by Laser + UCNP/ICG/RB-mal and checkpoint inhibitor.

Our Recent Relevant Publications

Y Li, et al, Nanomedicine, 18, 44-53, 2019
A Doughty, et al, Materials, 12, 779, 2019
M Wang, et al, Advanced Science, 6, 2019
Y Li, et al, Cancer Letters, 442, 429-438, 2019
B Zhou, et al, Nanoscale, 10(46), 2018

OCAST Oklahoma Center for the Advancement of Science and Technology

UNIVERSITY OF Central Oklahoma

Does Stopping Blood Vessel Generation Slow Tumor Growth?

Does Prolyl Oligopeptidase Inhibition Suppress Tumor Growth?

Victoria Christiansen, OUHSC, Warren Research HR18-046 Cancer

Project Narrative

Growth of a tumor requires the development of blood vessels to provide nutrients for the tumor. Stopping the growth of new blood vessels may slow or stop tumor growth. A protein named Proyl Oligopeptidase (POP) is part of one pathway that leads to new blood vessel growth. We have developed a compound that blocks the actions of POP, so that fewer new blood vessels are formed. That compound, named J94, does reduce the formation of blood vessels. J94 has also been tested in an animal experiment to examine tumor growth. This early study demonstrates a decreased growth rate of a colon cancer tumor in mice. These studies will test several doses of J94 in three different human tumor types in mouse models. We will also study the POP pathway of new vessel growth to find other steps that could be blocked to slow vessel growth, and possibly slow growth of a tumor.

Recent Accomplishments

- J94 tested in vessel-forming assay found to slow development of new vessels
- J94 tested in a mouse model of colon cancer demonstrated a slower growth of the tumor

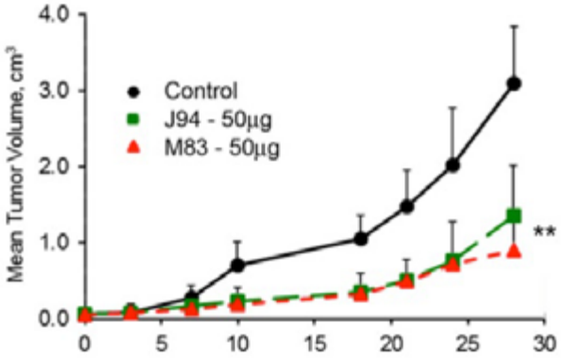


Fig. 2. In a mouse model of human colon cancer, J94 slows tumor growth.

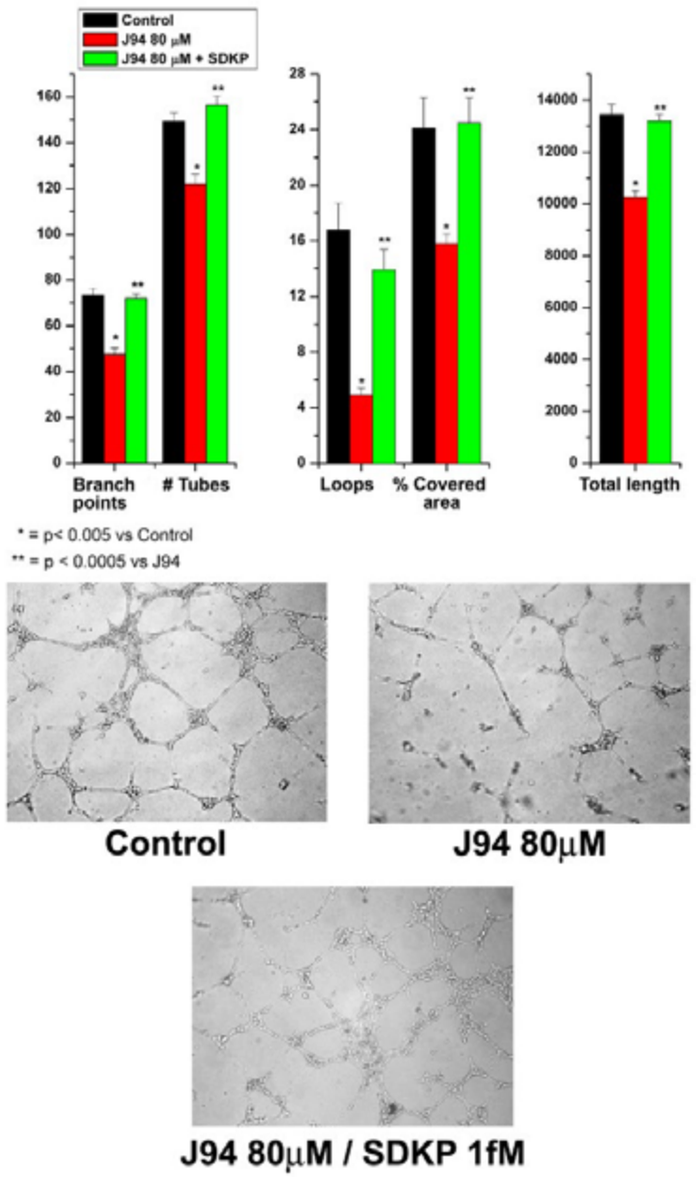
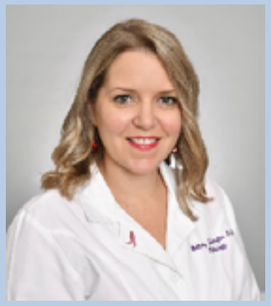


Fig. 1. In a vessel-forming assay, J94 reduces all measures of vessel formation.



The Role of Exosomes in Breast Ductal Carcinoma In Situ

Exosome microRNA Contents Are Altered During Breast Cancer Progression

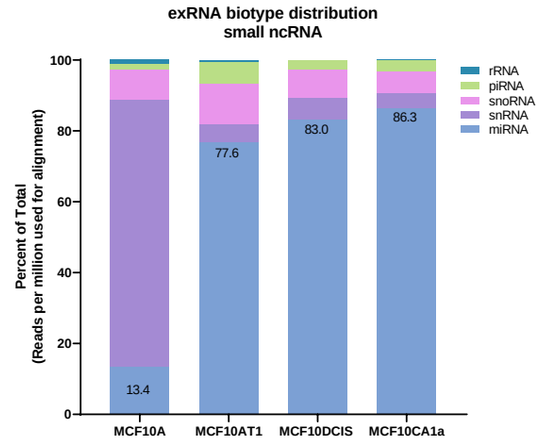
PI: Bethany N. Hannafon, PhD, OUHSC

OCAST Project HR17-052

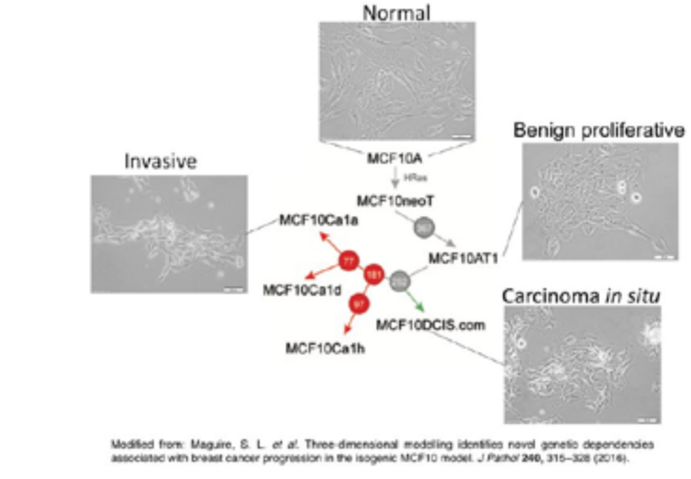
Research Area: Cancer Biology

Project Highlights

The progression of breast cancer involves the transformation of normal mammary epithelial cells to ductal carcinoma in situ (DCIS) and invasive breast cancer (IBC). This process is initiated by genetic alterations and characterized by changes to gene expression programs and microenvironmental alterations. However, the specific drivers of DCIS progression to IBC are not well understood nor has an indicator of progression been identified. Exosomes are small secretory vesicles that can contribute to cancer progression by transferring oncogenic factors, such as microRNAs (miRNAs), to surrounding cells in the tumor microenvironment, and enter the circulation to act at distant sites. miRNAs are short noncoding RNAs that regulate the expression of a target messenger RNA (mRNA). Altered regulation by miRNAs is implicated in cancer progression. In this study, we are seeking to characterize the exosome miRNAs in the MCF10 isogenic model of breast cancer progression in order to identify potential drivers of breast cancer.



Extracellular RNA biotype distribution in MC10A progression series.

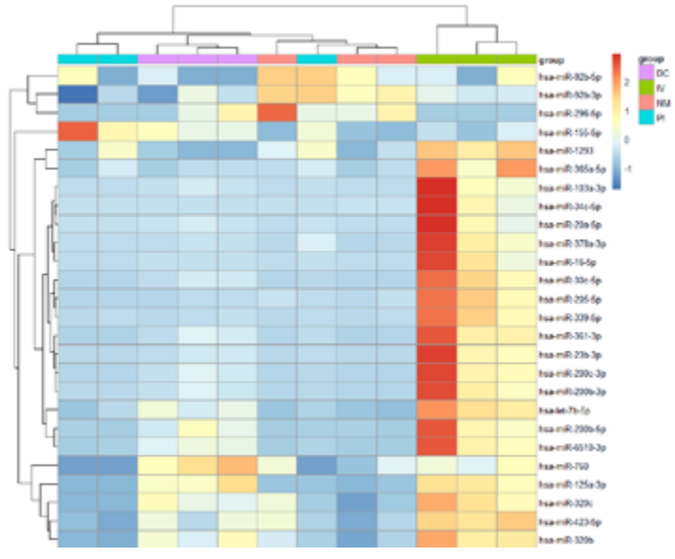


Modified from: Maguire, S. L. *et al.* Three-dimensional modelling identifies novel genetic dependencies associated with breast cancer progression in the isogenic MCF10 model. *J Pathol* **240**, 315–328 (2016).

Generation of the MCF10 progression series and representative bright-field images. This isogenic cell line series can be utilized as model of early breast cancer progression

Recent Accomplishments

- We have completed the Next Gen small extracellular RNA sequencing and are validating identified miRNAs in exosomes from additional cell lines and an animal model of DCIS progression.
- We are continuing to collect patient plasma samples to evaluate the extracellular RNA signatures in patients with DCIS and invasive breast cancer.



Hierarchical clustering analysis of Small RNA Next Generation Sequencing .

Targeted Therapy for Treating Metastatic Breast Cancer

Novel Targeted Protein-Drug Conjugates for Treating Metastatic Breast Cancer Combined with Immunostimulation and mTOR Inhibition

PI: Roger Harrison, University of Oklahoma **OCAST Project Number: HR19-148** **Research Area: Cancer Research/ Cancer Biology**

Project Highlights

Metastasis is still the main reason for death in breast cancer patients, and no cure exists. Therefore, the long-term objective of this project is to develop a therapy to successfully treat metastatic breast cancer with minimal side effects. The specific goal of the research proposed is to demonstrate that this long-term objective can be accomplished by targeting cancer drugs to breast tumors using the protein annexin V (AV) as the targeting vehicle. AV is selected as the targeting agent because it not only binds to a specific molecule (called phosphatidylserine) exposed on cancer cells, but it also binds to the surface of blood vessels in the tumor but not in normal blood vessels. The anti-cancer drugs that are selected as conjugates with AV are chlorambucil and DM1, also known as emtansine. One hypothesis that will be explored is that by using AV as the targeting agent for cancer drugs, the blood supply to the tumor will be cut off, creating hypoxia in the tumor; the addition of an mTOR inhibitor such as rapamycin will shut off the response of the tumor to hypoxia. A second hypothesis is that the attack on the cells lining the tumor vasculature—the endothelial cells—will lead to antigens being distributed in the bloodstream and throughout the body, and therefore the simultaneous administration of a checkpoint inhibitor antibody will amplify and stimulate the body's immune response to metastatic tumors. These studies are expected to lead to a much improved treatment of metastatic breast cancer and also to the treatment of other solids tumors. The studies on the use of immunostimulation coupled with targeting the tumor vasculature will lead to a better understanding of how to harness the immune system to treat metastatic cancer.

Recent Accomplishments

- The CHL and DM1 drugs were separately conjugated to the annexin V (AV) protein (approximately 10 molecules of CHL per AV molecule, and approximately 8 molecules of DM1 per AV).
- Conjugation of either drug to AV had a large effect on the efficacy of treatment of two types of mouse breast cancer cells and one type of human breast cancer cells, ranging from a factor of 50 to 900 times less for the IC50 (concentration needed to achieve 50% inhibition of viability).
- A preliminary test with the AV-CHL conjugate was performed for treating mice with 4T1 metastatic breast cancer. One day after inoculation of cancer cells in the mammary fat pad, daily treatment with the AV-CHL conjugate (dose of 0.5 mg/kg mouse weight) resulted in a cessation of growth over the first 9 days. A study to find the optimal dosage is in progress.

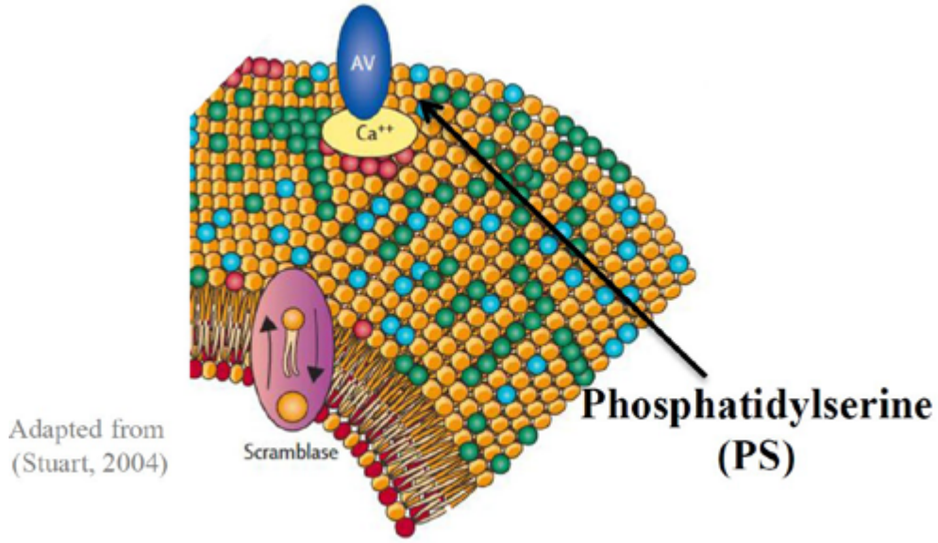


Illustration showing the binding of annexin V (AV) to the molecule phosphatidylserine (PS) exposed on the surface of a cancer cell. In this project, AV is conjugated to an anti-cancer drug. This conjugate is translocated across the cell surface into the interior of the cell.

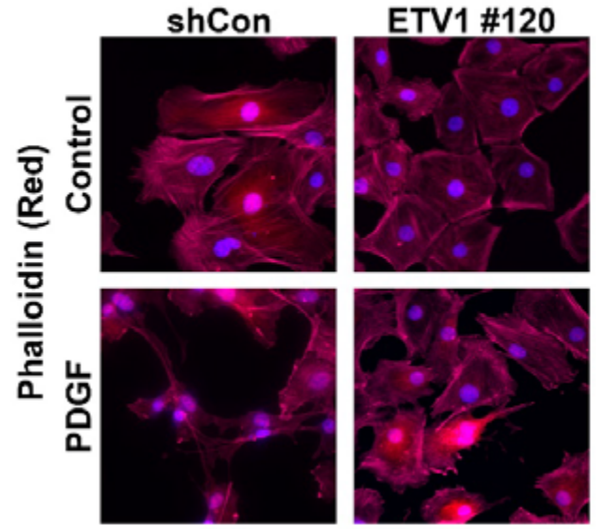
Altering the tumor micro-environment to promote immune surveillance

Rnd proteins promote a sentinel phenotype in fibroblasts

PI: Eric Howard, OUHSC OCAST Project Number: HR16-142 Research Area: Cancer Research/Cancer Biology

Project Narrative

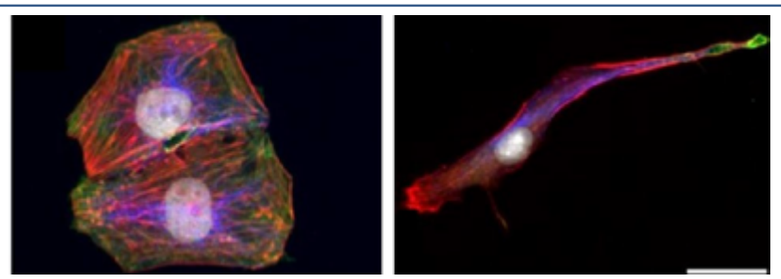
Solid tumors consist not only tumor cells, but also a variety of other cell types that influence tumor viability, growth, and invasion. Among those cells are fibroblasts and vascular smooth muscle cells, both of which are able to undergo a transition from a quiescent state to a highly contractile state. Contractile fibroblasts promote tumor progression through their ability to maintain a stiff mechanical environment, and contractile VSMCs maintain functional blood vessels. In response to specific cues, however, these cells undergo a transition to a migratory, non-contractile state, and we have found that this results in the production of factors that prime the immune system to recognize and eliminate the tumor. This project focuses on the mechanisms involved in this transition, with an emphasis on the regulation of the phenotypic switch. In conjunction with ongoing studies on immune stimulation, we hope to determine how altering the tumor micro-environment can promote immune-mediated tumor elimination.



Knockdown of ETV1 decreased VSMC migration. Transduced VSMCs were plated coverslips, and stimulated with PDGF. Cells transduced with control vector showed characteristic polarization, as well as the expression of immune mediators. Cells in which ETV1 was knocked down did not exhibit these phenotypic characteristics.

Recent Accomplishments

We have identified the transcription factor, ETV1, as a critical mediator of vascular smooth muscle cell transition. Knocking its expression down significantly blocked phenotypic switching in VSMCs. Using transcriptome analysis, we have also recently identified a network of mechanically regulated genes associated with the switching of VSMCs and fibroblasts to some of the phenotypes these cells are capable of becoming.



VSMCs transition from a contractile to a polarized, sentinel phenotype. Cultured VSMCs respond to PDGF stimulation by disassembling actin stress fibers and focal adhesions, and assuming a polarized state that facilitates directional migration. This migratory phenotype is also associated with the expression of immune mediators that alter the tumor microenvironment.

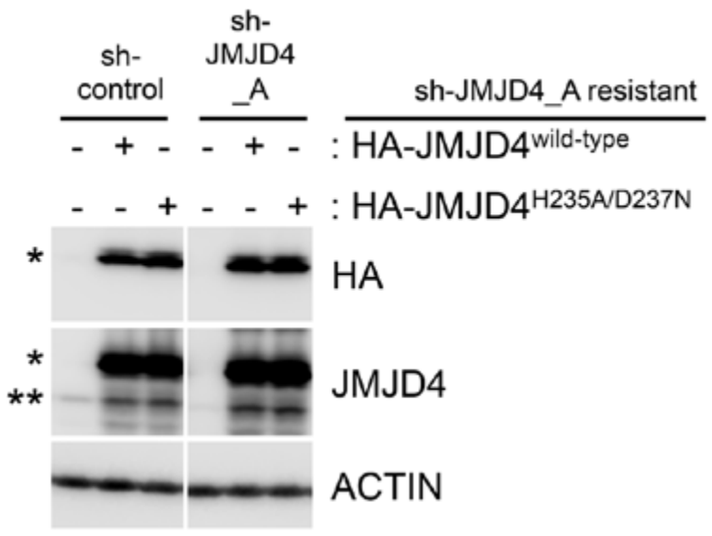
A protein that may be involved in the progression of pancreatic cancer.

Role of JMJD4 in Redox Regulation and Pancreatic Cancer

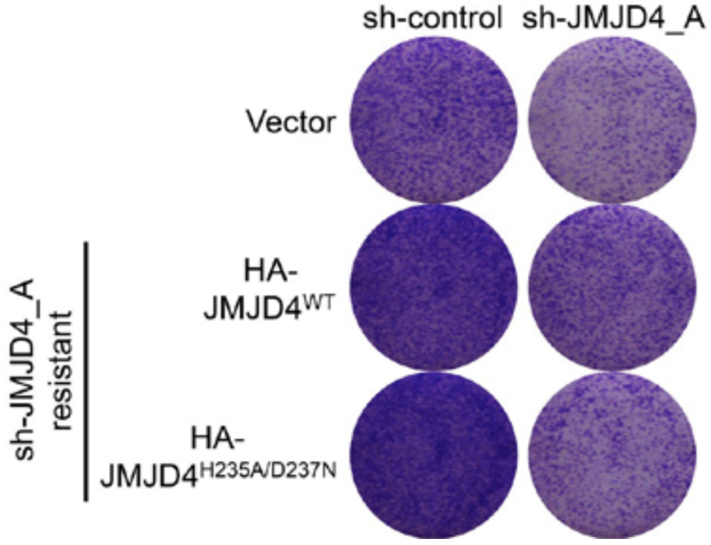
PI: Sangphil Oh, University of Oklahoma Health Sciences Center **OCAST Project: HR17-067** **Research Area: Cancer Research**

Project Narrative

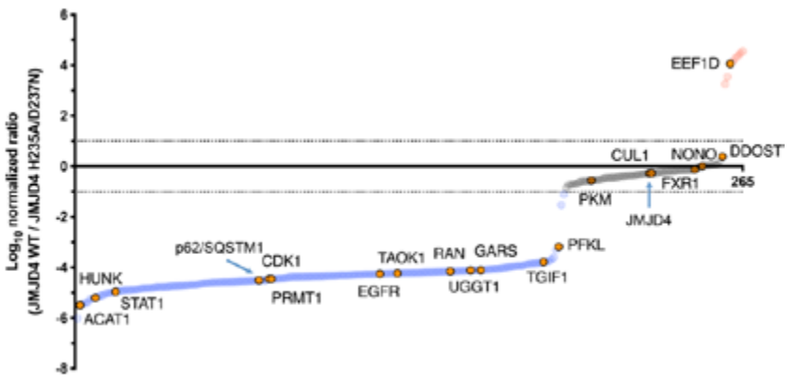
Pancreatic cancer is a very aggressive cancer with a survival rate of less than 5% in the US. Because pancreatic cancer is usually resistant to conventional chemotherapies, only very few treatment options are currently available after diagnosis. Thus, it is imperative to understand how pancreatic cancer develops and what makes these cells resistant to chemotherapy, which may identify new therapeutic targets in pancreatic cancer. The proposed research will focus on understanding how a protein, JMJD4, which is overexpressed in a subset of pancreatic tumors, regulates pancreatic cancer progression. Ultimately, this research may validate JMJD4 as a new target for pancreatic cancer therapy and may reduce the mortality rate of patients and the costs of health care, which will be beneficial to Oklahoma's economy.



Establishment of shRNA-resistant JMJD4 expressing constructs for rescue experiments



Growth defects caused by JMJD4 knockdown can be partially rescued with overexpression of wild-type JMJD4 in pancreatic cancer cell Panc-1



Visualization of JMJD4-interacting proteins identified by Mass Spectrometry analysis

Recent Accomplishments

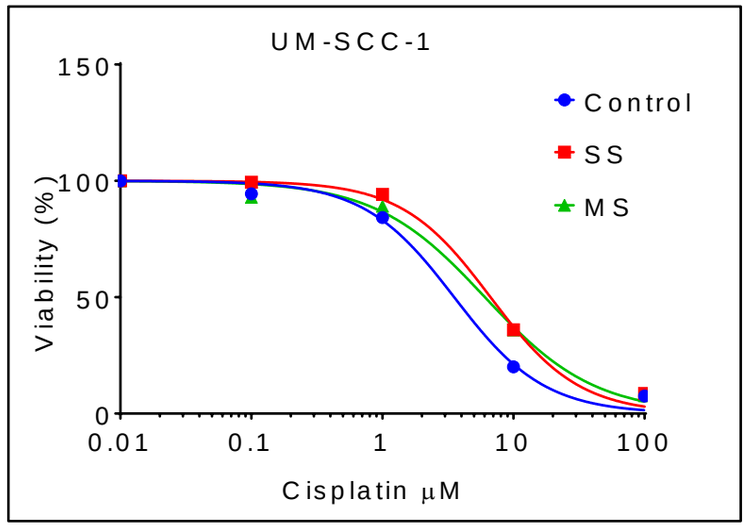
- ✓ We recently established system evaluating importance of functional JMJD4 in pancreatic cancer cell growth
- ✓ We screened JMJD4-interacting proteins that may explain how JMJD4 is involved in the progression of pancreatic cancer

Studying the effects of tobacco smoking on oral stem cells

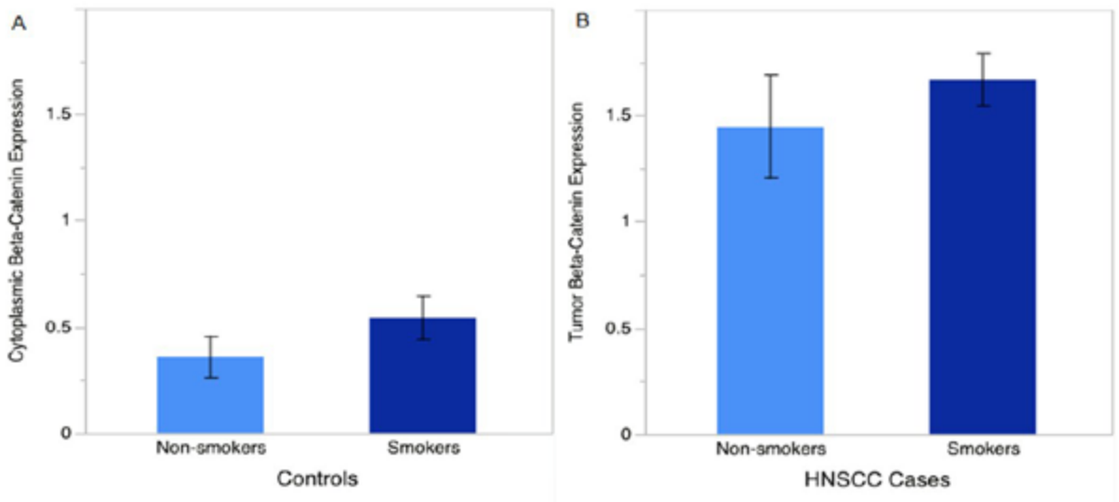
PI: Lurdes Queimado, OUHSC **OCAST Project: HR16-007** **Research Area: Cancer Research/Cancer Biology**

Project Summary

Smoking is the main cancer risk factor in the United States. Recently, we have shown that a single puff of active or passive (secondhand) tobacco smoke can induce significant DNA damage leading to mutations which can cause cancer. Stem cells are a small subset of cells with unique properties essential for tissue regeneration and tumor growth. We are investigating the effects of tobacco smoke on DNA damage, stem cells and response to chemotherapy. Our project provides the scientific data needed to advance tobacco control programs and might identify novel drugs that significantly improve the survival of patients with oral cancer. Ultimately, this will significantly reduce Oklahoma's tobacco-associated disease burden and allocated funds can then be directed towards other wellness enriching programs throughout the state.



Dose response curve showing that both active (MS) and passive (SS) smoking increase cisplatin resistance. $p<0.0001$



Tobacco smoking increases WNT activation in oral normal epithelium (A) and cancer tissues (B).

Recent Findings

- Exposure to tobacco smoke increases the expression of Wnt activators and multidrug resistance proteins.
- Exposure to active and passive (secondhand) smoke increases stemness and cisplatin resistance.
- Exposure to electronic cigarette aerosols reduce DNA repair capacity. Thus, it has potential to increase the cancer risk associated with other genotoxic exposures (Queimado et al., PNAS. 2018 Jun12;115(24):E5437-E5438).

Exosomes as a Surrogate for Determining Response to Immunotherapy

Non-Invasive Liquid Biopsy Approach for Using Exosomes as a Surrogate for Determining Response to Immunotherapy in Lung Cancer Patients

Rajagopal Ramesh, University of Oklahoma Health Sciences Center HR-18-088 Cancer Research

Introduction. Effective treatment of lung cancer remains a formidable clinical challenge. Molecularly-targeted therapies and immune checkpoint inhibitors have shown promise in lung cancer treatment. However, only a subset of patients benefit from these therapies due to tumor heterogeneity. Therefore, there is an urgent need to screen for biomarkers that will enable in predicting treatment response. Availability of biomarker for predicting therapy response will allow oncologists to make treatment decisions thereby offering alternate therapies and improving patient survival.

Objective & Aims. We in the present study propose a novel and innovative strategy for monitoring and predicting response to immunotherapy by utilizing a non-invasive liquid biopsy approach of isolating exosomes from urine and using them as a surrogate marker in lung cancer patients receiving immunotherapy, specifically Pembrolizumab (anti-PD1) therapy. We hypothesize that PD-L1 expression levels in urine-derived exosomes will define response to immunotherapy, specifically Pembrolizumab (anti-PD1) therapy. To test our hypothesis we propose two specific aims: **Aim 1**. (**a**). Examine expression of PD-L1 and other immune checkpoint proteins in urine-derived exosomes from lung cancer patients receiving Pembrolizumab (anti-PD1) as a single agent or in combination with chemotherapy and correlate with clinical response. (**b**). Evaluate expression of PD-1 and other immune check point proteins on T-cells from patients receiving Pembrolizumab (anti-PD1) as single agent or in combination with chemotherapy.

Aim 2. Investigate the immune-modulating properties of urine-derived exosomes *in vitro.*

Results. PD-L1 expression in exosomes of lung cancer patients receiving immunotherapy determined and is modulated pre- and post-treatment. Analysis of additional immune markers are in in progress.

Overall Impact. Completion of the studies will offer a non-invasive liquid biopsy using urine-derived exosomes as a biomarker source for monitoring and predicting treatment outcomes and abetting oncologists in making quick treatment decision.

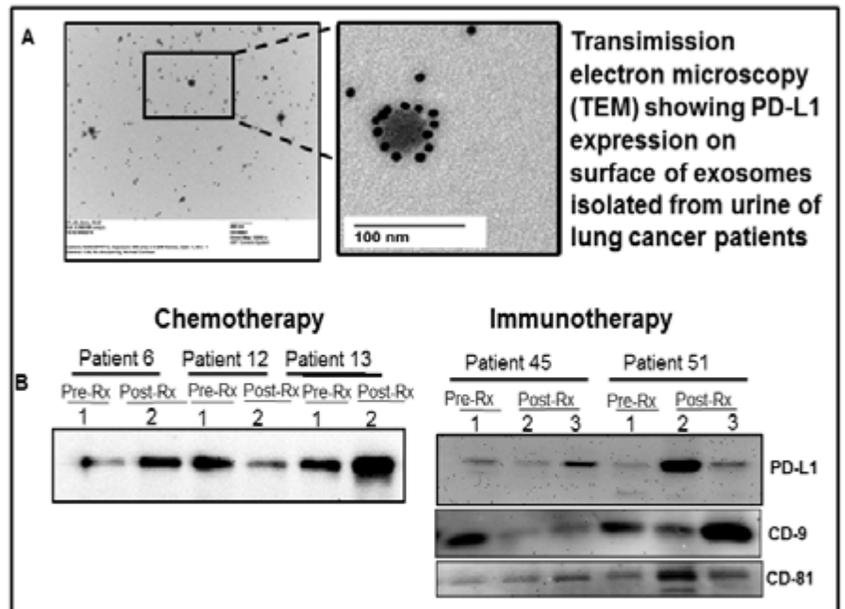


Figure 1. ***A,*** TEM showing PD-L1 expression in exosomes isolated from urine of lung cancer patients; ***B***, PD-L1 expression in exosomes from lung cancer patients receiving chemotherapy and immunotherapy pre- and post-treatment. Sample # 1 represents pre-treatment and Samples # 2 and # 3 represent post-treatment. CD9, and CD81 were used as markers of exosomes.

Recent Accomplishments

- **Publications (1); Abstract (3); Grant (2)**
- Amreddy, N., Ahmed, R., Munshi, A., **Ramesh, R**. Tumor-targeted dendrimer nanoparticles for combinatorial delivery of siRNA and chemotherapy for cancer treatment. Drug Delivery Systems: Methods in Molecular Biology Vol. 2059; pp: 167-189; 2019.
- Razaq, M.A., Pareek, V., Srivastava, A., **Ramesh, R.** Role of exosomal microRNAs (miRNAs) as predictors of response to treatment and prognosis in non-small cell lung cancer (NSCLC). 2019 World Conference on Lung Cancer, Barcelona, Spain, Abstract # P2.01-83, pg.705, 2019.
- **DOD-LCRP** # W81XWH-18-1-0637; W81XWH-19-1-0647

Identifying New Therapies for Prostate Cancer

Defining the role of the TMEFF2 transcript in androgen signaling in prostate cancer

Maria J. Ruiz Echevarria, PhD
Univ. of Oklahoma Health Sciences Center

Project Number: HR18-037

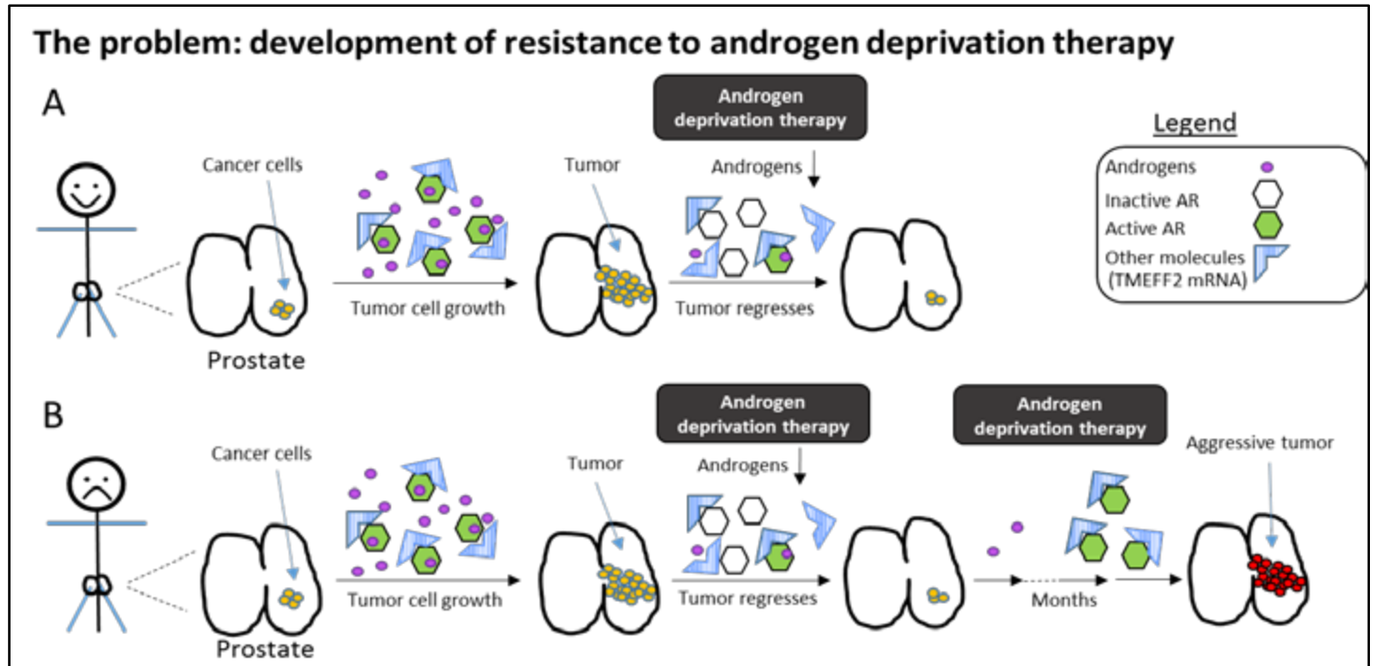
Cancer Research/Cancer Biology

Project Narrative

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men in the US, with nearly 88 men dying from it every day. PCa growth depends on androgens (i.e. testosterone) binding and activating the androgen receptor (AR), which is essential for tumor growth. Because of this dependency tumors are vulnerable to treatments that decrease AR activity, collectively known as "androgen deprivation therapy" (ADT). Although ADT is initially effective, eventually the tumor becomes resistant to the treatment and the disease comes back as a very aggressive and lethal form known as "castration resistant PCa".

The broad long-term goal of our studies is to define novel molecular mechanisms involved in modulating AR activity with prostate cancer progression that affects clinical outcome and ultimately resistance to therapy.

Based on our preliminary data, indicating that shRNA mediated silencing of the TMEFF2 tumor suppressor inhibits PCa cell growth and modulates AR levels and activity independent of TMEFF2 protein levels, we hypothesized that the *TMEFF2* transcript has a critical function in PCa as a regulator of AR signaling. Ultimately these studies *will contribute to improving the strategies in place to find a cure for PCa.*



(A) Androgens together with the androgen receptor (AR) and other molecules promote tumor development and growth. Reducing AR activity with androgen deprivation therapy leads to tumor regression. (B) Unfortunately, sooner or later, the tumor becomes resistant to the therapy and comes back in a very aggressive, lethal form.

Recent Accomplishments

- We have demonstrated that TMEFF2 targeted shRNAs suppress PCa cell growth through a mechanism that involves seed region complementarity to the 3' UTR of survival and androgen signaling regulatory genes. This results in potent inhibition of androgen mediated transcriptional response and cell growth.
- This inhibitory mechanism, that we have called AN-DISE, effectively kills castration resistant PCa (CRPC) cells that arise as a resistance to conventional therapies targeting AR signaling.
- Given the sensitivity of PCa cells, **AN-DISE represents a promising new therapeutic strategy for advanced PCa**

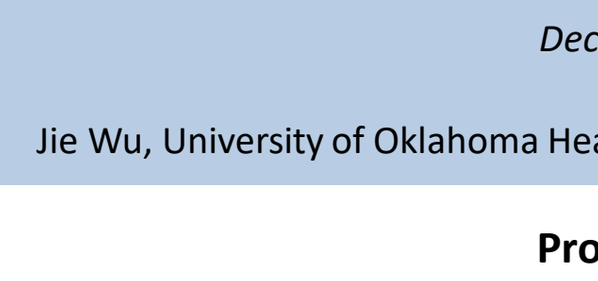
Decoding Mechanisms of Drug Resistance to a New Type of Cancer Drugs

Deciphering bypass mechanisms of resistance to SHP2 inhibition

Jie Wu, University of Oklahoma Health Sciences Center OCAST Project: HR19-026 Research Area: Cancer Research

Project Highlights

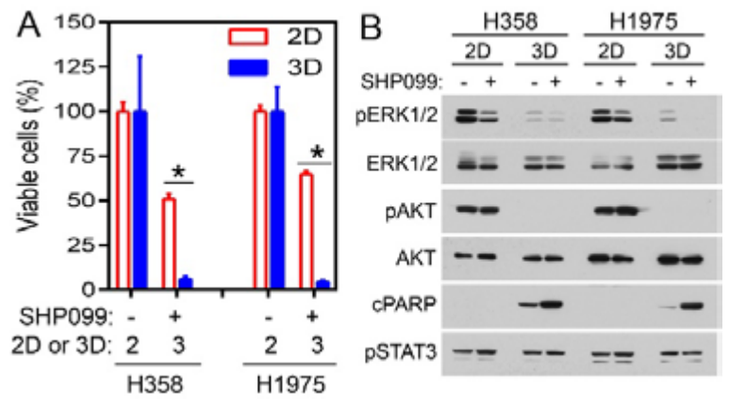
Shp2 is a unique enzyme critical for tumor growth. Recently, major breakthrough in developing Shp2-specific inhibitors has led to first-in-class clinical trials of these new type of cancer drugs. However, two laboratory observations raised urgent issues that must be addressed in order to use Shp2 inhibitors to develop an effective new cancer treatment. The first observation was that adherent cells had better tolerance to Shp2 inhibition. The second observation was that cancer cells displayed heterogeneous responses to Shp2 inhibition. Our study aims to uncover the mechanism by which cell adhesion confers tolerance to Shp2 inhibition and determine why some cancer cells are less dependent on Shp2. Understanding the mechanisms of resistance to Shp2 inhibition will allow us to identify and target the Shp2 bypass pathways that allow cancer cells to live and grow when the Shp2 activity is blocked. This will facilitate the development of novel combinational treatments to maximize the clinical benefits of Shp2 inhibitors in new cancer therapy.



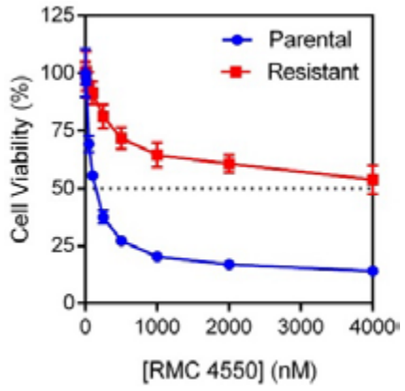
Analysis of active ERK1/2 in mouse lung tumors induced by Kras(G12D)-mutant. A and B were from the same mouse lung. A high level of active ERK1/2 was detected in A but not in B.

Recent Accomplishments

- We found variable levels of active ERK1/2 in Kras mutant-induced lung tumors, suggesting these tumors had heterogeneous properties.
- We have enriched the Shp2 inhibitor-resistant subpopulation from a cancer cell line, allowing us to start analyzing the differences between these two populations of cells.



Cancer cells in 3D spheroid cultures were significantly more sensitive to a Shp2 inhibitor than cells in 2D adherent cultures. Correspondingly, 3D spheroid cells had less active ERK1/2 and active AKT.



Comparison of the parental and enriched Shp2 inhibitor-resistant cell populations.

Understand and treat blood cancers

Define the role of Mpl in myelofibrosis

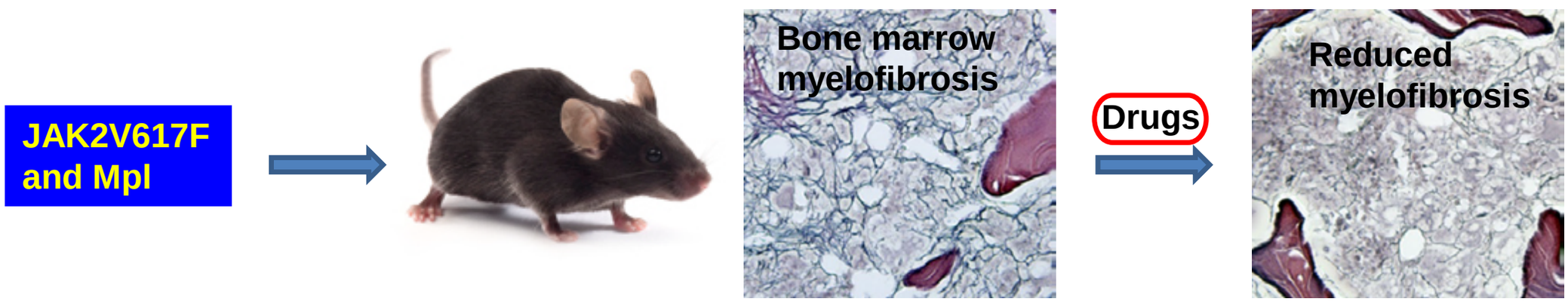
PI. Z. Joe Zhao, OUHSC **OCAST project: HR18-113 Research Area: Cancer Research/Cancer Biology**

Project Summary

Myeloproliferative neoplasms (MPNs) represent a type of chronic blood cancer characterized by over-production of red blood cells, white blood cells, and platelets. MPNs mainly affect older people with an average onset age of 55 years. One subtype of MPNs is myelofibrosis characterized by formation of scar-like tissues in the bone marrow. Myelofibrosis disrupts normal production of blood cells, leading to severe anemia and enlarged spleen and liver. So far, there is no effective treatment for myelofibrosis. Patients with myelofibrosis have a very poor prognosis with a median survival of 5 years after diagnosis. The major molecular lesion in MPNs is JAK2V617F, an acquired mutant form of the normal JAK2 gene. By using transgenic mouse models, we have identified two important genes, JAK2 and Mpl, which can alter the activities of HSCs and stem cell niches and thereby cause myelofibrosis when mutated or abnormally expressed. In this study, we will find out how JAK2 and Mpl cause myelofibrosis at the cellular and molecular level. This will allow us to develop drugs that restore normal function of HSCs and stem cells niche, thereby treating and preventing myelofibrosis.

Recent Accomplishments

1. We have generated a mouse model of myelofibrosis using by expressing JAK2V617F, the mutant form of tyrosine kinase JAK2 found in MPN patients. These mice have been used to test JAK2 inhibitors for treatment of myelofibrosis.
2. We identified an elevated level of TGF-beta1 in the bone marrow of JAK2V617F transgenic mice with myelofibrosis. We further demonstrated that TGF-beta signaling pathway inhibitor galunisertib effectively suppressed development of myelofibrosis in JAK2V617F transgenic mice.



Mouse model of myelofibrosis

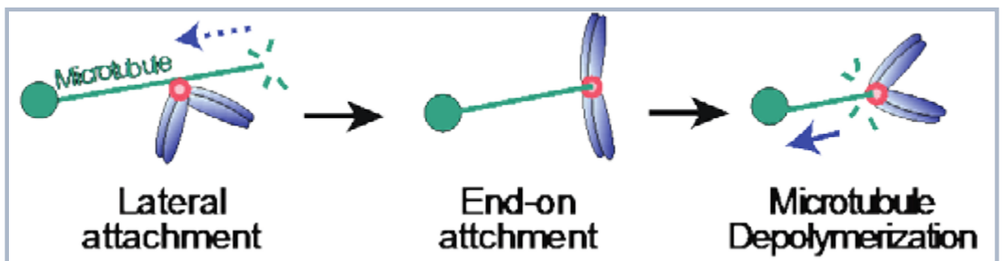
Understanding the machine that distributes chromosomes when cells divide

Orienting Chromosomes on the Meiotic Spindle

PI: Dean Dawson, OMRF **OCAST Project: HR17-115-1** **Research Area: Cell/Molecular Biology**

Project Narrative

We begin life as a single cell with 46 chromosomes, 23 from each parent. This cell divides to form cells. Two cells become four and four become eight – each with 46 chromosomes. By the time we reach adulthood our bodies are comprised of trillions of cells. To function normally, these cells must each carry that original chromosome set. How is this accomplished? Prior to each cell division, each of the 46 chromosomes is duplicated to produce a pair of identical chromosomes called sister chromatids. Then in a remarkable process called mitosis, those 46 pairs of sister chromatids is pulled apart from each other to opposite sides of the cell by small cables called microtubules. The cell then divides into two, with each daughter cell containing one of the chromosome sets. A similar process, called meiosis, is used to produce egg and sperm cells each with only 23 chromosomes, so that fusion of an egg and sperm produces a cell with 46. When this process of partitioning chromosomes fails, cells with the wrong chromosome number are produced. This can lead to birth defects or contribute to the formation of tumors. One error that can occur is when chromosomes fail to connect to the pulling cables (microtubules). This project focuses on a regulator (called Mps1) of this attachment process that controls the attachment of the chromosomes to the microtubule. We seek to learn how Mps1 does this. Learning this information could help us understand why the process sometimes fails – contributing to birth defects or cancer - and could help in the design of anti-cancer drugs inactivate Mps1 in tumor cells so they can no longer divide.



Cartoon showing the process by which chromosomes become attached to microtubules. Mps1 allows the chromosome stay attached to the microtubule as it shortens, pulling the chromosome to the correct place in the cell.

Recent Accomplishments

- We are developing a new assay to test the hypothesis that Mps1 promotes gliding of laterally attached chromosomes to microtubule ends.
- We have measured the rate of microtubule de-polymerization in *mps1* mutants to test the hypothesis that Mps1 controls this process. We found reduced depolymerization rates in the mutant.

Understanding the Glue between Cells; Sugar/Protein Connections

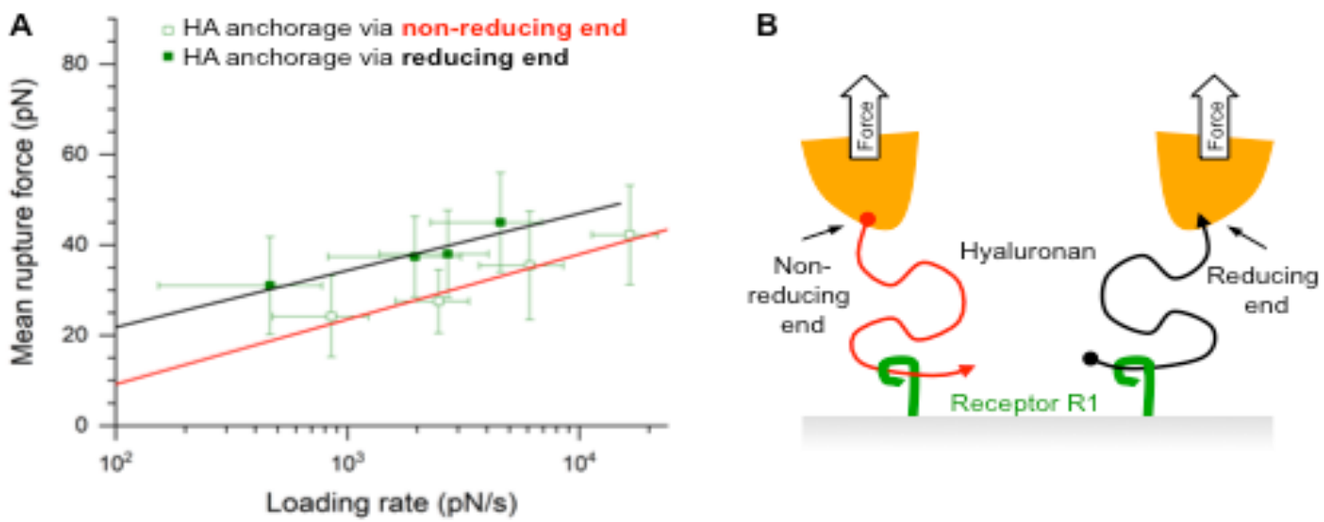
Tetherable Glycosaminoglycan Polymers for Insights into Matrix/Cell/Protein Interactions

PI: Paul DeAngelis, OUHSC, Dept. of Biochem. & Mol. Biol. OCAST Project HR18-104 Area: Glycobiology

Project Highlights

Key molecular interactions occur at the cell surfaces of all animal tissues thus define multicellular life. One type of cellular 'glue' relies on sugar polymers called glycosaminoglycans and their protein-binding partners that is critical for health and disease. However, we do not know the details of these interactions and their physical properties. Therefore, we are using custom-made sugar polymers with handles to 'pull' on the sugar while being bound by various proteins. Single molecule atomic force spectroscopy allows us to watch a single interaction so we can obtain an accurate molecular view.

Our work will help understand how tissues form as well as how some cells can home in on targets. Our ultimate goal is to design sugars that can modulate (inhibit or enhance) cell interactions and thus be used as selective therapeutics or biomaterial scaffolds in the future.



Recent Accomplishments

- We have evidence for **sliding** of sugar chains through binding protein pockets.
- We have identified that the **directionality** of the sugar chain is important for binding.
- The above results suggest that these sugar/protein **interactions may behave similarly to some DNA repair and binding proteins, but could use different mechanisms.**

The mechanical stability of sugar-protein bonds depends on the direction of applied force.

A: Single molecule force spectroscopy assays demonstrate that the bond between a sugar polymer and its binding receptor R1 [***green***] is **mechanically stronger when the sugar is pulled at the reducing end (*black line*) as compared to the non-reducing end (*red line*)**.

B: Schematics illustrate how the mechanics of single HA-receptor bonds were probed: customized sugar constructs made in this OCAST-HR project enable selective anchorage via the non-reducing end (***red line***; *left*) or the reducing end (***black line***; *right*), and thus provide exquisite control on the pulling direction via the gold probe tip (***orange***).

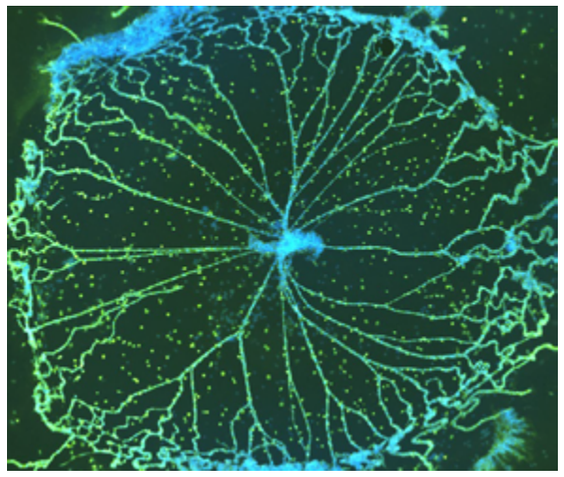
How Blood Vessels Respond to Inflammation

Investigation of the role of hypoxia in initiating hyaloid vessel regression

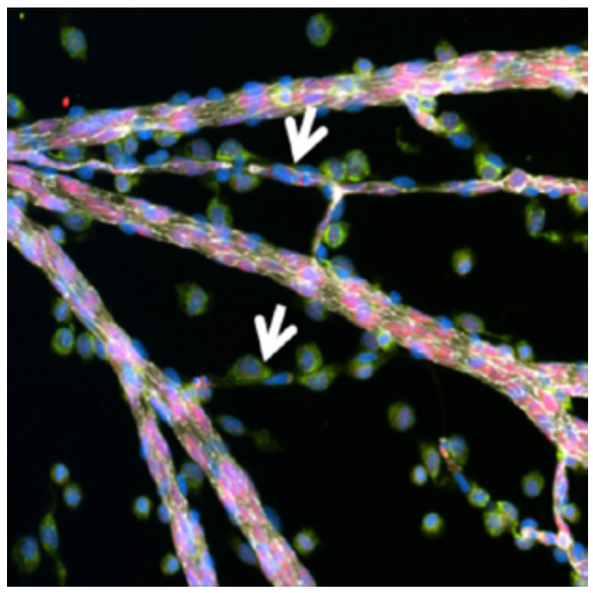
PI: Courtney Griffin, OMRF **HF18-014-1** **Cell/Molecular Biology**

Project Highlights

Inflammation is the process the body uses to fight infections using immune cells. We are interested in how inflammation affects blood vessels. Specifically, we investigate how endothelial cells, which line blood vessels throughout the body, sense and respond to inflammatory signals produced by immune cells. To study this, we focus on two distinct blood vessel networks in the mouse: pulmonary blood vessels in the adult lung and hyaloid blood vessels in the newborn eye. We rely on microscopes to visualize these tiny vessels and their endothelial cells during inflammation.



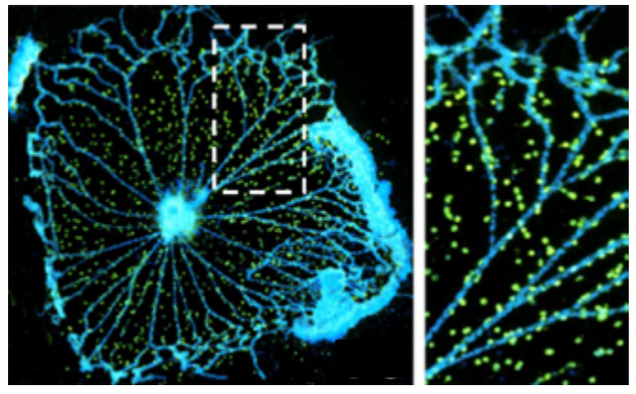
The hyaloid vessels (shown in green) are a network of blood vessels in the newborn mouse eye. Inflammation helps eliminate these vessels after the eye develops.



We use microscopes to observe how proteins such as VE-Cadherin (white) get downregulated in thin, regressing hyaloid vessels (white arrows).

Recent Accomplishments

- Found that anti-inflammatory treatments reduce regression of the hyaloid vessels
- Identified proteins in endothelial cells of the lung that help this organ respond to inflammation
- Developed mice with genetic mutations to help investigate how endothelial cells respond to inflammation



Macrophages (green) are immune cells that interact closely with hyaloid vessels (blue) and contribute to their regression. This photo shows hyaloid vessels undergoing regression 8 days after birth.

All photos were taken by Dr. Chris Schafer, who is supported by this postdoctoral fellowship

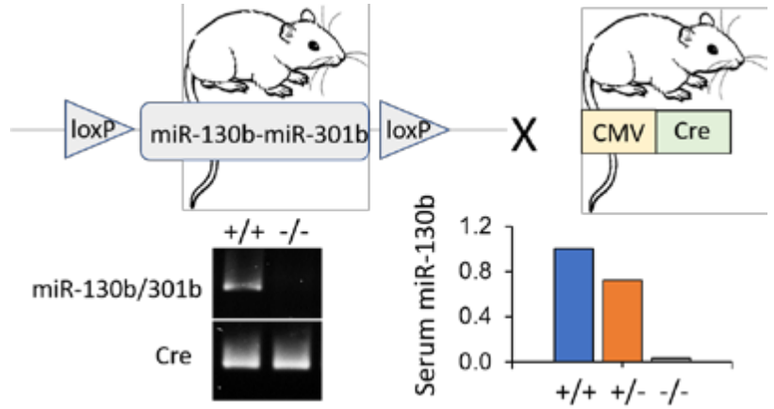
How a mother's adverse nutritional status during pregnancy impacts her child's lifelong risk of metabolic diseases

Fetal epigenetic programming of mitochondrial biogenesis in diabetes during pregnancy: the role of AMPK and microRNA-130b

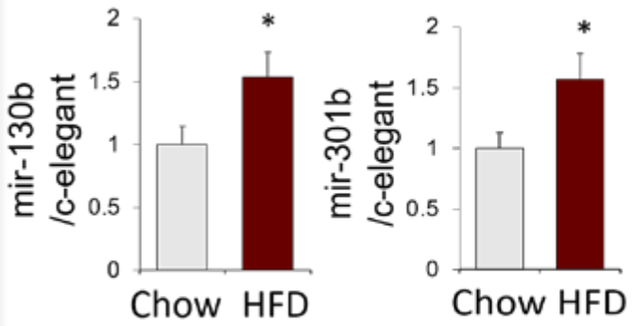
PI: Shaoning Jiang, University of Oklahoma **OCAST Project Number: HR19-133** **Research Area: Cell/Molecular Biology**

Project Highlights

A mother's health and nutritional status during pregnancy, such as diabetes, can impact her child's lifelong risk of diabetes and other metabolic diseases, which initiates a vicious cycle of "diabetes begets diabetes". How the environment in the womb "programs" the baby to develop diseases later in life remains unclear. The proposed work will investigate the roles of a particular microRNA family called miR-130b/301b, focusing on the roles in energy expenditure regulation. These studies will lead to better understanding potential molecular mechanisms underlying the long-lasting outcome, and identification of new biomarkers and therapeutic targets in preventing or reversing the poor health outcomes in offspring of mothers with diabetes.



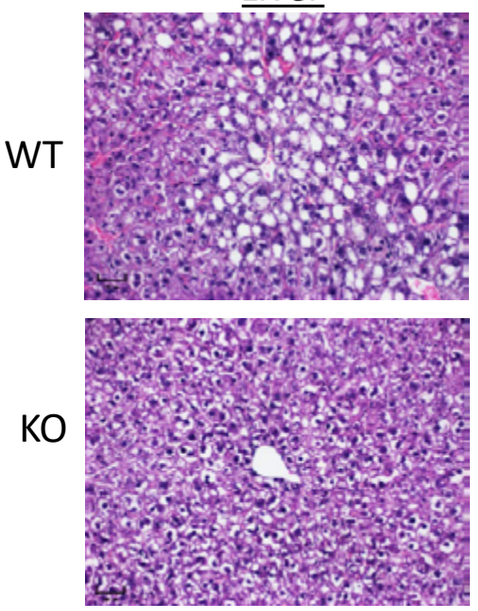
Generation of miR-130b/301b knockout mice



Maternal high fat diet increased miR-130b and 301b levels in mice

Recent Accomplishments

- We demonstrated that levels of miR-130b/301b were increased in a mouse model of maternal high fat diet
- We have generated the knockout mice of miRNA-130b/301b cluster
- We recently started characterizing the metabolic activities of miRNA-130b/301b knockout mice in response to high fat diet and analyzing the potential molecular targets of miR-130b/301b



Decreased lipid in the liver of knockout mice

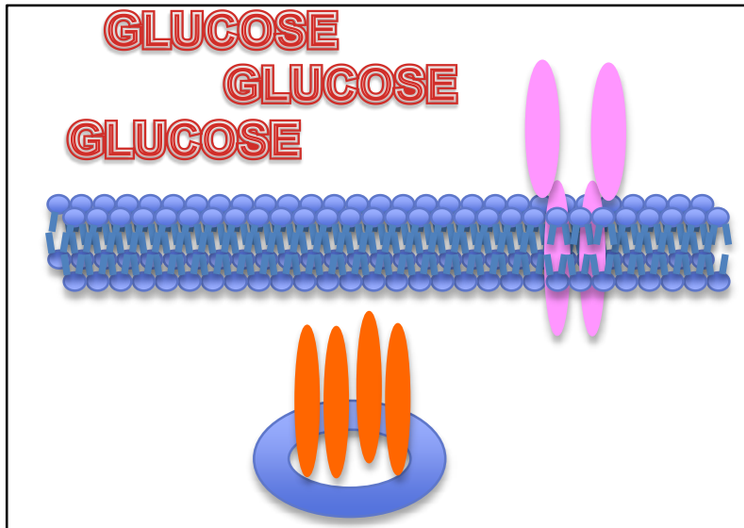
Increasing glucose uptake into skeletal muscle and adipose tissue can prevent type 2 diabetes

Mechanisms regulating GLUT4 expression in obesity

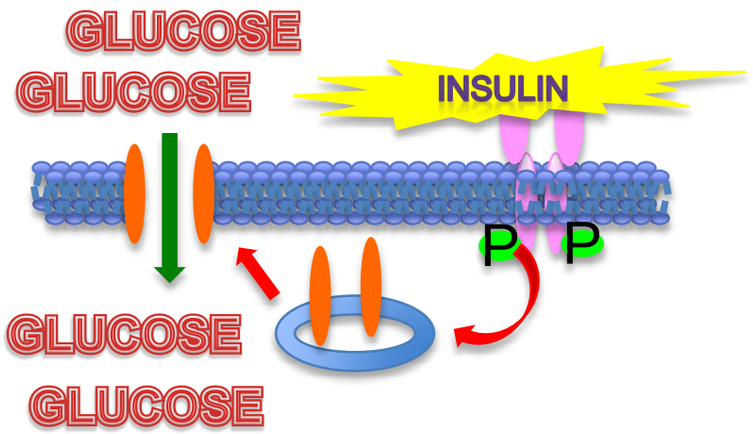
PI: Ann Louise Olson, OUHSC **OCAST Project: HR17-018** **Research Area: Cell/Molecular Biology**

Project Narrative

As a direct result of the obesity epidemic, the prevalence of Type 2 Diabetes is increasing around the world. There is an urgent need to understand how we can prevent the changes in metabolism that cause Type 2 diabetes as a result of obesity. Animal models and human clinical studies tell us that Type 2 diabetes can be prevented if we are able to increase glucose uptake into skeletal muscle and adipose (fat) tissue. The purpose of our study is to determine how we can increase the synthesis of the specialized glucose transporter isoform 4 (GLUT4) skeletal muscle and adipose cells. This glucose transporter protein allows tissues to take up glucose from the blood into the tissues. To achieve this goal, we are carrying out a comprehensive screen to identify the proteins that regulate the synthesis of GLUT4 protein in lean and obese mice. Completion of this research project will guide us in the development of pharmaceutical agents that increase GLUT4 expression in adipose tissue and skeletal muscle. This is critically important for treating and preventing Type 2 diabetes in at risk populations in Oklahoma and throughout the world.



Glucose cannot enter the cell until GLUT4 proteins (orange) move to the cell surface in response to insulin.



The amount of glucose that enters the cell increases with more GLUT4 proteins.

Ann L Olson, PhD

Professor of Biochemistry & Molecular Biology
College of Medicine

Preliminary Results

1. *cDNA library from obese adipose tissue revealed novel FoxO transcription factor binding to Glut4 promoter.*
2. *cDNA library from obese skeletal muscle is being screened.*

Understanding the causes birth defects and infertility

PI: Roberto Pezza, Oklahoma Medical Research Foundation

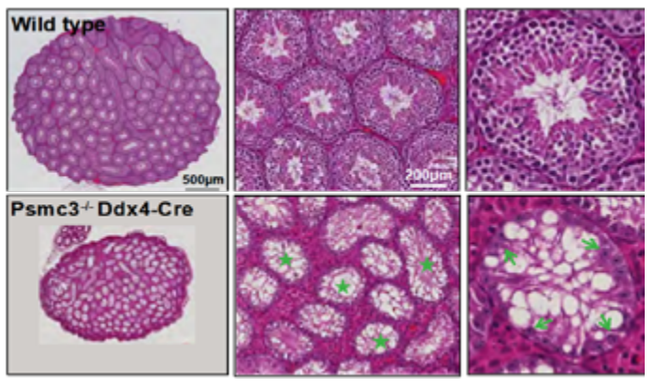
PI: Roberto Pezza, Oklahoma Medical Research Foundation

OCAST HR16-028

Research Area: Cell/Molecular Biology

Project Narrative

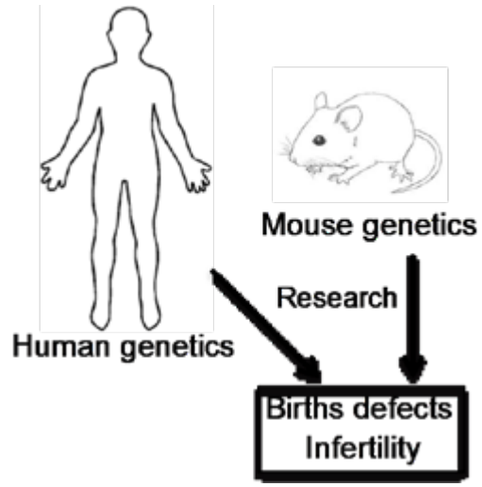
Birth defects, which affect approximately 1 in 33 babies in the United States, have immediate social and economical consequences. Birth defects can be a result of genetic errors passed from the parents to the baby. Indeed, oocytes or sperm with the wrong number of chromosomes are a leading cause of pregnancy loss and functional or developmental diseases such as Down, Klinefelter, Edwards, and Turner syndromes. Our work focuses in identifying and understanding the function of the genes responsible for the formation of gametes with extra or lacking chromosomes. We use the mouse as a genetic model and biochemical *in vitro* approaches to reveal the origin and the functional consequences of mutation in particular genes. Specifically, our results indicate that among the expression of 19 human genes that were different between fertile and infertile men, a gene named Psmc3 appears as a top candidate. More importantly, our results show that a mutation that deletes the Psmc3 gene in the mouse results in abnormal gonads and an increased number of gametes in which the number of chromosomes are altered. Our research has strong potential to accelerate approaches for the diagnosis, treatment, and/or prevention of birth defects.



Deletion of the Psmc3 protein results in testis developmental defects, cell apoptosis at early stages of development, absence of advanced gametes, and ultimately infertility.

Recent advances:

- We generated gonad-specific knockout mice. Using transgenic mice carrying Cre recombinase activated at different times during gamete development, we show that *in vivo* Psmc3 is required at premeiotic stages of gametogenesis (spermatogonia cells).
- We devised a purification protocol for recombinant Psmc3. The purified protein is active in DNA binding.



Our approach to understand the origin and effect of mutations responsible for birth defects and infertility.

Preserving vision during aging.

Nrf2-based therapeutics

PI:Scott M. Plafker, OMRF **OCAST Project: HR16-068** **Research Area: Cell/Molecular Biology**

Project Summary

Age-related neurodegenerative diseases are on the rise in the US and other Westernized countries. These diseases negatively impact longevity, quality of life, and the economics of healthcare. Thus, effective therapeutics and preventative measures are critically needed.

Two etiologies common to these diseases are that age is the leading risk factor and that the lesions manifested in each disease share multiple characteristics. Among these characteristics is oxidative damage, the harmful interaction of oxygen molecules with cellular components. Cells are equipped with defense systems to protect against oxidative damage but these systems decline as we age. The proposed studies are designed to determine how these defense systems in the eye change as we age and to use this information to advance therapies to preserve vision.

Recent accomplishments: We have discovered and are characterizing a new pathway by which cells become senescent. Senescence has been associated with pathologies linked to aging and with increased inflammation and frailty in the elderly. Current efforts are testing ways to block the onset of senescence and thereby maintain the youthfulness of cells.

Dr. Scott Plafker, head of the research project.

Cartoon of a human eye, highlighting the Macula region affected in the disease AMD.

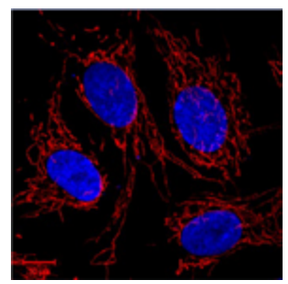


Image of retinal pigment epithelial cells labeled to show nuclei and mitochondria. The mitochondria produce the energy for these cells.

The surface of the eye is vulnerable to injury and diseases which can threaten eyesight.

Defense of corneal epithelial barrier integrity in homeostatic cell turnover and disease.

Allan F. Wiechmann, University of Oklahoma Health Sciences Center **OCAST Project: HR16-014** **Research Area: Cellular Biology**

Project Narrative

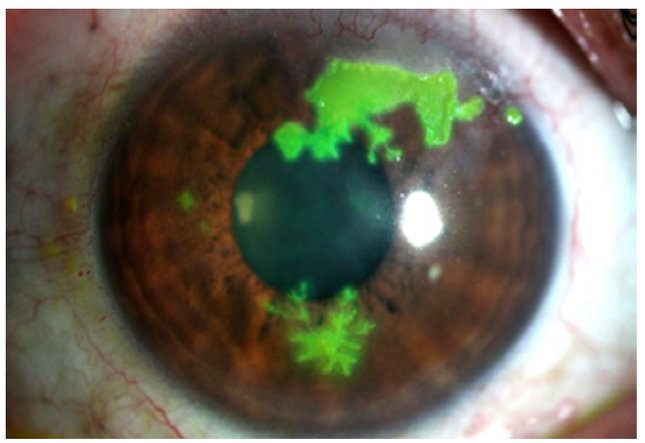
The transparent cornea of the eye is continually challenged by environmental assaults. The epithelial cell layer covering the corneal surface provides a crucial barrier to ocular infection. Deterioration of this epithelial barrier integrity leads to loss of sight and is a major health concern for millions of people. The proposed work will develop a corneal epithelium cell culture model that mimics the loss of corneal barrier function that is a hallmark of infection and loss of function in corneal diseases. This model will enable us to identify drugs that are designed to protect intercellular connections and thus barrier integrity in the corneal epithelium. The long-term goal of this research is to preserve and restore sight to people with corneal trauma and disease, thus enhancing quality of life and productivity.

The cornea of a healthy person is transparent to allow light to enter the eye to form visual images in the brain.

Epithelium from human donor corneas are grown in cell cultures to replicate the normal tissue architecture for study.

Recent Accomplishments

- We identified proteins in the eye that can degrade the integrity of the corneal surface epithelial barrier.
- We mutated the degradative enzymes in the corneal epithelium using CRISPR /Cas9 genomic editing.



Loss of the protective barrier at the corneal surface enhances the risk of ocular infections and loss of sight.

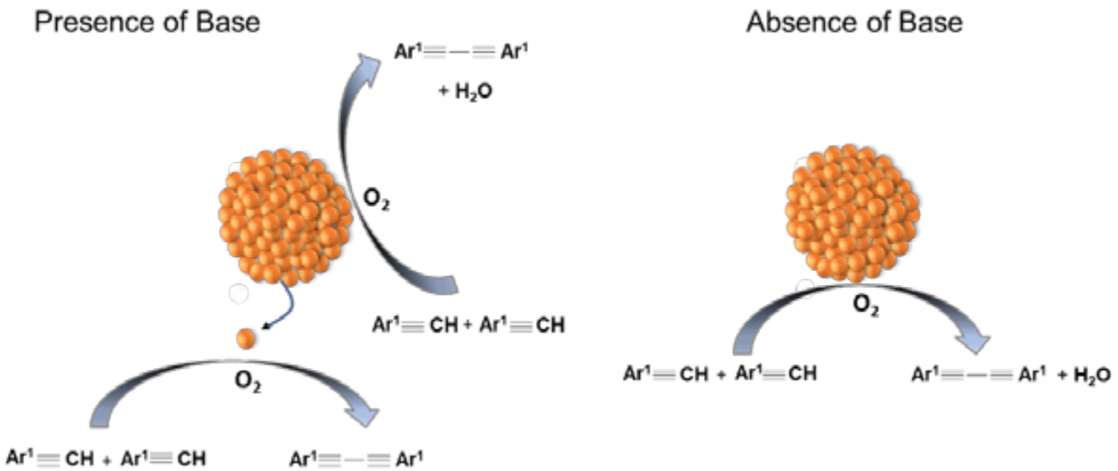
Development of novel nanocatalysts can help to reduce pharmaceuticals manufacturing cost

Copper Nanocatalyst as Efficient Heterogeneous Photocatalyst for Continuous Syntheses of Pharmaceuticals through Cross-Coupling Reactions

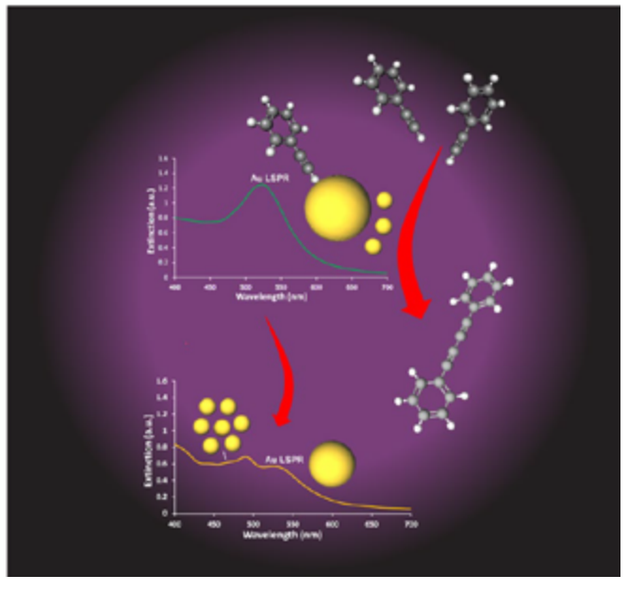
Dr. Marimuthu Andiappan, Oklahoma State University OCAST Project: HR18-093 Research Area: Chemistry & Biochemistry

Project Narrative

Carbon-Carbon (C-C) coupling reactions are widely used reactions in the pharmaceutical industry. These reactions are conventionally performed using batch processes. The objective of this project is to develop novel nanocatalysts that can be potentially used to perform C-C couplings via continuous processes. The continuous processes have the potential to (i) reduce pharmaceuticals manufacturing cost by up to 40-50 percent, (ii) improve drug product quality, (iii) reduce waste generation from pharmaceutical manufacturing processes, and (iv) reduce the manufacturing facility space by 10 to 100 times.



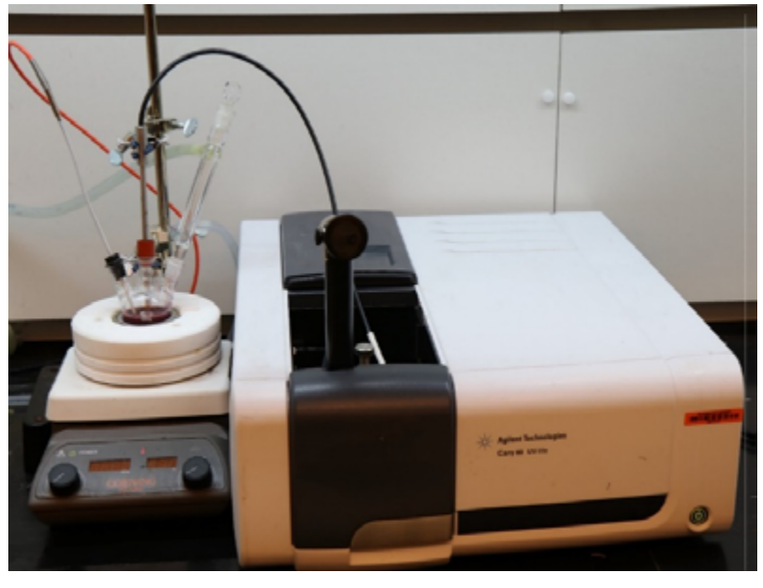
Cu_2O nanoparticle-catalyzed C-C coupling réactions, Andiappan and co-workers, *Green Chemistry*, **2019**, *21*, 5284.



Au nanoparticle-catalyzed C-C coupling réactions, Andiappan and co-workers, *Journal of Physical Chemistry C*, **2019**, 1*23*, 11539.

Recent Accomplishments

- We developed protocols for synthesizing gold (Au) nanocatalysts and cuprous oxide (Cu_2O) nanocatalysts of different sizes and shapes to catalyze C-C coupling reactions.
- We developed catalytic conditions under which the catalysts exhibit high long-term stability and desired product selectivity.
- We developed inexpensive and easily-transportable operando UV-Vis spectroscopy for monitoring the size of functioning nanocatalysts. This operando spectroscopy can be potentially used as in-line Process Analytical Tool in continuous manufacturing of pharmaceuticals.

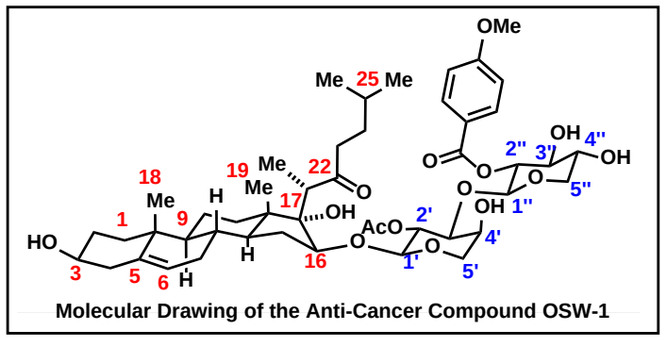


Operando UV-Vis spectroscopy as in-line process analytical tool (PAT) for monitoring the size of functioning nanocatalysts.

Making New Anti-Cancer Drugs That Only Target the Cancer Cells

Synthesis and Drug Development of ORP4 Protein Inhibitors: A New Route to Precision Anti-Cancer Therapeutics

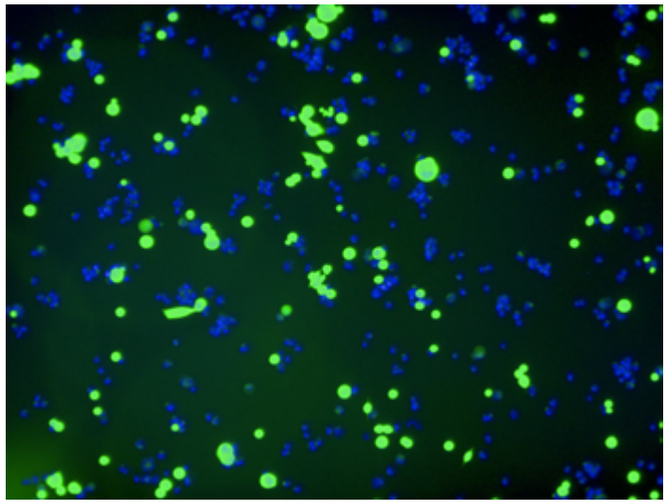
PI: Anthony Burgett, University of Oklahoma **OCAST Project: HR17-116** **Research Area: Chemistry and Biochemistry**



Molecular Drawing of the Anti-Cancer Compound OSW-1

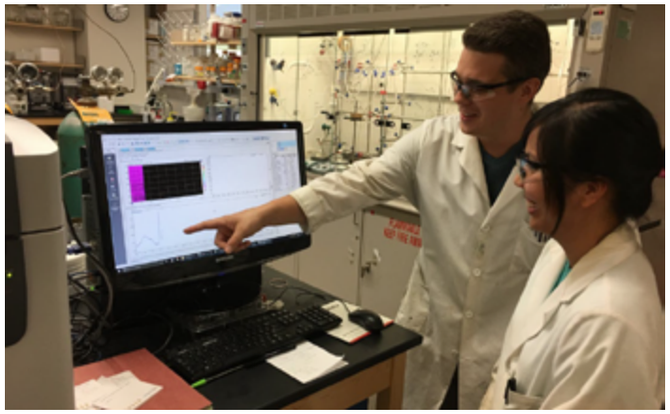
Project Narrative:

To better treat cancer, new and more effective therapies will have to be able to target only the cancer, without affecting the non-cancerous tissues of the body. This will produce better results with fewer unwanted side effects. For this cancer-only drug targeting to be developed, cancer specific targets must be identified, and new drugs must be discovered that work on these cancer specific targets. The protein ORP4 has recently been identified as a driver in developing some forms of cancer. Our lab is working on making and developing anti-cancer compounds that kills cancer cells through interacting with ORP4. If successful, these new ORP4-specific drug compounds can be developed as potential cancer specific therapeutics.


Cancer cells being killed by the OSW-1 drug compound

Recent Accomplishments:

- We gained new understandings of how the anti-cancer drug compound OSW-1 kills cancer cells through targeting the ORP4 protein.
- We have developed new chemistry that will allow for us to make new, better anti-cancer drug compounds based on the OSW-1 compound.


Mr. Cori Malinky and Ms. Anh Le, chemistry graduate students, purifying drug compounds

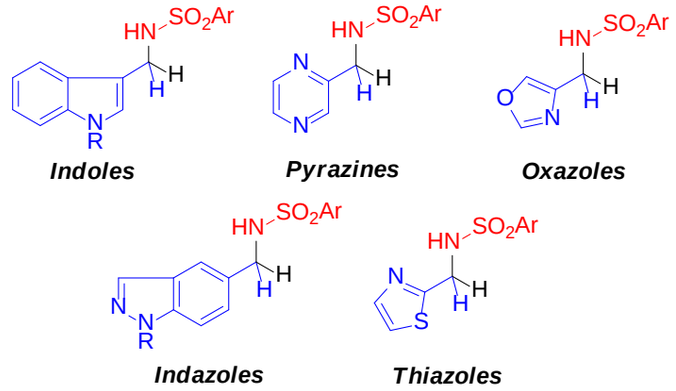
Using Visible-Light Activation to Develop New Tools for Drug Discovery and Production

Late-Stage C-N Incorporation to Bioactive Cores

PI: Angus A. Lamar, The University of Tulsa OCAST Project: HR18-013 Research Area: Chemistry and Biochemistry

Project Narrative

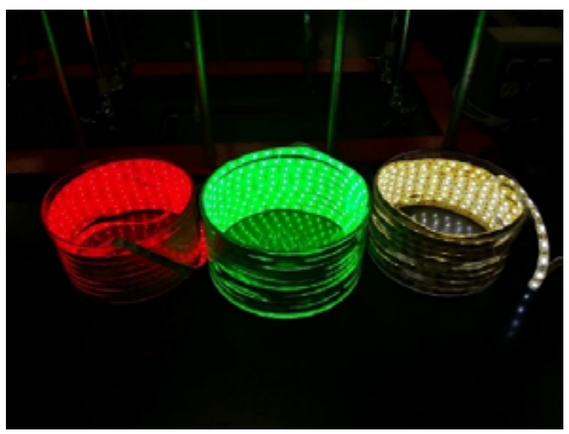
The science of medicinal chemistry depends upon versatile and well-understood reagents for the production of a library of complex molecules to test for bioactivity and/or pharmaceutical applications. Synthetic pharmaceuticals generally contain a higher percentage of nitrogen than natural products (in fact, about 84% of small-molecule pharmaceuticals contain at least one C-N bond). In order to avoid the challenges that arise from the presence of nitrogen-containing functionality during the synthesis of drugs, *the late stage incorporation of C-N bonds into bioactive cores remains a crucial challenge*. Our research aims to establish a reliable and well-understood set of tools to be used to selectively and directly modify bioactive frameworks using novel N-sources. We have recently developed a visible-light activated, non-metal promoted approach for nitrogen installation that involves a distinctly unique reactive intermediate. This unique species has thus far provided new points of entry for the installation of challenging functionality into complex molecules at sites that were previously inaccessible. The results from our proposed research plan will have a significant positive impact on the broad mission of the OCAST Health directive to ease the burden of illness and disability and promote healthy life by improving the discovery and efficiency of production of many of the molecules which facilitate such goals.



Additionally: pyrrolopyridines, imidazopyridines, quinolines, imidazoles, pyrrazoles, furans, thiophenes, pyridines, pyrimidines

Key features of our approach:
Mild conditions (20-50 °C, no harsh acid) and visible-light
Promoted by I_2
Operationally simple procedures
Current library of >70 novel compounds

Installation of an *N*-sulfonyl unit to heteroarene cores using our visible-light activated approach.

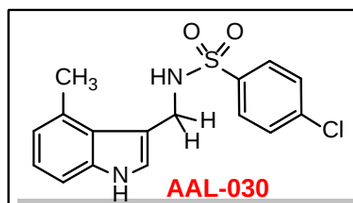


Left: Red LED visible-light photochamber
Middle: A green LED photochamber
Right: A white LED photochamber

Recent Accomplishments

1) In the past year, we have developed new methods to incorporate *N*-sulfonyl units into relatively complex molecules at sites that were previously inaccessible.
   a) Lamar et al., *Molecules* **2018,** *23* (8), 1838.
   b) Lamar et al., *Organic & Biomolecular Chemistry,* **2019**, *17*, 8391.

2) We have also developed new ways to halogenate arenes and heteroarenes using visible-light (LED photochambers shown on the left) activation of photocatalytic dyes.
   a) Lamar et al., *ACS Omega*, **2018**, *3*, 12868.
   b) Lamar et al., *Organic Letters*, **2019**, *21*, 4229.
   c) Lamar et al., *Tetrahedron*, **2019**, *75*, 130498.



Two-step (one pot) synthesis from commercially available reagents

IC_{50} (μM)

Compound	H293	HeLa	NCI-H196
AAL-030	**47.8**	**53.5**	**43.5**
ABT-751	209.1	117.5	139.7
Indisulam	229.1	100.6	155.8

Cell Type

ABT-751 and Indisulam are well-known anticancer agents

Initial hit (AAL-030) with potential as an anticancer agent following further optimization and SAR studies.

High BCO2 Protein in Your Body Will Make You Sensitive to Flu

β, β-carotene 9', 10'-oxygenase 2 (BCO2) in acute respiratory distress syndrome

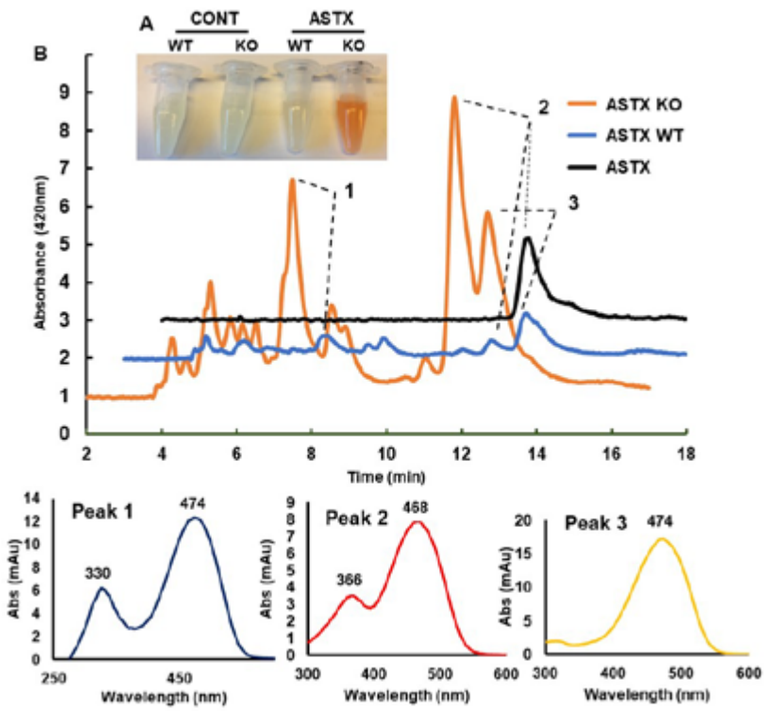
PI: Dingbo Lin, Oklahoma State University **OCAST Project: HR17-114** **Research Area: Biochemistry**

Project Highlights

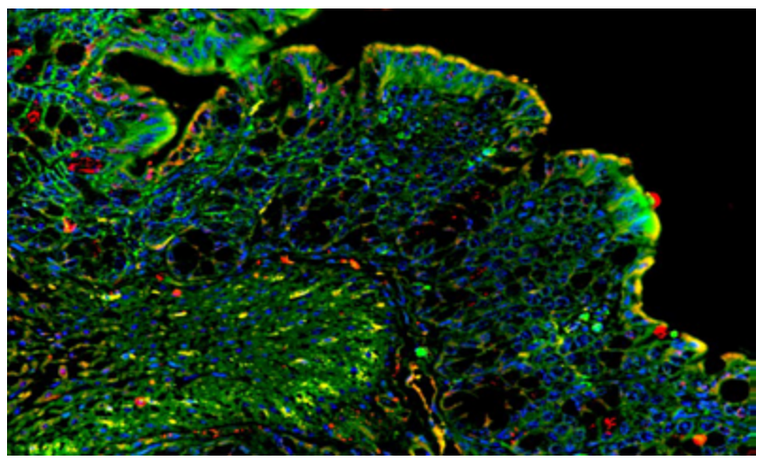
Seasonal influenza viruses cause annual epidemics. However, it is not well known about precisely how the cellular antioxidant systems are overwhelmed during the influenza virus infection process. This gap in knowledge retards the development of effective therapeutic strategies, especially during outbreak of new influenza. β-carotene oxygenase 2 (BCO2) is a mitochondrial inner membrane protein mediating inflammation and oxidative stress. Lack of BCO2 makes the mouse more resistant to seasonal flu. Thus, the overall goal of this research is to dissect the role of BCO2 in host innate immune response to influenza by testing BCO2 in pulmonary alveolar epithelial cell apoptosis in mice infected with influenza A virus; and by examining the mechanism by which BCO2 regulates mitochondrial function in influenza A virus infection in vitro. We expect that the results of this research would have a significant impact for the public by providing new understanding of the role played by BCO2 as they relate to influenza and potential treatment targets.

Recent Accomplishments

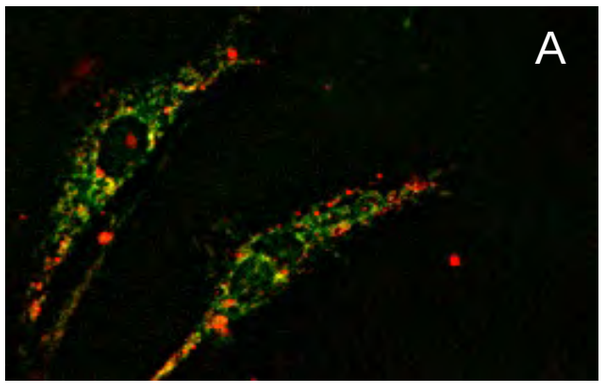
1. BCO2 deletion induces the immune T cell activation which may enhance your resistance to flu;
2. Increased intake of carotenoids, a group of colorful fat-soluble pigments also strengthens your immune system;
3. BCO2 deletion results in MAVS oligomerization and subsequently activation type 1 interferon signaling
4. Overexpression of BCO2 diminishes MAVS-interferon signaling;
5. BCO2 deletion associated host immune response to flu might link to the gut microbiome homeostasis.

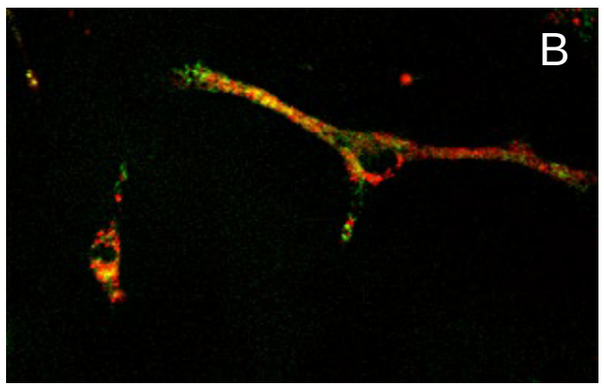


Depletion of BCO2 gene causes carotenoid accumulation in the liver mitochondria (A, far right panel), which can be detected by traditional HPLC (B) (Blue and red traces, three peaks are shown underneath the blot)



Beauty of the gut cell-cell tight junction (orange) that keeps your gut health



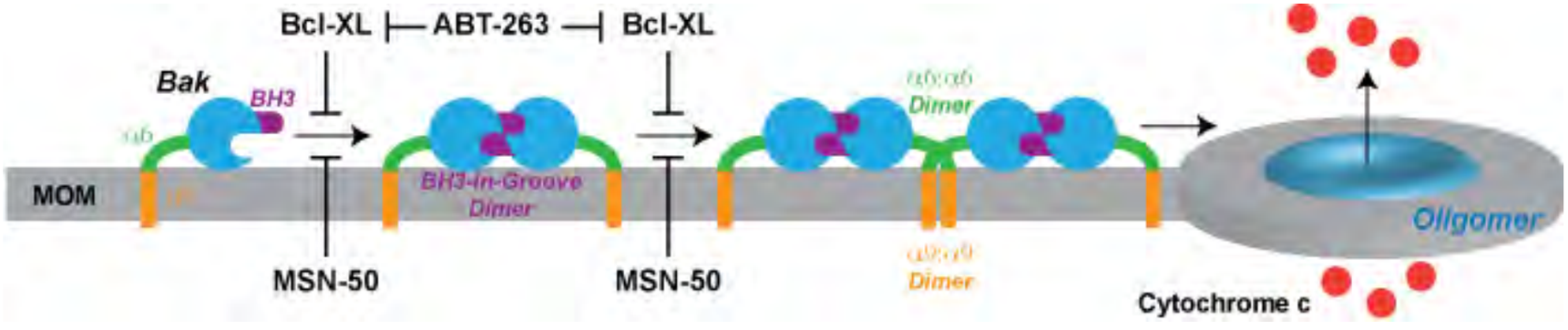


Mitochondrial stress (orange color) in the mouse embryonic fiberblasts in wild type (A) and BCO2 deficient mice (B)

Deadly pore formation in mitochondrial membrane: mechanism and therapeutic potential

Structure, Function and Inhibition of Apoptotic Bak Oligomers in Mitochondria

PI: Jialing Lin, University of Oklahoma Health Sciences Center **OCAST Project: HR16-026** **Research Area: Chemistry and Biochemistry**



Project Summary:

The long-term goal of this project is to elucidate the molecular mechanisms for regulating programmed cell death. Understanding these mechanisms is the key to the development of effective treatments for a wide variety of human diseases such as cancer, stroke and heart attack, in which cell death is either inhibited or accelerated. Despite sequence and structural homology, pro-death Bak protein forms large pores in the mitochondrial outer membrane to induce cell death, whereas pro-survival Bcl-XL protein binds Bak to inhibit the deadly pore formation. This project will identify the key structural features of Bak pore and the molecular mechanisms for its assembly in the membrane, and decipher the molecular mechanisms by which Bcl-XL and small molecules inhibit Bak pore formation. We will use a multidisciplinary approach, including molecular biology, biochemistry, cell biology and structural biology, to characterize the molecular interactions of these proteins and small molecules and the functional consequences. The anticipated outcomes from this rigorous investigation are the mechanistic details for (1) how Bak damages the mitochondria to kill the cell, (2) how Bcl-XL inhibits Bak to preserve the mitochondrial integrity and save the cell, and (3) how small molecules bind Bak or Bcl-XL to regulate their respective pro-death or pro-survival activity. The impact of these significant outcomes to the cell death field will be vital, because they will reveal or validate relevant target sites and provide lead compounds for developing the next generation of drugs to more effectively combat cancer, stroke and heart attack.

Figure: Model for Bak oligomeric pore formation and regulations by Bcl-XL and small molecules ABT-263 and MSN-50. Active Bak forms the BH3-in-groove interface and then the α6:α6 and α9:α9 interfaces resulting in a tetramer and then a higher order oligomer that perforate the mitochondrial outer membrane (MOM) to release Cytochrome c. Bcl-XL and MSN-50 inhibit either or all Bak dimerizations and perhaps Bak interactions with the MOM to block the pore formation. ABT-263 neutralizes Bcl-XL so it cannot inhibit Bak.

Recent Accomplishments:

NIH Centers of Biomedical Research Excellence (COBRE) Grant, 9/6/2017-5/31/2022.

Papers:

(1) Ma et al. Dynamic PGAM5 multimers dephosphorylate BCL-xL or FUNDC1 to regulate mitochondrial and cellular fate. Cell Death Differ. 7/31/2019. Epub ahead of print.

(2) Lin. Tightening a deadly pore former. Nat. Chem. Biol. 15(4):316-317. April 2019.

Narrow Open Tubular Column for Ultra-High Efficiency Liquid Chromatographic Separation

Interpreting a Focusing Effect Caused by Eluent and Sample Matrix Mismatch and Developing a Simple and Economic Approach to Perform Gradient Liquid Chromatographic Separations

PI: Shaorong Liu, University of Oklahoma, Norman, OK ***OCAST Project: HR17-022*** ***Research Area: Chemistry/Biochemistry***

Project Narrative

Historically, an efficient approach to improve the separation efficiency is to reduce the mono-disperse particle size that are packed in the column. Nowadays, Narrow open tubular (NOT) columns enable us to perform high-efficiency liquid chromatographic separations. While we understand that sharp peaks can be obtained due to the presence of a focusing effect caused by gradient elution, we were puzzled by exceptionally sharp peaks that appeared when the separation was performed using an isocratic eluent. In this works, we found that a focusing effect had occurred during elution. We designed experiments and confirmed the presence of such a focusing effect. We further determined that the focusing effect was caused by an eluent and sample matrix mismatch and developed a model to explain why this effect led to the sharp peaks. On the basis of this insightful understanding, we developed a simple and economic approach to perform gradient narrow open tubular liquid chromatographic separations.

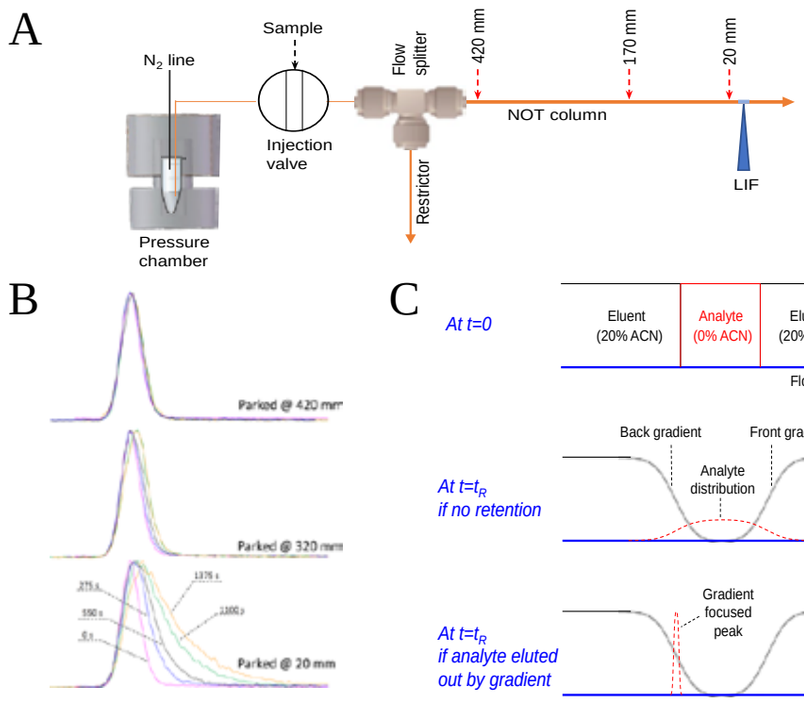
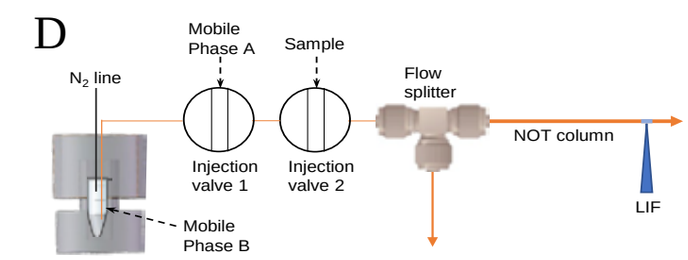
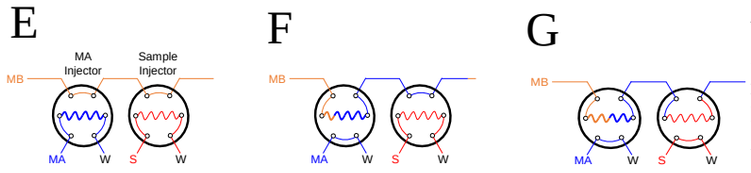


Fig. A - Apparatus for performing NOTLC separation. The NOT column had a 2-μm i.d. and was trimethoxy(octadecyl) silane derivatized. Injection valves were VICI 6-port valves. At the 5 cm from the effluent outlet of NOT column, a detection window was made by removing the polyimide coating. A laser-induced fluorescence detector (LIF) was used to monitor the resolved analytes. Fig. B - Effect of distance on peak focusing. The peaks were color-coded to indicate the fluorescein parking time. The distance on top of each peak group indicated the position (in Fig. A) the peaks were parked. C - Schematic of peak focusing mechanism. (Top) Right after the analyte was injected (at t=0). (Middle) At $t=t_R$, two gradients before and after the analyte zone were formed, and non-retained analytes would stay in between the two gradients. (Bottom) For a retained analyte, all analytes would be eluted out by the back gradient and get focused.

Recent Accomplishments

We have identified a focusing effect and used this effect successfully to interpret the exceptionally sharp peaks obtained from “isocratic” NOTLC. Inspired by this insightful understanding, we have developed an economic approach to perform gradient NOTLC separations. With only a pressure chamber, two valves and NOT column system , the high-efficiency gradient separation can be successfully achieved. We are optimistic that this NOTLC technology will be compatible with MS instrumentation and become a powerful analytical technique for analytical chemistry.





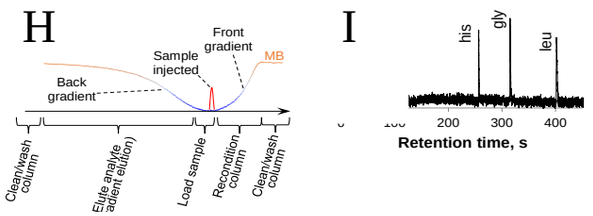


Fig. D - Apparatus for performing picoflow gradient NOTLC separation. On the basis of the original isocratic NOTLC system, a mobile phase A (MA) injection valve (valve 1) was added between the pressure chamber and the sample injection valve (valve 2). The mobile phase A was prefilled in V 1 and the mobile phase B was refilled in vial in high pressure chamber.
Fig. E – G - The operation of the simple and economic approach to run gradient NOTLC. The orange line indicates the mobile phase B (MB), the blue line means MA, and the red line represents the sample. Firstly (fig. E), MA and sample are loaded respectively in valve 1 and valve 2. Then (fig. F) MA is injected into the system and a portion of MA is allowed to pass through injection valve 2. Finally (fig. G) the sample is injected into the MA plug.

Fig. H - A schematic presentation of the gradient elution process.
Fig. I – A typical chromatogram obtained using the simple picoflow gradient generator. Sample: histidine, glycine and leucine (each at 0.1 μM).

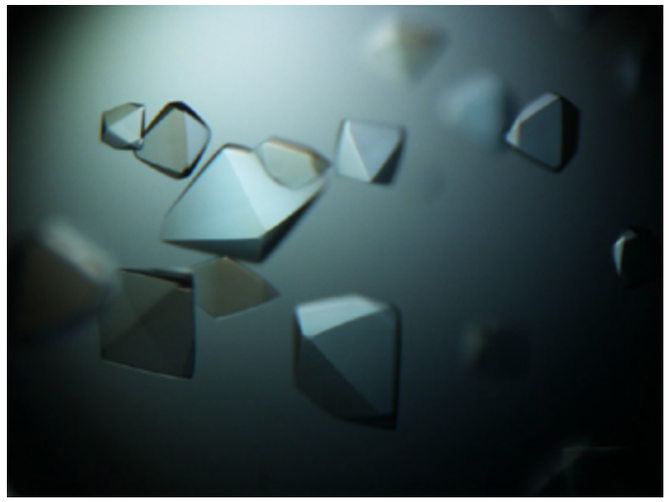
Using powerful X-rays to visualize cardiovascular hormones and guide the development of new drugs for migraine headache and heart attack

Rational design of potent and selective peptide ligands for the CGRP and Adrenomedullin receptors

PI: Augen Pioszak, OUHSC **OCAST Project: HR16-005** **Research Area: Biochemistry**

Project Narrative

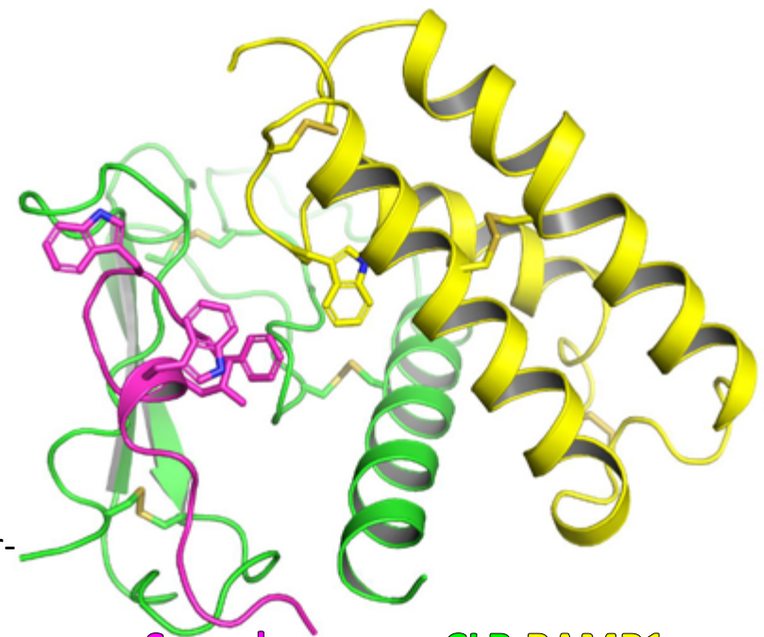
The hormones CGRP and AM control blood vessel relaxation in the brain and heart. Too much CGRP can cause migraine headache whereas extra AM seems to be beneficial for heart attack patients. CGRP and AM interact with receptors on the surface of cells to exert their control. This interaction process is analogous to a key fitting a lock. In previous work in our lab we used powerful X-rays to obtain 3-D pictures of CGRP and AM fitting their receptors, which significantly advanced our understanding of how these hormones work. In this project we propose to use the knowledge gained from these pictures to guide the design of new "super-hormones" that fit the receptors better than the natural hormones. The resulting super-hormones may lead to new therapeutics for the treatment of migraine headache and heart attack, which would benefit Oklahomans who suffer from these conditions.



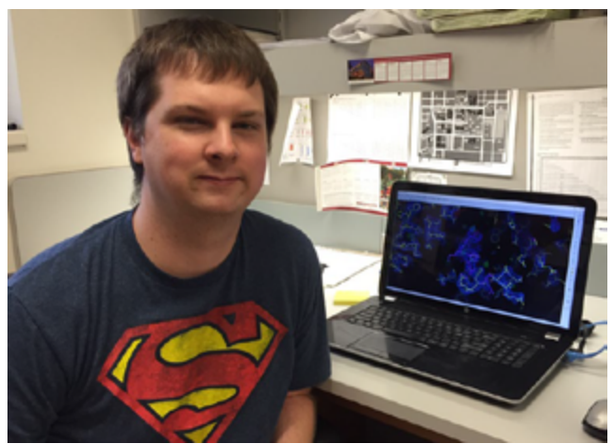
Shooting powerful X-rays through these crystals of a hormone-receptor complex allows us to visualize how the hormone fits the receptor like a key in a lock

Recent Accomplishments

- Designed several "super-hormone" antagonists and agonists for the human CGRP and AM receptors
- Determined high-resolution crystal structures that confirm how two of the "super-hormones" work
- Demonstrated activity of the "super-hormones" in human primary cells



X-ray-derived picture showing how one of our designed superhormones binds the human CGRP receptor



Jason Booe, a graduate student in the lab, analyzes the resulting image of the hormone and receptor on a computer.

Understanding bacterial immune systems can help us improve biological tools .

Leader DNA motifs specify distinct mechanisms for spacer insertion in different subgroups of CRISPR type II-A systems

Rakhi Rajan, University of Oklahoma OCAST Project: HR17-124 Research Area: Chemistry and Biochemistry

Project Narrative

Bacteria are constantly under attack from viruses. Many kinds of bacteria have evolved immune systems to fight off these infections, the most recently discovered being CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). The CRISPR immune system fights off infections by saving short pieces of the viral DNA and using them as a guide to fight off future infections from the same virus. The current project is focused on understanding how the CRISPR system is able to extract and store these short pieces of DNA. We do this by isolating individual parts (protein and DNA) of the CRISPR system and seeing how they interact with DNA of different sequences. A greater understanding of these parts of CRISPR will lead to new tools to expand capabilities to insert desired DNA pieces at any genomic region of interest. This has the potential to impact not only the academic community, but industries such as medicine and agriculture, benefiting Oklahoma's medical facilities and economy.

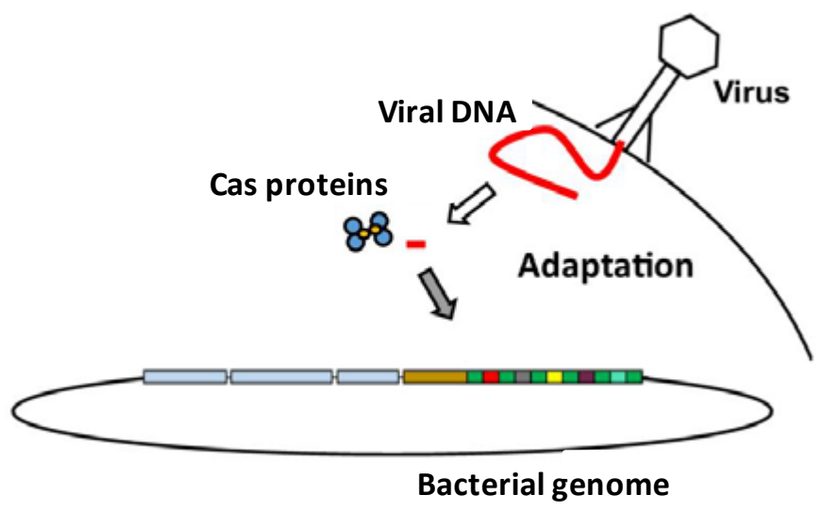


Figure 1. Schematic of CRISPR-associated (Cas) proteins saving a piece of viral DNA in the bacteria's own genome for later use.

Recent Accomplishments

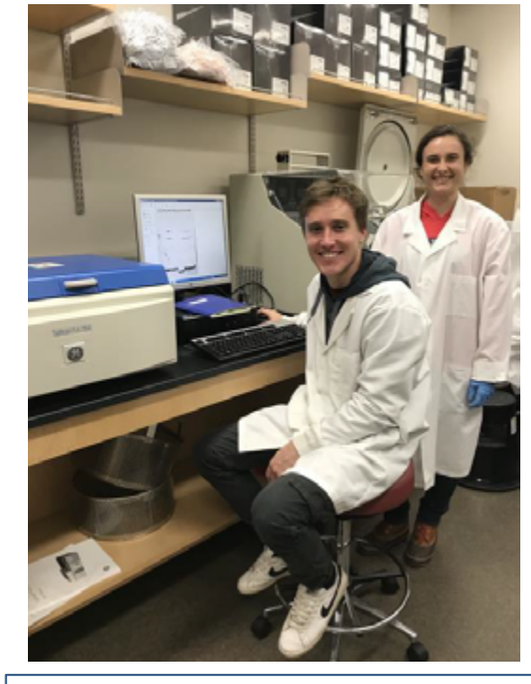


Figure 3. Rajan laboratory members Mason Van Orden and Sydney Newsom analyzing gels showing spacer insertions

- We have developed two sets of CRISPR proteins that can attach:
  - a single end of a spacer into a target DNA strictly based on 12 base pair conserved DNA sequence
  - both ends of a spacer into a target DNA based on a 40 base pair conserved sequence
- We have used a technique called multi-angle light scattering to show that only the CRISPR proteins that assemble as shown in Figure 2 can insert spacers into a target DNA
- Towards determining an atomic resolution structure of Cas1 protein from the bacterium *Lactobacillus paragasseri*, we have obtained crystals, collected X-ray diffraction data, and are currently processing the data to build the structure

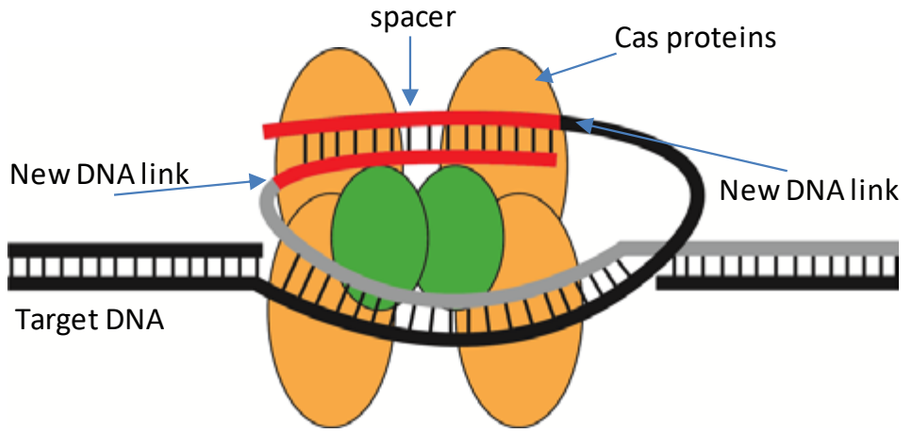


Figure 2. Cartoon representation of how Cas proteins can take short pieces of viral DNA (spacer) and insert them into existing bacterial DNA (target DNA) for future use in an immune response.

Towards the Design of New Neuro- and Cardio-Protective Drugs

Rational Development of Selective and Potent Inhibitors to Pro-apoptotic Bax Protein

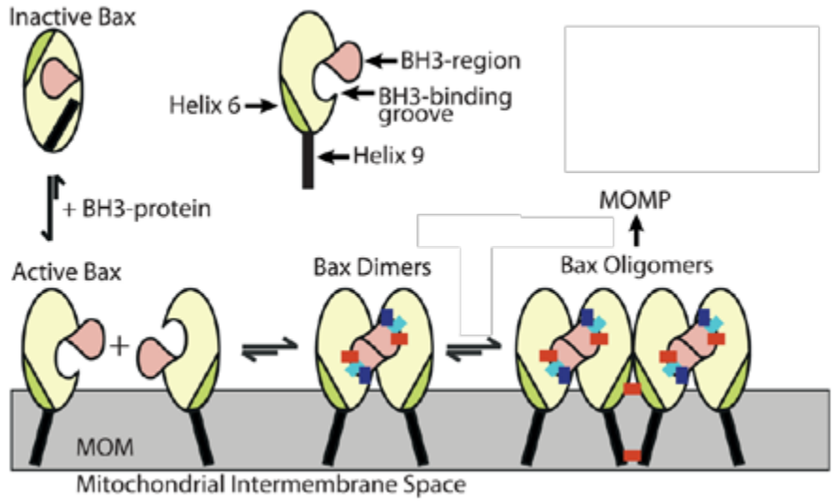
Yihan Shao, University of Oklahoma HR18-130 Chemistry and Biochemistry / Computational Biology

Project Narrative

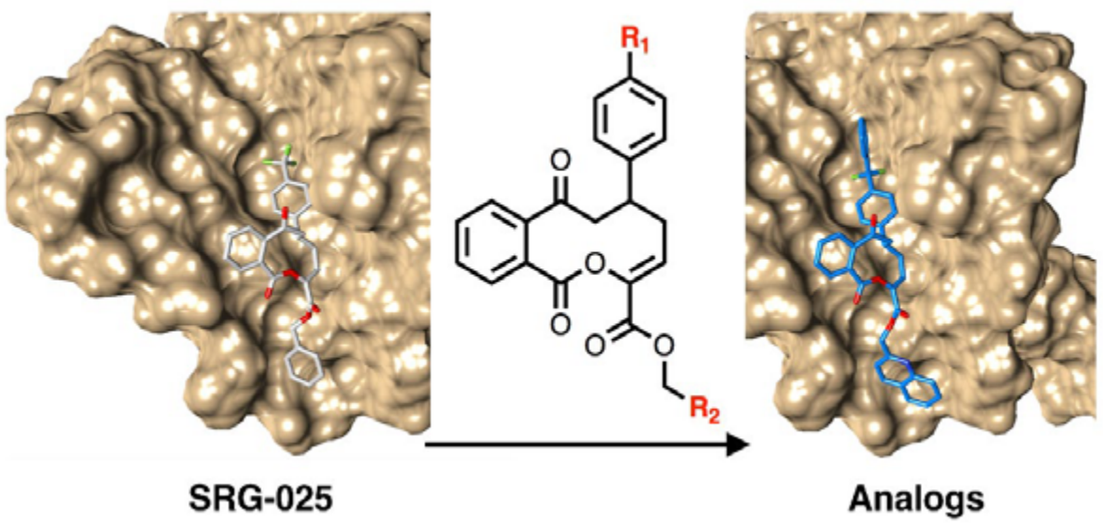
Bax is an important protein whose function is to kill cells. It binds together into homo-oligomers and making a hole in the mitochondria outer membrane. Once a hole is formed, it triggers a cascade of events and leads to cell death.

We need effective Bax inhibitors. After a stroke, brain injury or heart attack, brain or heart cells die too quickly. Bax inhibitors slow down the death of nerve and heart cells, thus saving lives. The best Bax inhibitors now work in the 1—10 μM range, and our **long-term goal** is to help develop better Bax inhibitors in the 10—100 nM range.

We will achieve this goal through a multidisciplinary teamwork. My group use computational tools (docking and binding free energy calculations) to model the ligand-Bax binding and to design new ligands. Best candidate compounds are synthesized in Dr. Indrajeet Sharma's lab (OU Chemistry) and then their bioactivities are tested in Dr. Jialing Lin's lab (OU HSC).



Scheme: Bax Oligomerization and Pore Formation



Recent Accomplishments

- SRG-025, an oxacycle compound from Dr. Sharma's lab, was identified as a template for Bax ligand design
- SRG-025 derivatives with aromatic groups (for example, R_1=benzyl and R_2=quinoline) displayed increased bioactivity than SRG-025 in Bax-mediated dye-release liposomal assay experiments.
- New SRG-025 derivatives are being designed and synthesized to further improve the solubility and bioactivity.

Developing Non-Addictive Pain-Killers and Anti-Itch Agents

Design and Synthesis of Collybolide Analogues as Probes in Kappa-Opioid Pharmacology

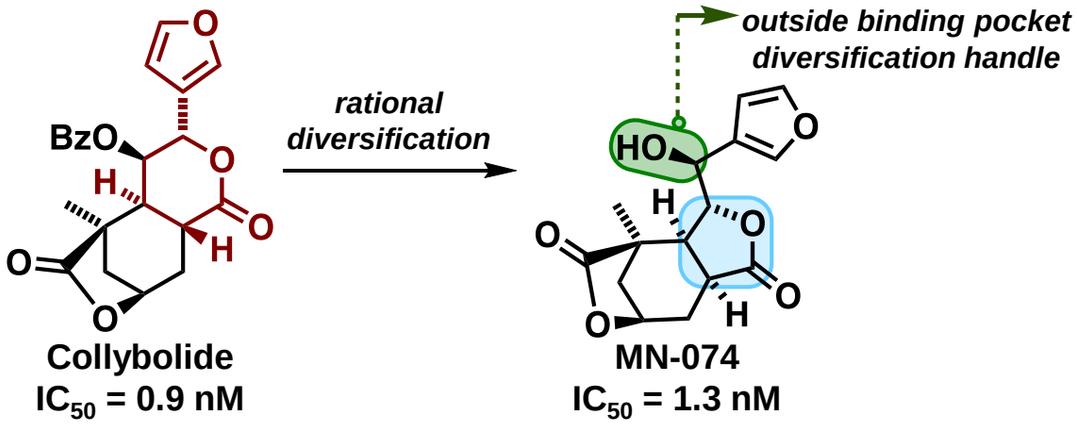
PI: Indrajeet Sharma, University of Oklahoma **OCAST Project: HR16-095** **Research Area: Chemistry & Biochemistry**

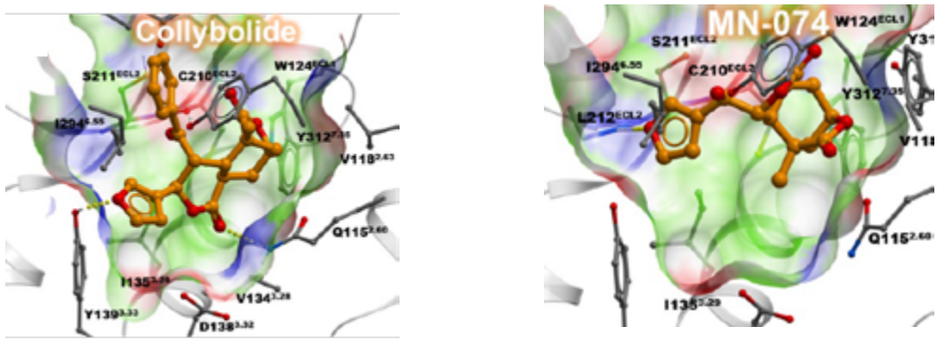
Project Summary

While Mu-opiate analgesics such as morphine are effective for severe pain, their use is limited due to unwanted side effects such as tolerance development, abuse liability, respiratory depression, and itching. Studies suggest that selective kappa-opioid receptor (κOR) agonists biased towards G-protein signaling over *β*-arrestin recruitment could lead to novel therapeutics for treating intractable itch and pain with reduced side effects. In the quest for new biased κOR ligands, we identified collybolide natural product, which is a highly selective biased κOR agonist having analgesics and anti-itch properties. This work will lead to a better understanding of the κOR pharmacology to develop potential therapeutics for the treatment of pain and itch with reduced side effects.

Recent Accomplishments

- Identification of a lead compound MN-074, a highly selective biased κOR agonist as collybolide, but has improved pharmacological properties such as solubility and metabolic stability. This will lead to the development of therapeutics for pain and itch with reduced side-effects..
- This project is recently funded by the NIH–NIDA grant.





Current Liabilities:

- Metabolic instability ($t_{1/2}$ = 2.6 min in microsomes)
- Low aqueous solubility

Improved Properties:

- Higher metabolic stability ($t_{1/2}$ = 45 min in microsomes)
- Improved aqueous solubility

Deadly Diarrhea: Exploiting The Decision-Making Process In Killer Bacteria

Two-component Signal Transduction In The Human Bacterial Pathogen Clostridioides difficile

PI: Dr. Ann West, University of Oklahoma **OCAST Project: HR18-110** **Research Area: Chemistry & Biochemistry**

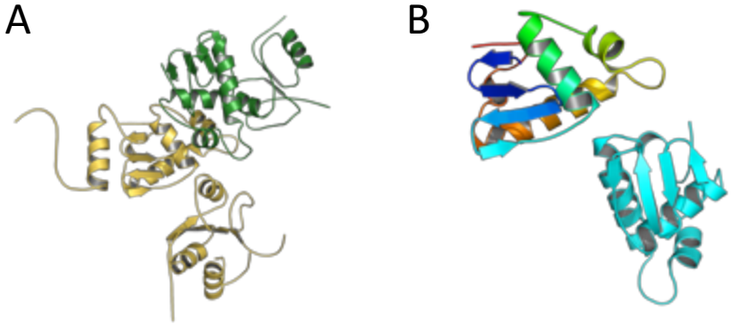


Figure 1. Crystal structures of both (A) full-length RR_1586 and (B) the DNA-binding portion of RR_1586.

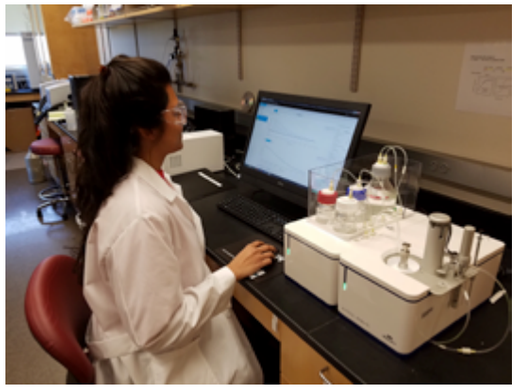


Figure 2. Graduate student Kriti Shukla collecting data of RR_1586 binding to a gene that is important for infection.

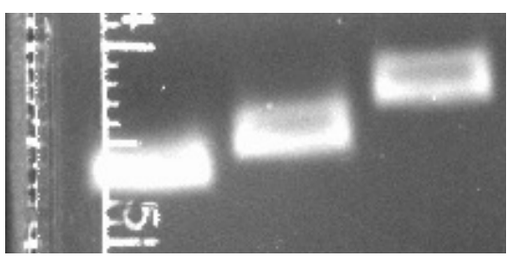


Figure 3. The three fluorescently glowing bands show successful control experiments for detecting if genes are being turned on in the bacterium.

What we do: Just as nerve cells carry information from your extremities to your brain, special proteins called response regulators carry information within bacteria. Each response regulator waits for a different message and then turns on or off a unique set of genes. We study the messages received and the genes regulated by response regulators in the bacterium *C. difficile.*

Why we do it: We are working toward a "peaceful resolution" to the perpetually escalating arms race between bacteria and the healthcare industry. Because common antibiotics are not fully effective in killing *C. difficile*, we are looking for ways to trick response regulators into shutting off the genes that cause deadly diarrhea.

How we do it: First, we test a response regulator to see which genes it binds in a test tube. Then we monitor those genes inside the bacterium to see if the response regulator turns them on or off. We use other tools to determine the structure of response regulators from crystallized protein. These structures provide valuable insight regarding how response regulators work and provide unique clues as to how they can be exploited for therapeutic intervention.

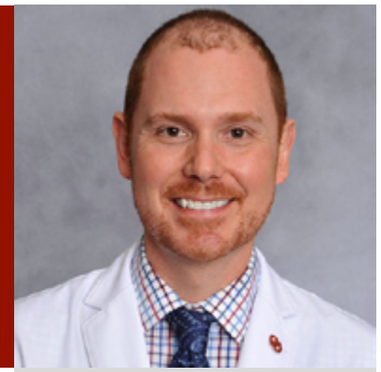
Recent accomplishments: This work focuses on a response regulator called RR_1586.

- We have determined two crystal structures of RR_1586 (Figure 1).
- We have new data showing RR_1586 binding to a gene that is important for infection (Figure 2).
- We have developed experiments for testing genes inside the bacterium (Figure 3).

Changes in blood cell epigenetic patterns both diagnose knee osteoarthritis and predict how quickly it will progress.

Differential leukocyte epigenetic and transcriptomic patterns as diagnostic and predictive biomarkers in knee osteoarthritis

PI: Matlock A. Jeffries, MD, OUHSC OCAST Project: HR16-066 Research Area: Genomics & Gene Expression

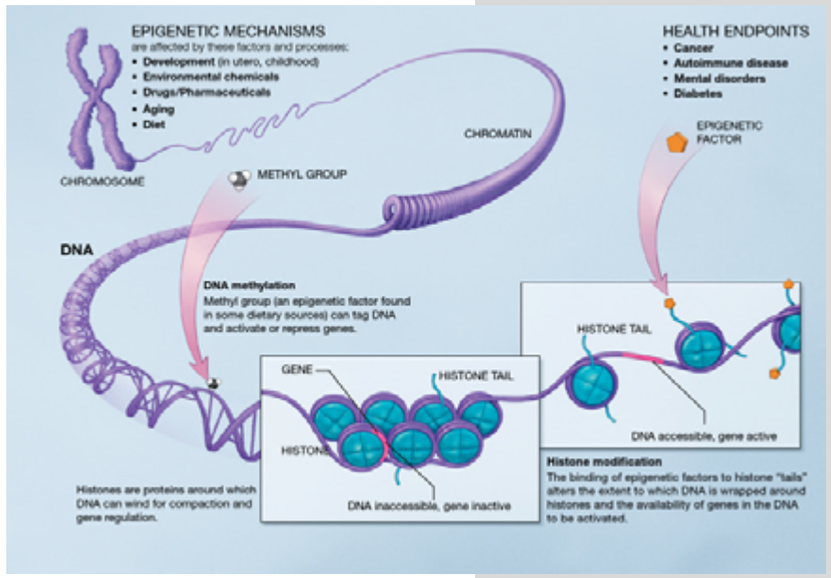


Dr. Jeffries, Principal Investigator

Project Narrative:

Osteoarthritis (OA) affects nearly half of the population aged 60 and over. There is no treatment for people with this disease, which over time leads to severely diminished physical activity, quality of life, and overall health. Reduction in OA patients' ability to exercise predisposes them to a variety of chronic diseases. Over the past several years, we have begun to understand that OA is not simply disease of joints, but rather of all-over-the-body inflammation.

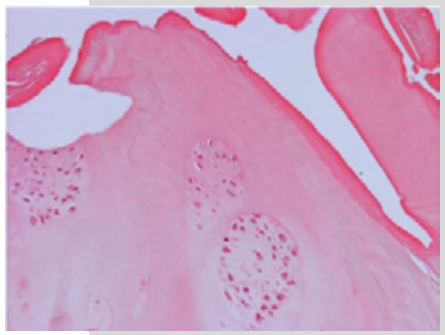
Our project aims to recruit 150 patients with early knee OA. Patients will donate a blood specimen and microbiome specimens, record their OA symptoms, and have knee x-rays every 6 months for 2 years. We will examine both the kinds and amounts of inflammatory cells present in their blood when they first enter the study and as time goes on and their OA worsens. Furthermore, we will look at epigenetic patterns within specific blood cells at each of these time points. Epigenetics is the study of markers which allow the environment to "interact" with genes by turning them on and off. At the end of our study, we will examine the xrays of our patients and separate them into rapid progressors and nonprogressors. We will then go back and determine if there were particular blood cell population or epigenetic patterns that were unique to each group. If successful, this project will produce the first accurate, blood-based diagnostic and prognostic test for people with knee OA, and may offer insights into the ways in which blood-based inflammation may be contributing to OA. This may ultimately lead to the development of new treatments for individuals with OA, and significantly benefit the economy of Oklahoma.



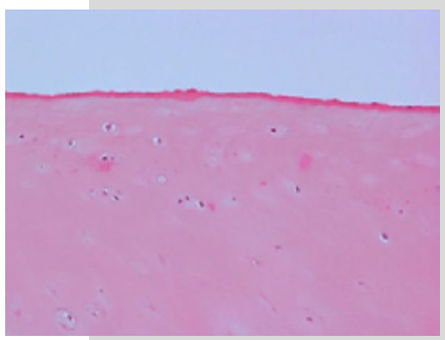
Introduction to epigenetics.

Recent Accomplishments:

- We have accomplished the first goal in this research project by confirming our earlier findings that peripheral blood cell epigenetic changes can predict future OA progression in a large set (around 150 patients) from the national Osteoarthritis Initiative study.
- We are continuing to recruit patients for our Oklahoma OA cohort, and have recently expanded to include patients from the Oklahoma Joint Reconstruction Institute.
- We have formed a new collaboration with one of the largest OA studies in the country, the Johnson County OA Project in North Carolina, who will also be providing samples for us to analyze.



Microscopic view of eroded human OA cartilage.



Microscopic view of smooth non-OA cartilage.

Decreased food intake can change the genome function that can lead to beneficial effects

Role of DNA methylation in Dietary Restriction mediated Cellular Memory

Archana Unnikrishnan, University of Oklahoma **OCAST Project: HR17-098** **Research Area: Genomics & Gene Expression**

Project Highlights

Dietary restriction (DR) has been shown to delay the onset and progression of most age-related diseases (e.g., colon cancer) as well as improving most physiological processes that decline with age. Therefore, dietary restriction is believed to retard aging and has become the 'gold standard' by which other manipulations that increase lifespan are compared. Many mechanisms have been proposed for the life-extending action of dietary restriction; however, it is still unclear as to the molecular basis of dietary restriction's action. An important facet of dietary restriction that has been largely overlooked by the research community is that dietary restriction can have early effects that create a cellular memory, which persists even when the restriction is discontinued. These studies suggest that dietary restriction could be increasing lifespan and retard aging through a novel mechanism that involves a molecular signal(s) that arises shortly after the implementation of dietary restriction and has an impact on the animal over its lifespan even after dietary restriction is discontinued. The most likely molecular process by which dietary restriction could increase lifespan and retard aging after being discontinued would be through DNA methylation. Alterations in DNA methylation at specific genes is critical during development and is a mechanism by which the transcriptional potential of cells can be altered for the life of an organism.. The purpose of this project is to determine the effect of dietary restriction on DNA methylation in the colon that will allow us to test the following hypothesis: *DR induces cellular memory in colon epithelium by inducing changes in DNA methylation (5mC) at specific genomic regions that regulate the expression of genes, which are potentially important in stem cell function*. If we show that a short period of DR is sufficient to impart life-long beneficial effects, this would be an important discovery because short-term DR would be a more compliant approach translationally than the rigorous life-long regimen.

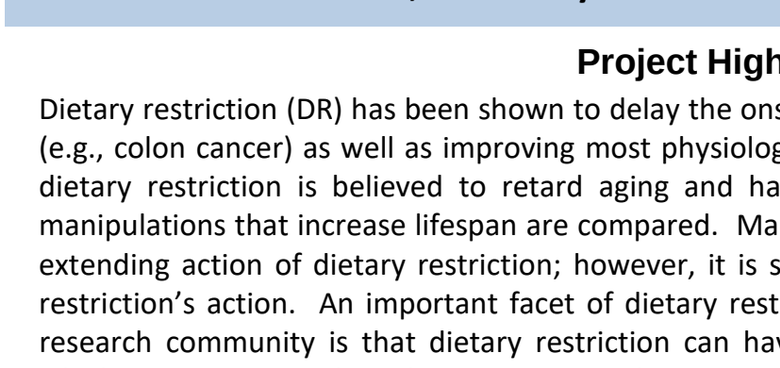


Figure 1: 24 month old C57BL/6 mice fed either *ad libitum* (control) or dietary restricted diet throughout life.

Recent Accomplishments

- Whole genome methylation analysis showed that total 5mC and 5hmC levels are similar in the colon and intestinal stem cells in the baseline condition.
- Single cell transcriptomic analysis of young and old whole crypts showed that young mice had more stem, paneth and goblet cells.
- We are currently analyzing global methylation and single cell transcriptomic data in colons of mice exposed to short term dietary restriction.

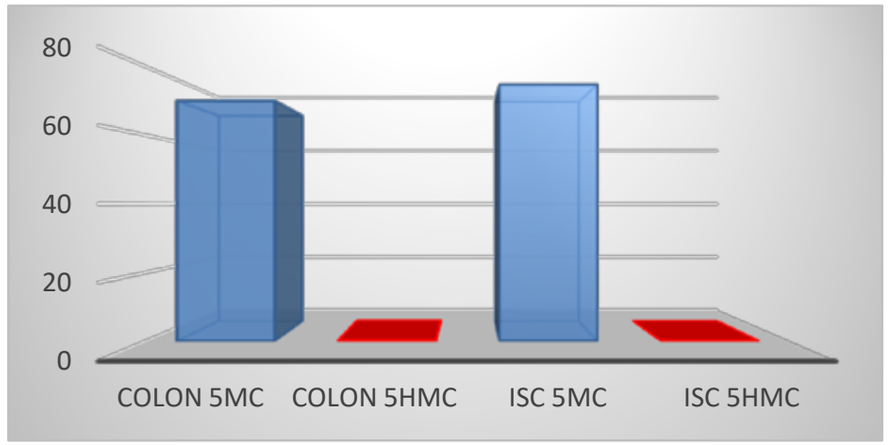


Figure 2: Total methylation levels (5mC and 5hmC) measured in colon and intestinal stem cells (ISC). 5MC- 5 methyl Cytosine; 5HMC – 5 hydroxy methyl cytosine.

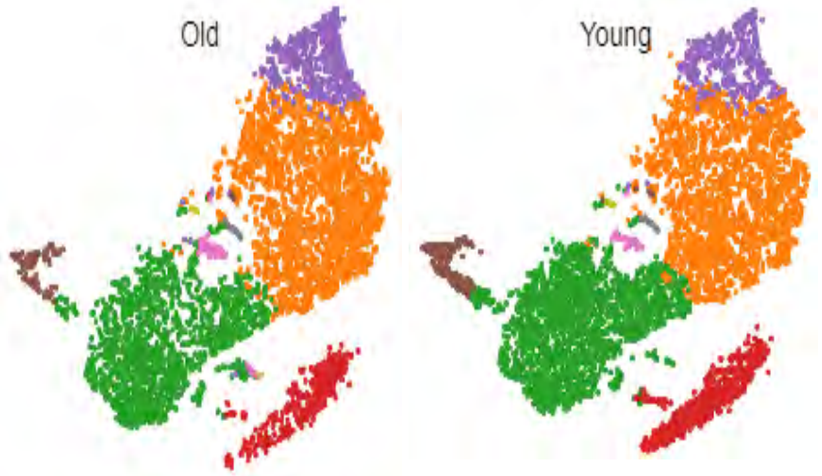


Figure 3: Whole crypt cells from the intestine of young (6 months) and old (24 months) mice were dissociated by mechanical disruption and subjected to scRNA-Seq with the chromium version 3 platform. The plot shows the different sub population of cells in the crypt and how it differs between young and old. Green- Stem cells, Red-Paneth Cells, Brown-Goblet cells, Orange- Enterocytes, Purple – Fat cells.

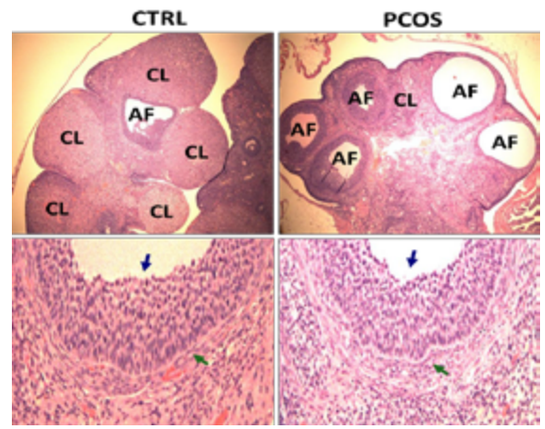
An autoimmune pathophysiological and molecular mechanism in Polycystic Ovarian Syndrome

An antibody to a Pituitary Receptor May Induce Ovary Cysts and Infertility 07/01/2017-06/30/2020

PI: Hongliang Li, Department of Medicine, OUHSC **OCAST Project: HR17-123**

Project Highlights

Polycystic ovary syndrome (PCOS), a metabolic and reproductive associated disease, defined as hyperandrogenism with reproductive dysfunction including menstrual disorders, anovulation, infertility, and polycystic ovaries. Our studies previously showed a high percentage of PCOS subjects with autoantibodies directed toward the second extracellular loop (ECL2) of gonadotropin-releasing hormone receptor (GnRHR). We immunized rats with GnRHR-ECL2 peptide and the resultant GnRHR-autoantibodies (GnRHR-AAb) induced insulin resistance in energy storage and peripheral tissues. In the present study, we investigated that chronic elevated GnRHR-AAb induces reproductive dysfunction by increasing LH secretion and androgen production. This is associated with increased insulin resistance and a compensatory hyperinsulinemia. The enhanced insulin signaling in reproductive tissues likely increases local androgen production and provides a novel pathophysiological basis for PCOS.

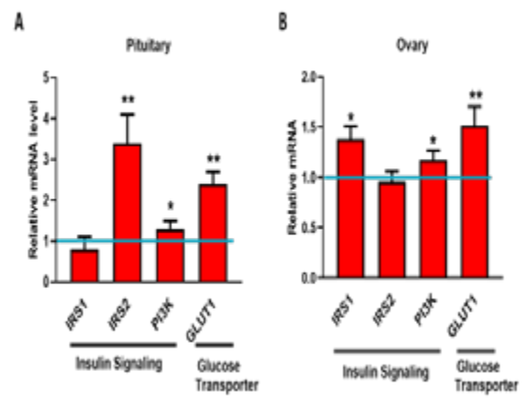


Representative images of HE staining of ovarian

Recent Accomplishments

Yankai Guo, a research scholar from our lab

- GnRHR-AAb titers and GnRHR stimulating activity in the GnRHR group were significantly higher than the control group.
- The GnRHR group exhibited an increased frequency of LH pulses and elevated serum concentration of testosterone.
- The GnRHR group exhibited a lower frequency of proestrus and estrous phases, increased atretic follicles, decreased corpora lutea, loosely packed granulosa cells, and thecal cell hyperplasia in ovarian tissue compared with controls.
- In the GnRHR group, the mRNA expression levels of insulin signaling genes in reproductive tissues were significantly increased compared to controls.



Expressions of insulin signaling pathway detected by qRT-PCR in reproductive tissues

Keeping a check on B and T cell development

Regulation of RAG2-chromatin interactions during V(D)J recombination

Karla K. Rodgers, PhD, OUHSC HR18-072 Biochemistry/Immunology

Project Highlights

The capability of the adaptive immune system to defend against infection arises from its vast repertoire of antigen-binding receptors, such as antibodies and T cell receptors produced by B and T cells, respectively. In a unique process, known as V(D)J recombination, the functional antigen-binding genes are assembled during B and T cell development, which in turn generates the repertoire of diverse binding specificities. Proteins known as RAG1 and RAG2 initiate V(D)J recombination by catalyzing site-specific DNA cleavage within the antigen receptor genetic loci. However, mistakes in this process can result either in immunodeficiency diseases or in genomic instability that can lead to lymphomagenesis. Mechanisms that normally regulate the RAG proteins are not yet well defined. Our recent evidence indicates that association of RAG2 with chromatin is regulated through the opposing effects of specific modified chromatin versus post-translational modifications on RAG2. We propose that conformational changes induced in RAG2 prevents erroneous interactions with chromatin and helps to maintain a proper balance between genomic integrity with continued development of B and T cells.

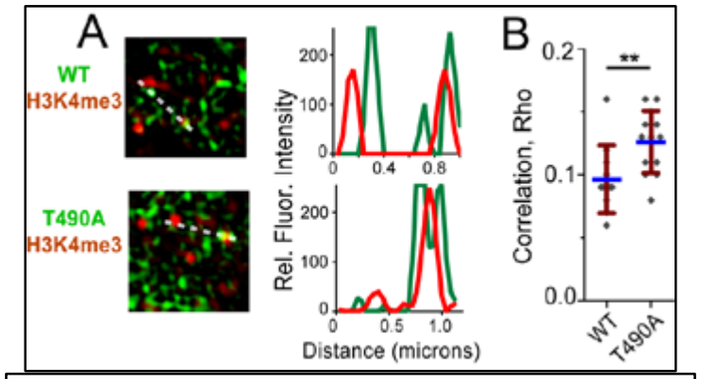


Fig. 2. The RAG2 protein and a specific modification to chromatin (referred to here as H3K4me3) can be visualized in intact pre-B cell nuclei using Superresolution microscopy. We can determine the extent of spatial overlap between the protein and chromatin **(A&B)**. Mutation of a single residue in RAG2 (T490A) leads to increased, and likely unregulated interactions with chromatin.

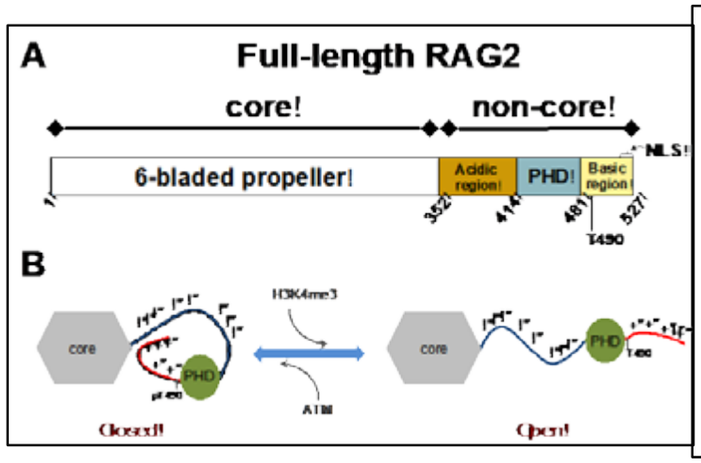


Fig. 1. Full length RAG2 schematic and model. (A) RAG2 contains a core region and non-core region. Non-core RAG2 contains a plant homeodomain (PHD) and a nuclear localization signal (NLS). (B) Model for closed and open forms of RAG2, where H3K4me3 (specific modified chromatin) promotes formation of the open conformer. Conversely, kinases, such as ATM, promotes the closed conformer through phosphorylation. In the closed form a basic region (including the NLS) is less accessible.

Recent Accomplishments

- We have completed and published a study that shows the RAG2 mutant T490A is not properly regulated in its interaction with chromatin, as compared to the wild type RAG2 protein.
- In current work, we are identifying post-translational modifications in RAG2 that can modulate its interaction with chromatin.

A novel diagnosis tool to find biomarkers from lupus patient blood

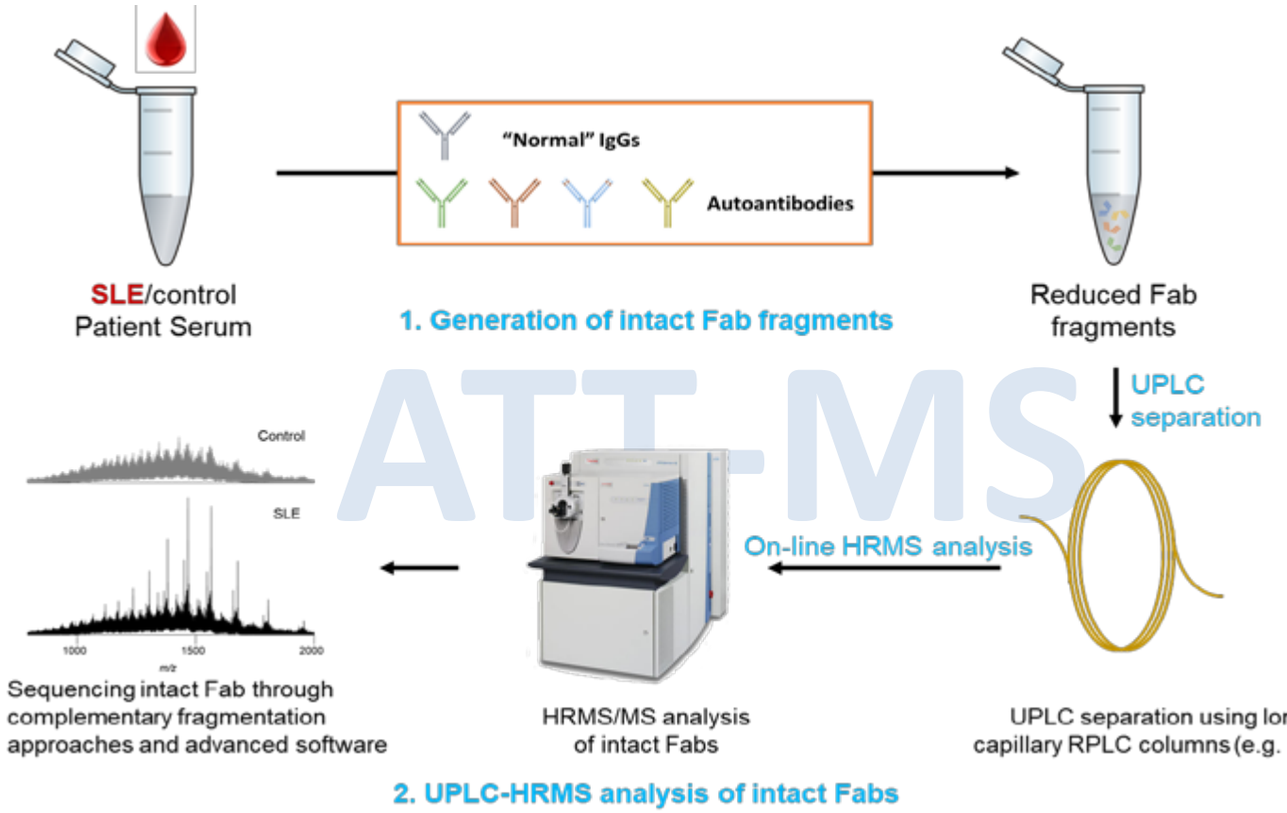
Characterization of serum autoantibody biomarkers through an antigen-targeted top-down mass spectrometry (ATT-MS) platform

PI: Si Wu, University of Oklahoma **OCAST project: HR 16-125** **Research Area: Immunology**

Project Highlights

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disorder estimated to affect at least 1.5 million Americans. In SLE, autoantibodies accrue early in disease development, and thus, hold great potential as biomarkers for autoimmune disease diagnosis.

In collaborating with Dr. Kenneth Smith from OMRF, we are developing an ATT-MS platform on high throughput serum autoantibody analysis, providing new strategies in autoimmune disease prognosis, intervention, and prevention, which may lead to novel high diagnostic value biomarkers.



Accomplishments

- ✓ We developed the first top-down proteomics platform for human serum autoantibody analysis
- ✓ Top-down analysis provides a "bird-eye" view of the complexity of human serum autoantibodies
- ✓ It holds the potential to lead to the discovery of novel biomarkers for the diagnosis and treatment of SLE

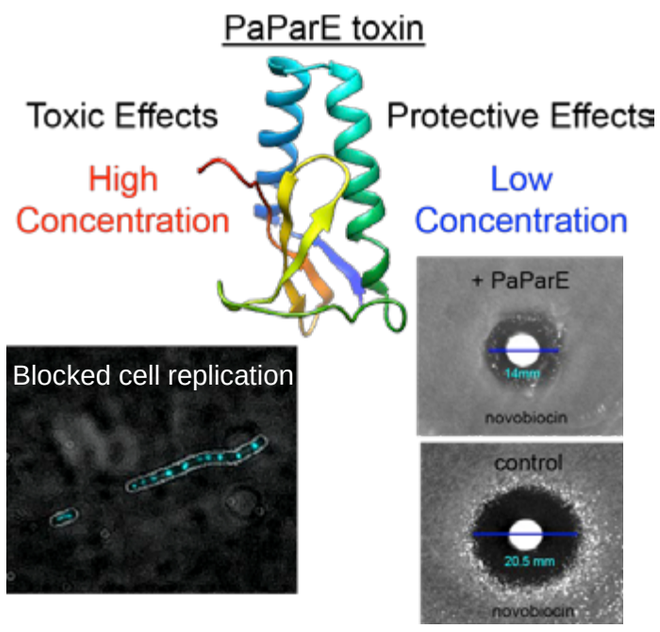
Preventing the progression to chronic bacterial infections

Targeting bacterial cell metabolism by manipulating toxin-antitoxin systems

PI: Christina Bourne, OU Dept. of Chem and Biochem OCAST Project: HR17-099 Research Area: Infectious Disease

Project Narrative

Chronic bacterial infections drive increases in health care costs, complicate patient outcomes, and increase the likelihood of the development of antibiotic-resistant mutations. These infections arise when the initial bacterial growth is able to shift metabolism to a "survivor" state in response to treatments. One mechanism that shifts bacterial metabolism is protein switches that, when complexed are "off", but when separate are "on" and allow the cell to survive antibiotics (a trait called "tolerance"). Our group is seeking fundamentally different ways to treat these infections by blocking the survival imparted by these switches in the hopes of preventing recurring and chronic infections. We have focused on protein switches in the bacteria *Pseudomonas aeruginosa*, a pathogen of serious concern to human health. To achieve our goal we first need to understand the molecular basis of the unique interactions within the switch, which is the focus of this project. At the completion of our project we expect to have determined a compelling new strategy to improve existing antibiotic treatments, thus preventing the progression to chronic infections.

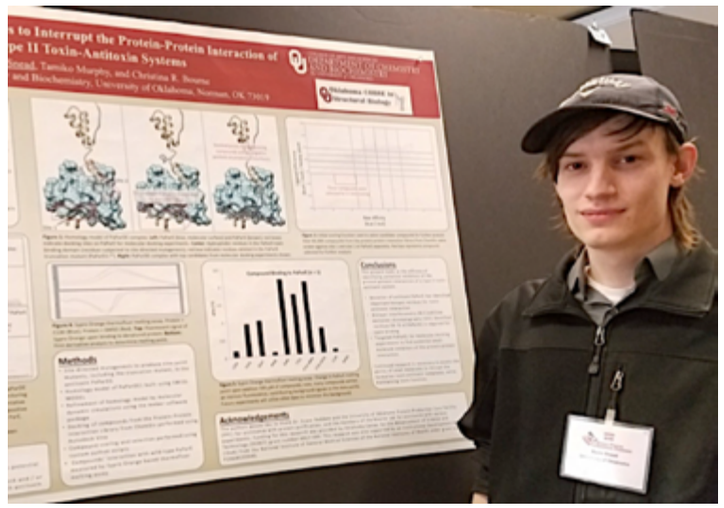


Low levels of expression of PaParE1 in *E. coli* decreases the effectiveness of anti-gyrase antibiotics, shown here in a disk diffusion assay. However, high expression levels cause toxicity. (Published in 2019)

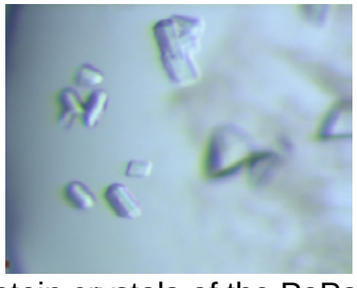
Recent Accomplishments

- Published two manuscripts describing this work in 2019 (doi: 10.111/mmi.14165 & 10.1002/mbo3.902)
- Provided support for one graduate student and one part-time staff scientist
- Identified minimal antitoxin needed to prevent interaction (a surprisingly small truncation)
- Protein crystals of the complex have been obtained; these diffract to 1.6 Å using our home source X-rays; phasing efforts are under way.

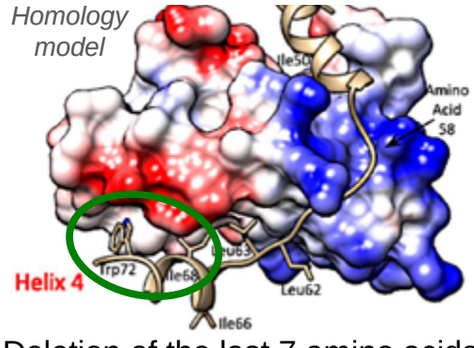
These activities will allow us to localize the important interacting region the antitoxin uses to complex the toxin – these will be the interactions we seek to block!



Graduate student Kevin Snead presenting a poster on this work at the Great Plains Infectious Disease Conference (2018).



Protein crystals of the PaParDE1 complex.



Deletion of the last 7 amino acids completely prevents association (the K_D for this complex is ≅160 pM)

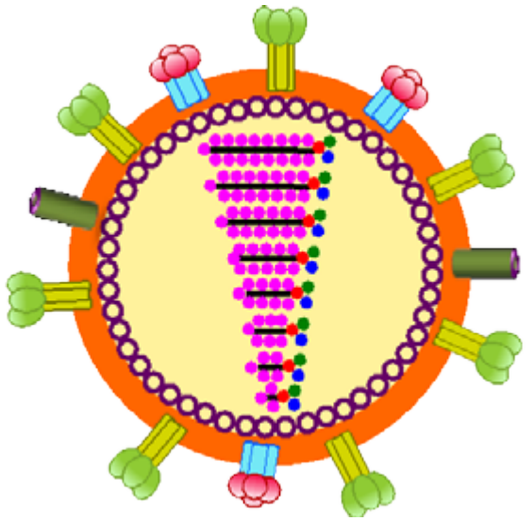
Discovery of a New Anti-Flu Gene

Role of Plakophilin 2 in Limiting Influenza A Virus Infection

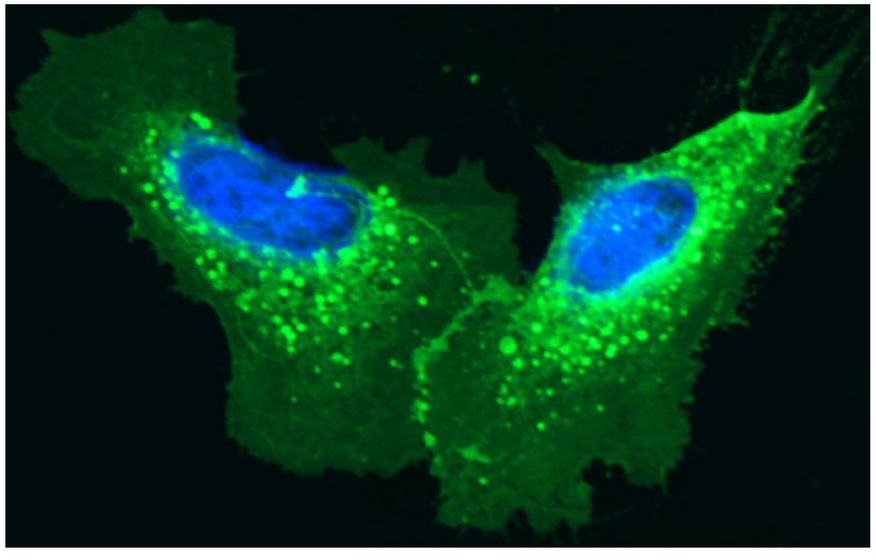
PI: Shitao Li, Oklahoma State University **OCAST Project: HR17-045** **Research Area: Infectious Disease**

Project Narrative

Influenza A virus (flu virus) is a human respiratory pathogen that causes seasonal epidemics and occasional global pandemics with devastating levels of morbidity and mortality. Currently, the antivirals have reduced efficacy because new viral strains are often resistant to conventional treatments. Thus, there is an urgent need to find new therapeutic targets and develop an antiviral drug based on these new targets. This study aims to elucidate the mechanisms of how the host protein plakophilin 2 restricts flu infection. Knowledge gained from this study will be promising for the design of the future generation of antiviral therapies.



A schematic of flu virus particle



Flu virus infected human lung cells.
Green: viral M2 protein; Blue: cell nucleus

Recent Accomplishments

- We have identified and characterized plakophilin 2 as an anti-flu gene.
- We found that the plakophilin 2 binds flu polymerase and inhibits viral replication in cells.
- We found that expression of plakophilin 2 inhibits viral infection.

Testing a combination treatment of flu infection, a common respiratory problem due to influenza virus.

A novel combination therapy for influenza pneumonia

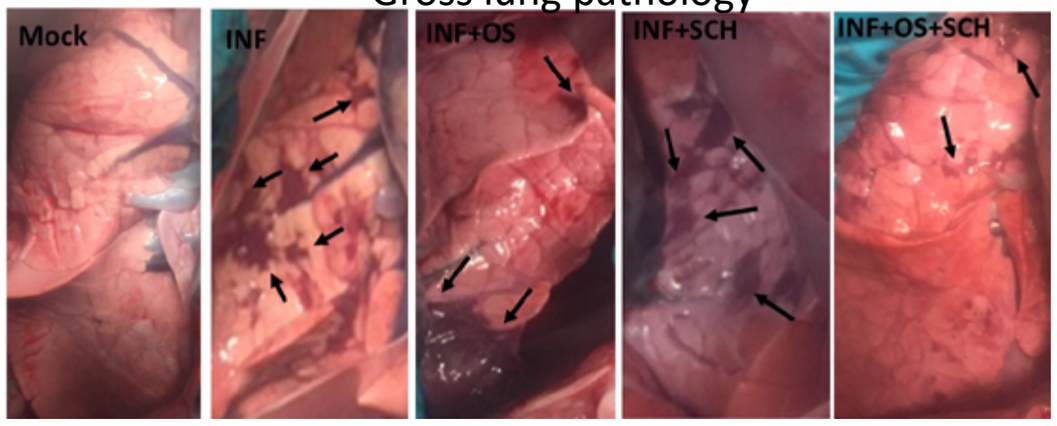
PI: Teluguakula Narasaraju Oklahoma State University OCAST project: HR16-109 Research area: virology

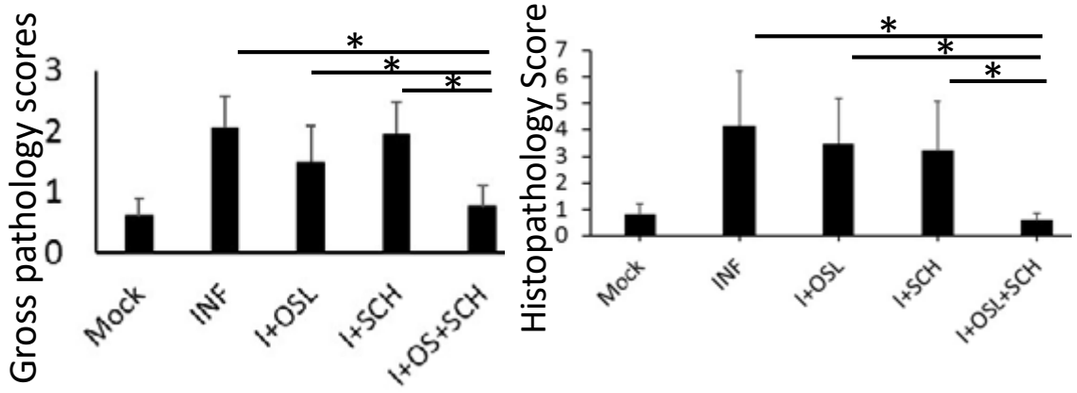
Abstract: Currently, influenza accounts for up to 500,000 deaths per year globally. The present study will be conducted to test the role of neutrophils in influenza pathogenesis. We have found that a combination of a drug that blocks a neutrophil recruitment together with an antiviral agent reduces lung damage and protects from influenza infection in mice. This proposal tests the protective effects of these drugs in a pig-influenza pneumonia model.

We tested an efficacy of combination therapy in pig-influenza model. We have used a neutrophil blocker (SCH527123) alone and in combination with the antiviral drug, oseltamivir. Gross lung pathology of the lungs demonstrate significant decrease in lung damage in combination treated piglets compared to either of the drugs treated alone. We have established a pig-model of influenza pneumonia. As shown in the Figure, Pigs infected with influenza display severe gross lung pathology with wide-spread hemorrhagic lesions (black arrows). Microscopic histopathology and biochemical analysis of acute lung injury have shown significant improvement in combination treated piglets compared to other groups.

Based on these results, we submitted a concept proposal to AstraZeneca, Sweden for a collaborative project.

Gross lung pathology





The challenge of making an RSV vaccine that is both effective and safe

A novel virus-like-particle based RSV vaccine to generate broad and durable protection

PI: Tom Oomens, College of Veterinary Medicine, OSU

OCAST Project: HR18-079

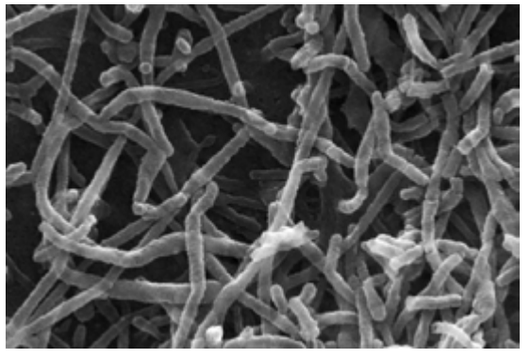
Research area: Infectious Disease

Project highlights

Respiratory syncytial virus (RSV) is a major viral cause of lung disease in children and elderly worldwide. Despite its medical importance and decades of effort, there is no licensed vaccine. One of the biggest challenges has been to design a vaccine that not only induces a broad and protective immune response but is also safe for the vaccine recipient.

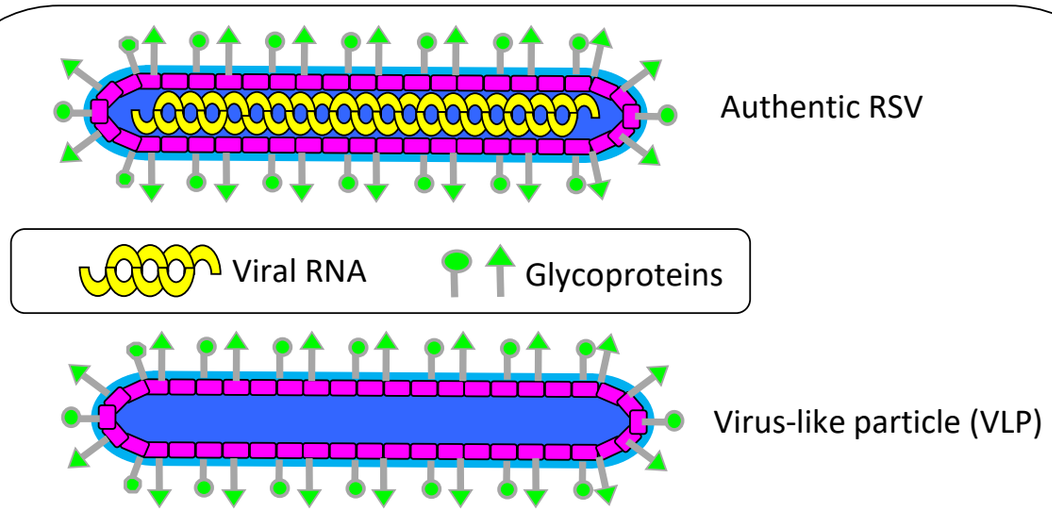
Our major goal is to develop vaccines with stringent safety profiles. In this project, we generate virus-like-particles (VLPs) to serve as vaccines. VLPs are particles that look like the real virus and hence stimulate the recipient to develop a protective anti-viral immune response. However, VLPs are engineered such that they lack the genetic material of the virus and are unable to replicate inside a vaccine recipient, making this approach safe. Recent successes with other VLP vaccines, such as the human papilloma virus (HPV) vaccine that protects from cervical cancer, are highly encouraging. In this project, we test novel RSV-based VLPs in a mouse model, to assess their ability to safely induce an effective immune response.

If successful, the VLP vaccine may overcome the long-standing RSV vaccine problem in balancing safety and effectiveness, and may lead to a platform for generation of safe RSV vaccines. With close to 200,000 annual deaths worldwide in children alone, a safe RSV vaccine could have an enormous impact.



Electron microscopy image of respiratory syncytial virus (RSV)

Breathing problems due to RSV. RSV infects the respiratory tract and can lead to severe lung disease in infants and the elderly.



Schematic comparing an authentic RSV particle (top) to an RSV-based virus-like-particle (VLP)(bottom). The VLP mimics the authentic virus and induces an effective immune response, but cannot replicate and is safe because it lacks the genetic material (RNA).

A Low-Cost High-Impact Route to Kill MRSA With FDA-Approved Antibiotics

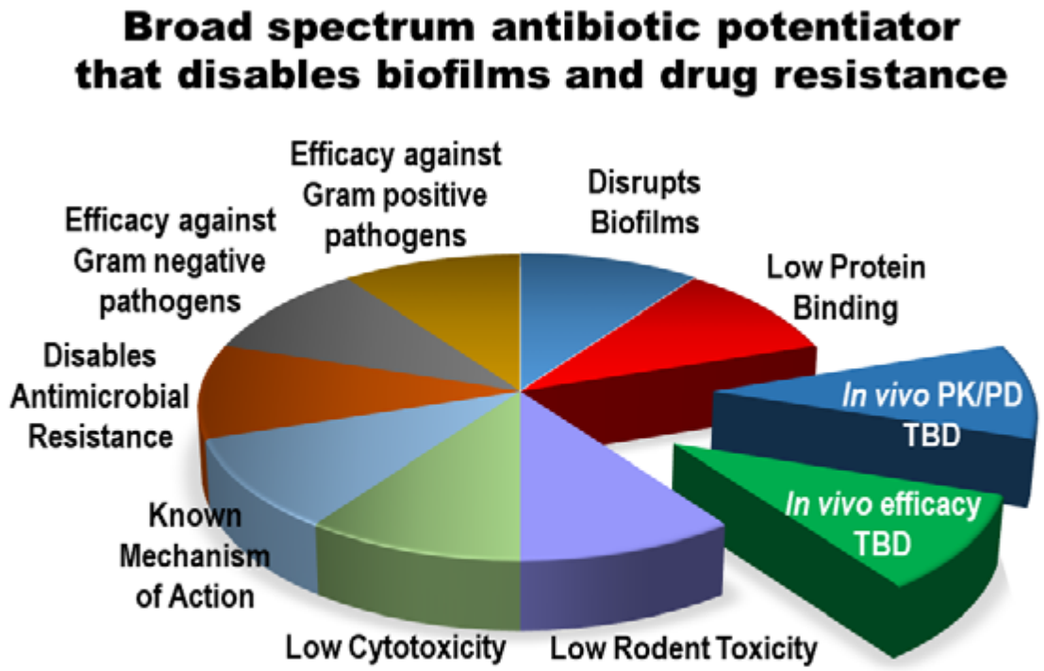
Potentiating Beta-Lactams to Treat MRSA Infections

PI: Charles V. Rice, University of Oklahoma **OCAST Project: HR16-084** **Research Area: Infectious Disease**

Project Summary

Bacterial pathogens cause serious hospital- and community-acquired infections. Antibiotic therapy is useful, but incomplete treatment and resistant strains often result in severe chronic infections. Leading causes of chronic infections are biofilms that are often impenetrable to antibiotic medications and can be composed of multidrug resistant pathogens. In the absence of a robust pipeline of new antimicrobial drugs, existing drugs and regimens must be re-evaluated as combination(s) with potentiators that overcome biofilms and/or antibiotic resistance.

Novel compositions of BPEI + antibiotics have been developed as a single, dual-function potentiator to: 1) inhibit biofilms, and 2) disable resistance mechanisms. Anti-microbial synergy with common antibiotics has been achieved. Anti-biofilm properties in laboratory strains and clinical isolates have also been achieved. Furthermore, the potentiator retains potency in serum while having minimal cytotoxicity.



Lam AK, et al. “Antibiofilm Synergy of β-Lactams and Branched Polyethylenimine against Methicillin-Resistant Staphylococcus epidermidis.” *Biomacromolecules*. **2019**. doi: 10.1021/acs.biomac.9b00849

Hill MA et al. “BPEI-Induced Delocalization of PBP4 Potentiates β-Lactams against MRSA.” *Biochemistry*. **2019**; 58(36): 3813-22.

Lam, A. K. et al. “Cationic Branched Polyethylenimine (BPEI) Disables Antibiotic Resistance in Methicillin-Resistant Staphylococcus epidermidis (MRSE).” *ChemMedChem*, **2018**. 13(20), 8-14.

Foxley MA et al. “Targeting Wall Teichoic Acid In Situ with Branched Polyethylenimine Potentiates β-lactam Efficacy against MRSA.” *ACS Med Chem Let*. **2017**; 8 (10), 1083-1088.

Bacteria have gained resistance to the last-resort drug daptomycin, prompting the need for new antibiotics.

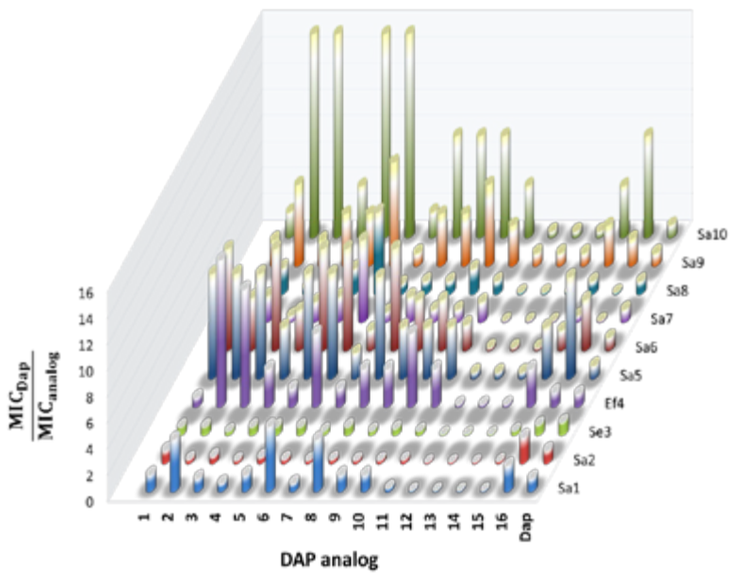
The development of daptomycin analogs

PI: Shanteri Singh, University of Oklahoma **OCAST Project: HR19-080** **Research Area: Infectious Disease**

Project Highlights

Daptomycin is an antibiotic of last-resort used to treat infections of drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE) that pose a high risk for metastatic complications and death. Despite its potent bactericidal activity against many Gram-positive bacteria, there have been increasing reports of daptomycin resistance, rendering daptomycin ineffective in treating infections. Therefore, it is crucial to develop new daptomycin analogs capable of treating infections caused by daptomycin resistant strains. The proposed work is intended to generate novel daptomycin-analogs using an innovative approach. The results of the proposed work may lead to potentially new daptomycin leads against daptomycin resistant infections.

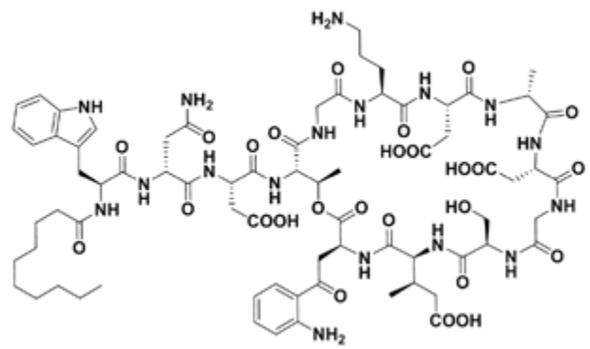
Electron micrograph image of MRSA (courtesy of the National Institute of Allergy and Infectious Diseases.)



Activity screen of daptomycin analogs

Recent Accomplishments

- The innovative chemoenzymatic strategy we proposed uses an enzyme for the late-stage derivatization of daptomycin to generate daptomycin analogs.
- The activity assay of 16 newly generated daptomycin analogs revealed some of our analogs to be potent against daptomycin resistant *Staphylococcus aureus* as well as daptomycin resistant *Enterococcus*.



Structure of daptomycin

Lead me, follow me and walk with me: analyze your gait motion from a robot

A Mobile Platform for Clinical Gait Analysis

PI: Guoliang Fan, Oklahoma State University OCAST HR18-069 Research Area: Data Science/Clinical Platform

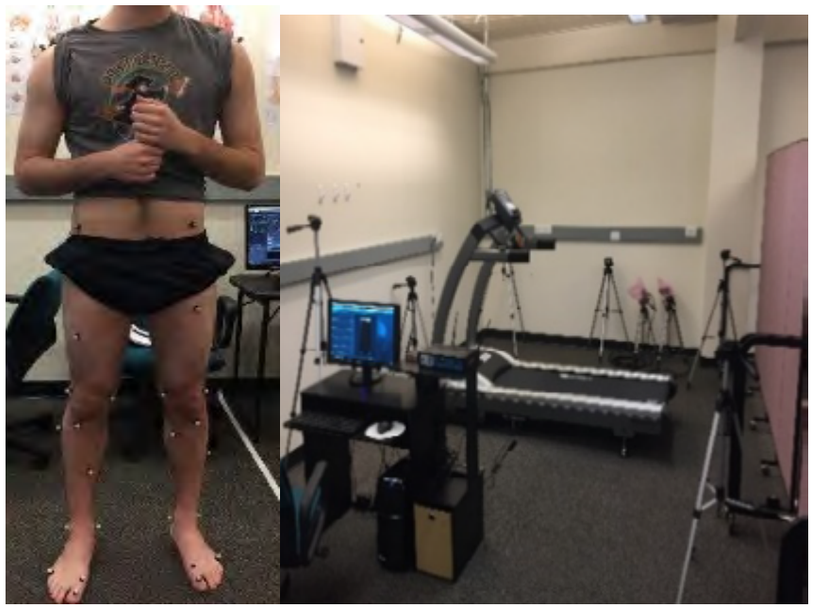
Project Narrative

This project seeks an innovative approach to develop a mobile platform for low-cost clinical gait analysis, which can (1) autonomously follow a walking subject in a free and natural setting and from three different perspectives; (2) reliably estimate full-body gait kinematics represented by skeletal joint angles and points from depth sequences under different perspectives, (3) robustly extract relevant biomechanical parameters for gait imbalance assessment. The proposed system is targeted on the limitation and constraints of the current motion capture (Mocap) systems, including "gold standard" optical Mocap systems, vision-based gait analysis tools, pressure sensor mats and wearable sensor networks. On the one hand, the proposed system is designed to minimize the interference and constraints to the subject's motion pattern, and at mean time, to maximize the subject's walking space and freedom and to support multi-view motion capture. On the other hand, the system is affordable (less than $2000), portable and fully integrated with a small footprint. The proposed mobile Mocap system has a great potential to be applied for many motion-related clinical applications, gait disorder analysis, physical therapy and musculoskeletal surgery rehabilitation. The main system hardware includes a vision-guided robot, a RGB-D sensor, and a laptop. In this project, the focus is on the *enhancement*, *enrichment* and *engagement* of robot's vision capability for multi-view motion capture and extraction of biomechanical gait parameters. The proposed research is translational in the sense that it bridges the gap between lab and clinic by creating a new mobile solution to advanced clinical gait assessment.

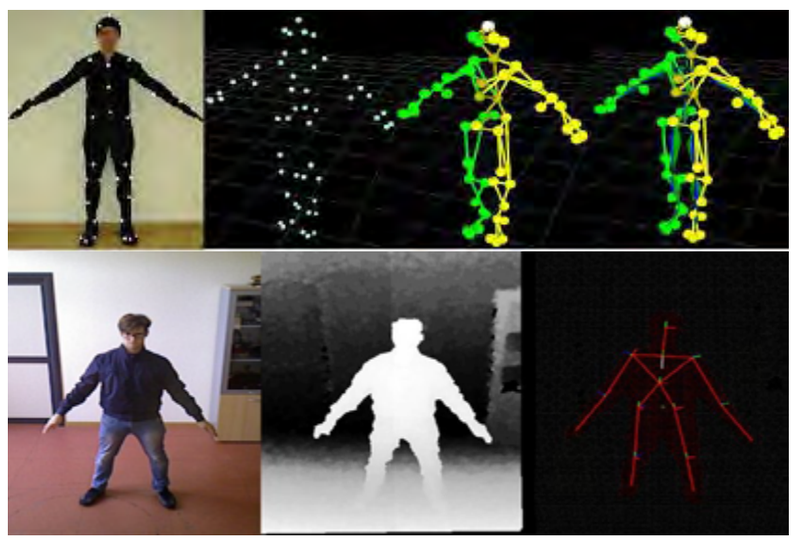
The dual-senor mobile system developed in the PI's lab.

Recent Accomplishments

- We developed adaptive Kalman filtering-based approaches to improve the quality of skeleton data from a depth sensor.
- We proposed a deep learning-based motion manifold for skeleton recovery to handle noise, outlier and occlusion.
- We developed a human tracking algorithm that allows a robot follow and track a human subject for motion capture on the fly.
- We implemented an Android-based interface on a tablet for system initialization, debug, control and data visualization.



The marker-based motion analysis system in the collaborator, Dr. Jeromoe Hausselle's lab (MAE, OSU)



Optical Marker-based Mocap (above) and markerless Mocap by a depth sensor (below).

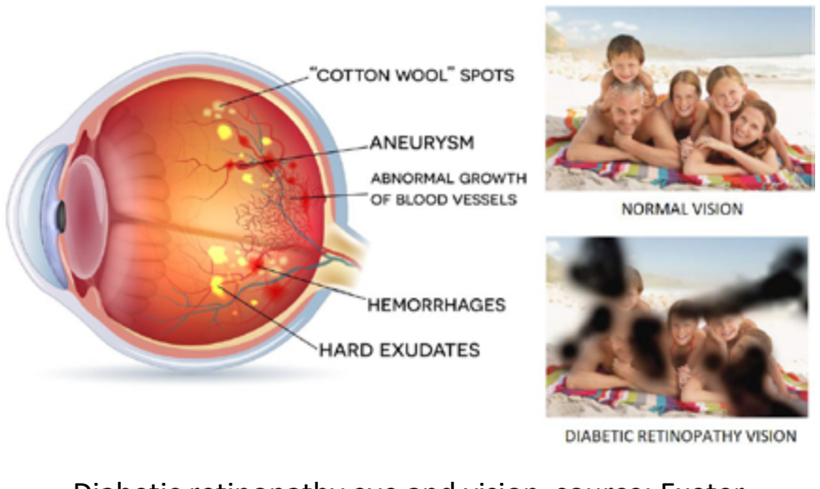
Can we use a Patient's data to predict Diabetic Retinopathy?

Validating a clinical decision support algorithm developed with big data to diagnose, state, prevent, and monitor a patient's diabetic retinopathy

William D. Paiva, Ph.D., Oklahoma State University **OCAST Project HR18-087** **Health Data Analytics**

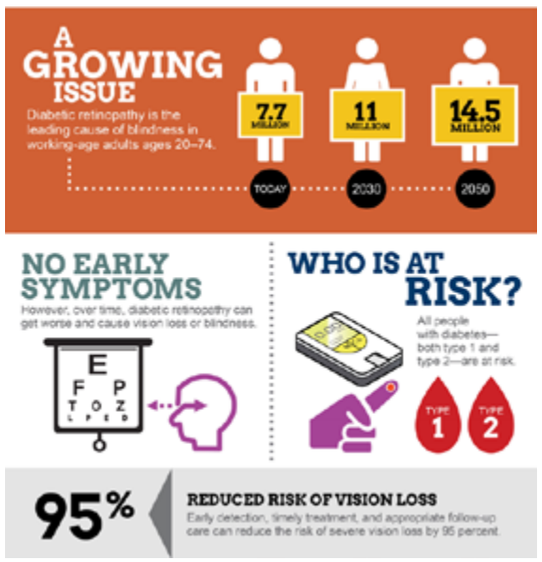
Project Narrative

Diabetic retinopathy (DR), a complication of diabetes, is a leading cause of blindness among working-aged adults globally. In its early stages, DR is symptomless, and can only be detected by an annual eye exam. Once the disease has progressed to the point where vision loss has occurred, the damage is irreversible. Consequently, early detection is quintessential in treating DR. Two *barriers* to early detection are poor patient compliance with the annual exam and lack of access to specialists in rural areas. This research is focused on developing and validating new, cost-effective predictive technologies that can improve early screening of DR. Our overall objective is to develop and implement an entire suite of tools to detect diabetes complications in order to augment care for underserved rural populations in the US and internationally.



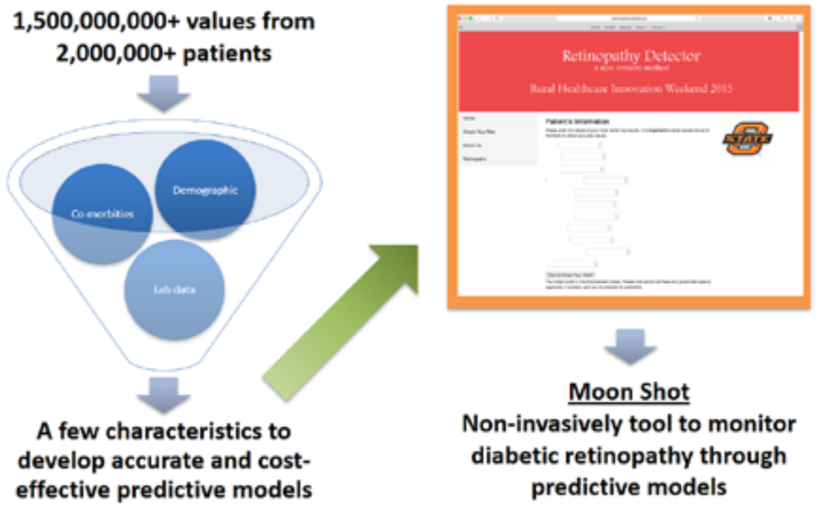
Diabetic retinopathy eye and vision, source: Exeter Eye and Retina Labs

Recent Accomplishments



Diabetic retinopathy infographics, source: National Eye Institute

- We developed a clinical decision support system to detect DR using demographic, comorbidity, and lab data, which performs with an accuracy of 92.7%.
- We recently simplified the model by identifying 3 essential laboratory tests for DR diagnosis technology while maintaining a similar level of accuracy.
- The critical variables remain the same based on data from the Harold Hamm Diabetes Center.
- We are getting more data from the University of Kansas Medical Center for external validation.



Diabetic retinopathy prediction: from science to practical tool

Seeing the Unseen Radiation in Patient with XACT Imaging

X-ray-induced acoustic computed tomography (XACT) is a new and promising imaging modality to guide and improve the radiotherapy

PI: Liangzhong (Shawn) Xiang, University of Oklahoma **OCAST Project: HR19-131** **Research Area: medical imaging**

Project Highlights

Cancer remains a leading cause of death globally and a major public health concern. As one of the main treatment options for cancer, radiation therapy is received by roughly two-thirds of all cancer patients. Radiation dosimetry is crucial to the continued success and improvement in cancer treatment, ensuring that a correct and accurate dose is delivered to the desired location. However, current clinical practice only allows for the planning and/or verification of delivered dose through simulations with phantoms; an *in vivo* and in-line verification of the delivered dose remains absent in the clinic.

This research seeks the development of a completely novel *in vivo* dosimetry that allows for direct online measurement of dose delivery by photon beam radiotherapy which will improve the precision of radiotherapy for cancer patients.

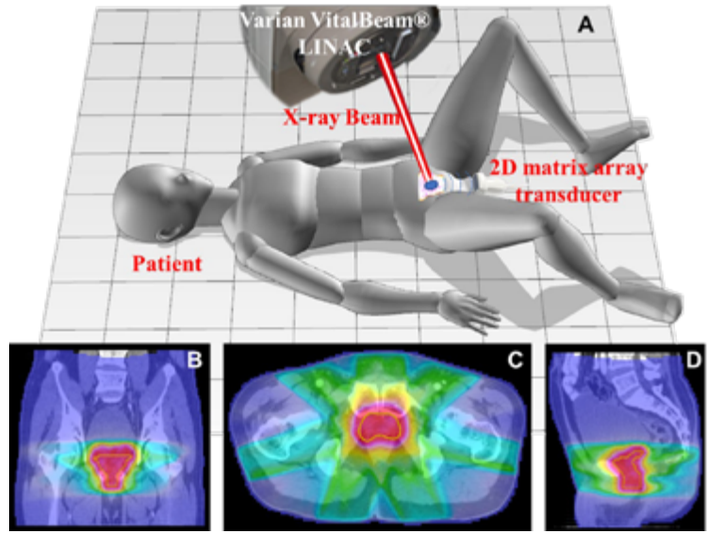


Fig 1. Schematic diagrams of a prostate cancer patient set-up and transperineal ultrasound array configuration.

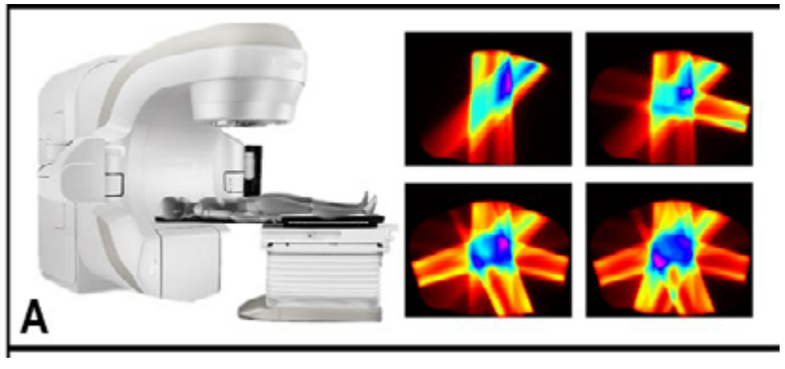


Fig 2. The workflow of the XACT imaging for real time radiation therapy guidance for prostate cancer. The process of the radiation dose distribution acquisition is shown.

Recent Accomplishments

- We completed the computer simulation on XACT imaging for in vivo dosimetry on prostate cancer.
- We recently started working on the development of the XACT imaging system including the hardware components.

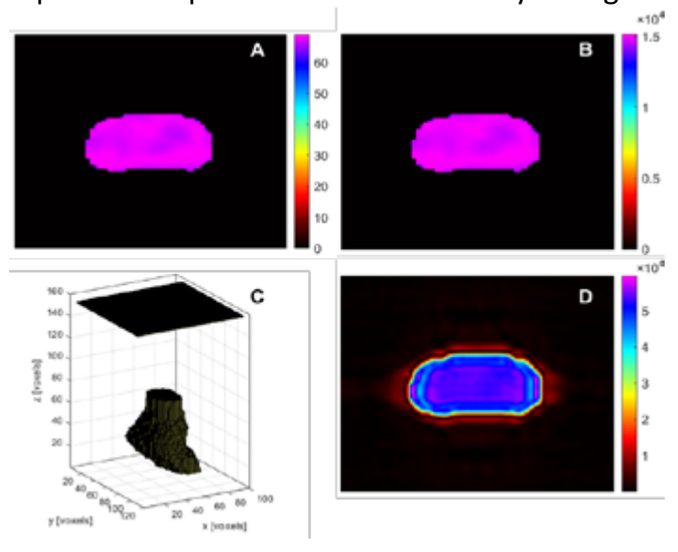


Fig 3. A simulation on XACT imaging as a tool for *in vivo* dosimetry. (A) prostate dose distribution. (B) the initial XA pressure distribution. (C) the 3D digital model of the prostate and the 2D matrix array transducer. (D) the reconstructed XACT imaging.

A membrane protein's role in memory forming processes in the brain

The role of AMPA receptor-interacting protein Prrt1 in synaptic plasticity

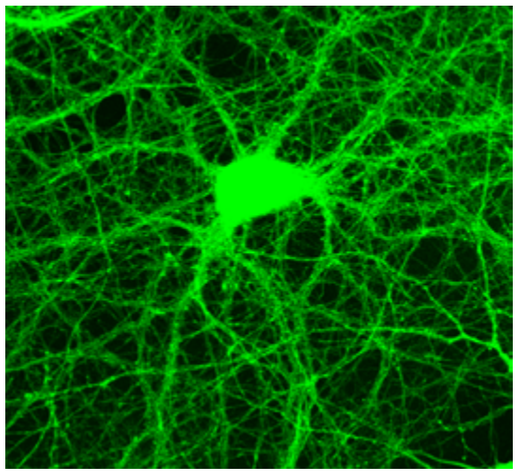
PI: Mohiuddin Ahmad, University of Oklahoma Health Sciences Center

OCAST Project:HR16-116

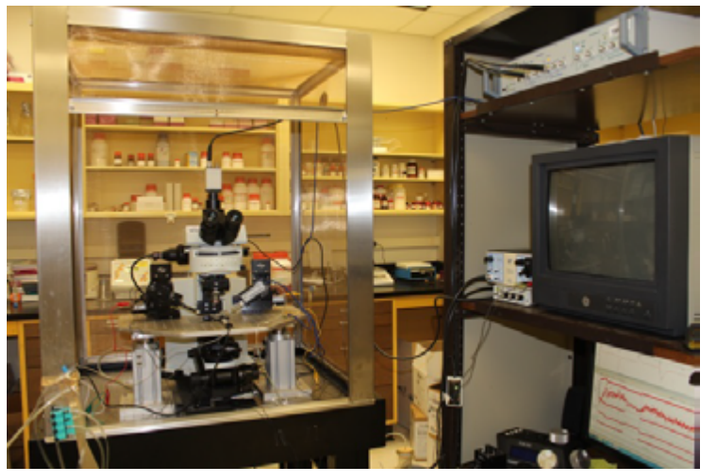
Research Area: Neurobiology

Project Summary

The human brain contains billions of cells which are connected through trillions of connections called synapses. The formation of memories involves long-term strengthening or weakening of these connections. We have identified that a membrane protein called PRRT1 is important for activity-dependent changes in the strength of synapses. Our work elucidated the mechanisms by which PRRT1 regulates these changes to allow correct formation of memories. The results of the work will lead to a better understanding of how memories are formed, and provide important information for future investigations on the weakening of memories in old age and Alzheimer's disease.



Brain cells (neurons) forming processes and connections in a culture dish



Electrophysiology equipment for recording the strength of brain connections

Recent Accomplishments

- We identified that PRRT1 regulates the stability of modified receptors and controls their localization on the cell membrane.
- We uncovered the mechanism by which PRRT1 regulates the activity-dependent weakening of synapses. *Troyano-Rodriguez et al., Mol Cell Neuro, 2019*

Can nutritional interventions prevent neurodevelopmental disorders?

Regulation of ZIP12- a candidate gene for neurodevelopmental disorders

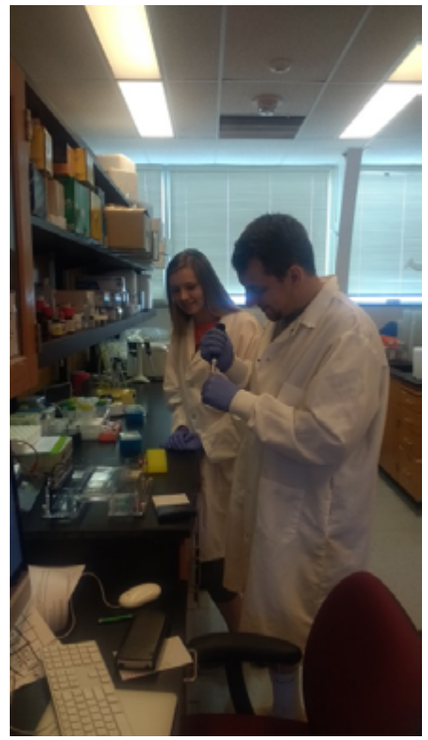
PI: Winyoo Chowanadisai, Oklahoma State University **OCAST Project: HR16-060** **Research Area: Developmental Neuroscience**

Project Summary

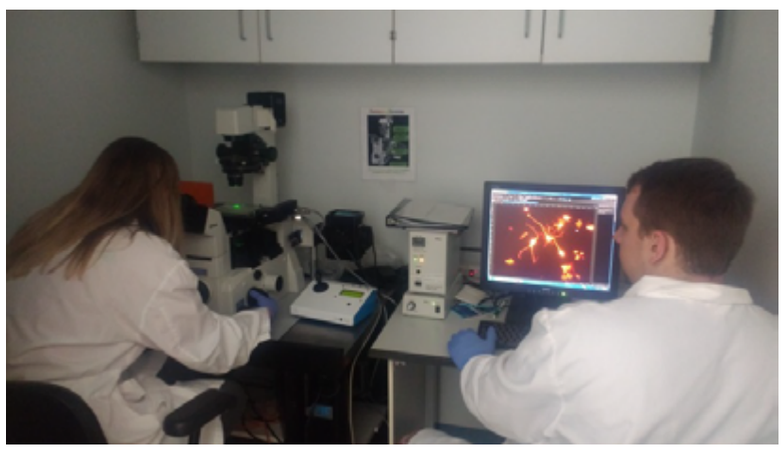
Both nutrients and genes are important for brain development. Impaired gene function and nutrient deficiencies can lead to neurodevelopmental disorders such as neural tube defects and autism. The genes that control how zinc enters neurons and the brain are critical parts of brain development and function. The proposed work includes determining how this specific gene is important for brain development and function.

- ZIP12 KO cells due to Cas9/CRISPR genome editing have increased carbonyl groups introduced into proteins as a form of oxidative damage
- Human subjects with polymorphisms in the ZIP12 (SLC39A12) gene have a detectable brain MRI phenotype, according to a recent genetics study

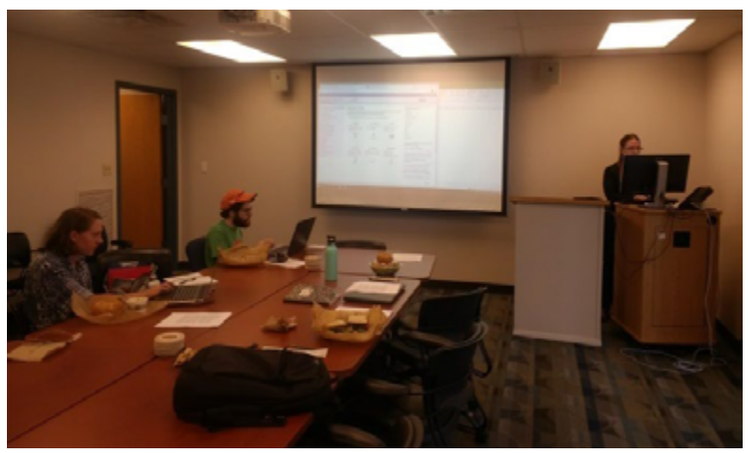
Understanding these processes may identify which neurodevelopmental disorders are responsive to zinc. Ultimately, uncovering the connections between zinc and the brain may lead to nutritional strategies for preventing neurodevelopmental disorders.



Morgan Strong, a OSU graduate student, and Matthew Hart, an undergraduate student, are working with DNA.



Students viewing cultured cells at a microscope



Emily Chambers, a OSU graduate student, is leading Danielle Davis, an undergraduate student, and Evan Hermann, a research assistant, through genetic dataset analyses.

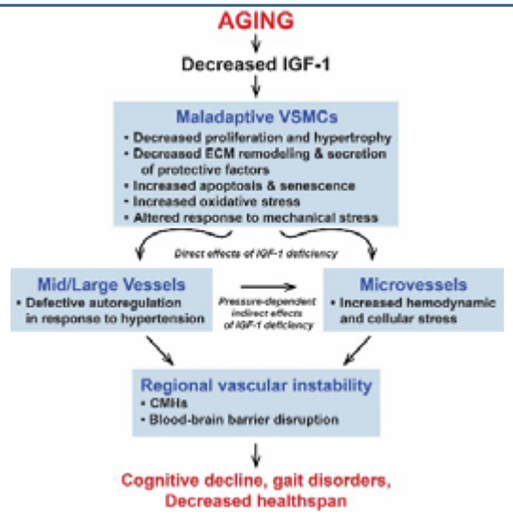
Vascular Smooth Muscle Cells in Brain Aging

The role of vascular smooth muscle cell plasticity in age-related cognitive decline

Shannon Conley, OUHSC HR18-118 Cell Biology

Project Narrative

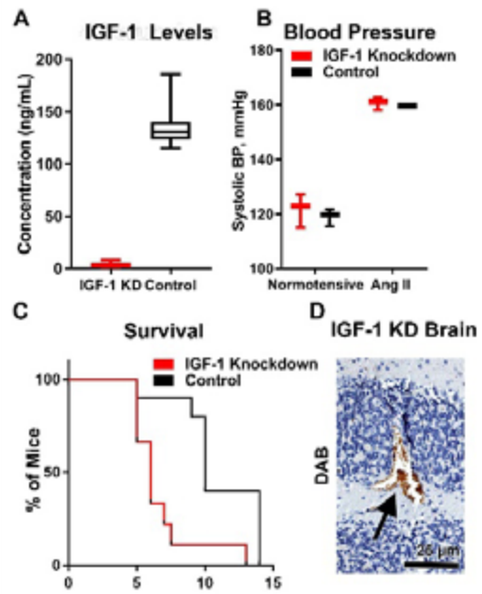
Cerebral microhemorrhages (CMH) result from rupture of small intracerebral blood vessels and progressively impair neuronal function. The incidence of CMH dramatically increases with age and hypertension and is a major cause for age-related cognitive decline and mobility deficits. CMHs occur due to increased blood vessel fragility but underlying mechanisms are unknown. Blood vessel integrity requires plasticity of vascular smooth muscle cells (VSMCs). In response to stress, VSMCs exhibit an adaptive switch from a highly contractile state to a protective anti-fragility state characterized by cell growth and proliferation as well as extracellular matrix remodeling. Insulin-like growth factor (IGF)-1 has vasoprotective effects and promotes adoption of the protective anti-fragility phenotype. However, circulating IGF-1 levels are dramatically decreased with age. Our **hypothesis** is that impaired ability of VSMCs to adopt a protective anti-fragility state switching due to deficient IGF signaling has a fundamental role in increased development of CMH and associated neurological and mobility deficits with age. We have two aims. The first is to evaluate wither IGF-1 signaling on VSMCs contributes to vascular fragility in aging. Our second aim is to evaluate transcriptional mechanisms associated with Tbx15/18 to understand whether they play a mechanistic role in IGF-1 mediated adoption of protective VSMC states. The studies proposed here will significantly enhance our understanding of the role of IGF-1 deficiency in the development of CMH in aging, critical given the increased aging population and need to understand root causes for cognitive decline and mobility deficits.



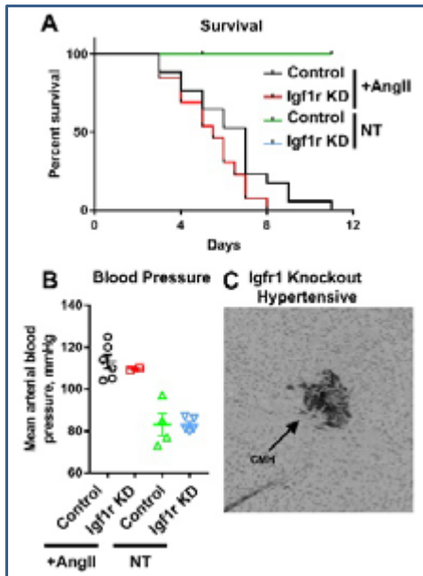
Proposed experimental paradigm

Recent Accomplishments

- A reduction in IGF-1 leads to development of microhemorrhages in the brain and changes in adaptive matrix remodeling in the vasculature.
- Initial studies suggest that knockdown of Igf1 receptor in smooth muscle cells leads to the development of cognitive decline and microbleeds.



Global IGF-1 knockdown plus hypertension impairs survival and promotes CMH. A. IGF-1 knockdown leads to reduced circulating IGF-1. **B.** Hypertension is induced by implantation of a minipump delivering Angiotensin II. **C.** Hypertensive IGF-1 knockdown animals have reduced survival compared with controls and develop CMH **(D)**.



VSMC-specific Igf1 receptor knockdown (Igf1r KD) leads to decreased survival and development of CMH in a hypertensive model. Hypertension was induced in VSMC-specific Igf1r knockdown and control animals by delivery of Angiotensin (AngII) with normotensive controls (NT) **(B)**. Death due to neurological complications accelerated in Igf1R KD **(A)** Histological analysis revealed the development of CMH throughout the brain in Igf1r KD animals; example shown in **(C)**, quantification is ongoing.

Excess weight gain and changes in the brains of female rats after removal of ovaries

Neuroimmune activation and weight gain in a rat model of postmenopausal obesity

Kathleen S. Curtis, Oklahoma State University – Center for Health Sciences **OCAST HR18-089** **Neurobiology**

Project Highlights

More than 30% of adults in Oklahoma are obese, and obesity increases with age. Given the aging of our population and diseases associated with obesity, such as diabetes and high blood pressure, better understanding and management of obesity is critical. Unfortunately, though some progress has been made in understanding how the brain controls eating and body weight, obesity remains a problem throughout the US. This issue is a particular concern for post-menopausal women, since body weight increases after menopause. In fact, almost 40% of women over 60 are obese. Body weight also increases in laboratory animals after removal of the ovaries (ovariectomy); therefore, we are using female rats to examine changes in the brain with post-ovariectomy weight gain. This weight gain does not require special diets or genetic manipulations, so the changes are associated with obesity, rather than with other causes. Importantly, the post-ovariectomy weight gain occurs rapidly and predictably (Fig. 1), so specific factors, including neuroimmune signals, in specific brain areas can be linked to the *development* of obesity (Fig. 2). These studies will allow us to identify factors that change early during post-ovariectomy weight gain, or at particular phases of the weight gain, rather than with established and extreme obesity. Ultimately, the information will help to target these factors in attempts to manage—or prevent—obesity.

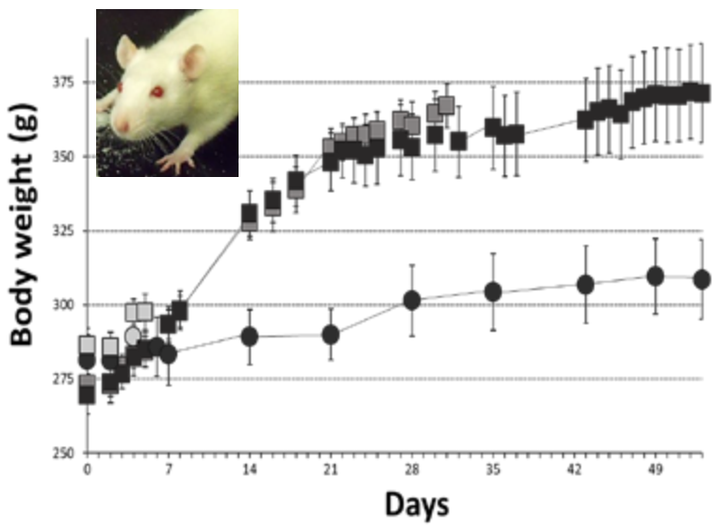


Fig. 1 Body weight of female rats with (circles) or without (squares) ovaries.

Recent Accomplishments

- **Rats rapidly and reliably gain weight after surgical removal of ovaries (Fig.1)**
- **Development of post-ovariectomy weight gain is associated with temporally- and regionally-specific neuroimmune activation (Fig. 2).**
- **Post-ovariectomy weight gain is transiently reduced by voluntary exercise (Fig. 3).**

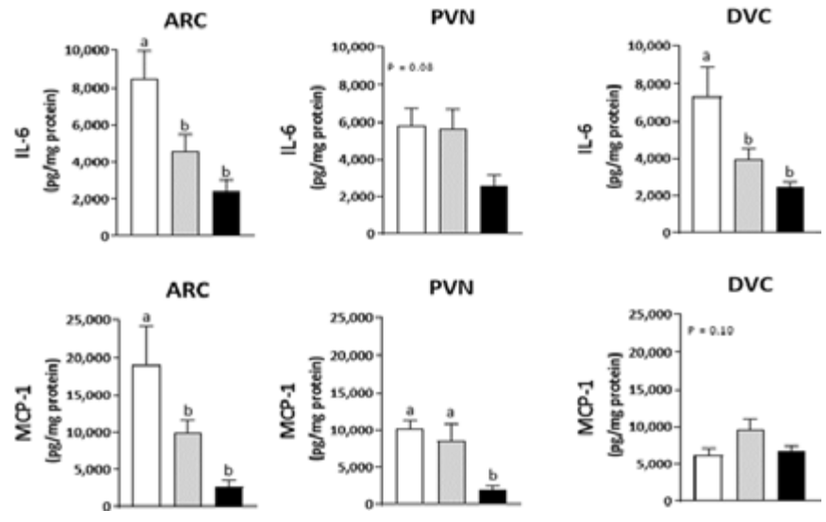


Fig. 2 IL-6 and MCP-1 levels is specific brain areas 5 days (baseline; white bars), 4 wks (gray bars) or 8 wks (black bars) after ovariectomy. ARC = arcuate nucleus; PVN = paraventricular nucleus; DVC = dorsal vagal complex

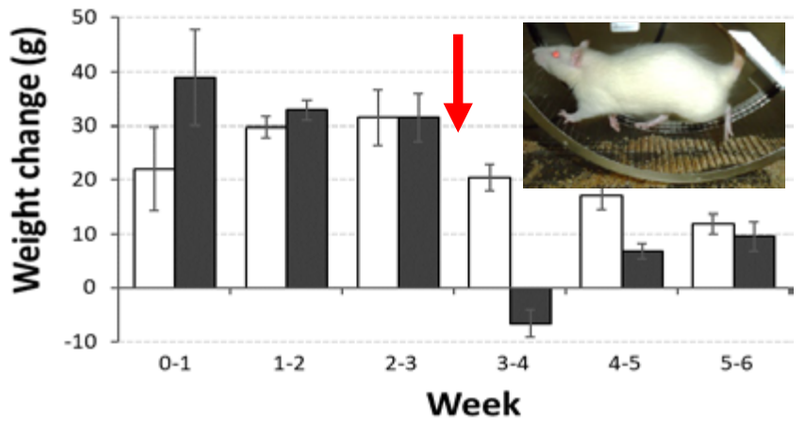


Fig. 3. Change in body weight of female rats with access to running wheels immediately (white bars) or 3 wks after (black bars) ovariectomy. Arrow = switch from running to sedentary condition (R>>>S) or from sedentary to running condition (S>>>R).

Advancing therapeutic options for treating mood and anxiety disorders using a novel anti-inflammatory agent.

PI: Randall L. Davis, Oklahoma State University Center for Health Sciences

OCAST Project: HR18-033

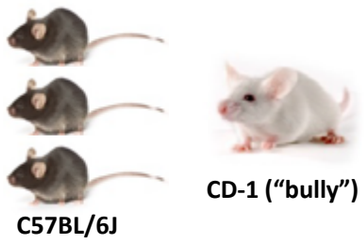
Research Area: Neurobiology

Project Highlights

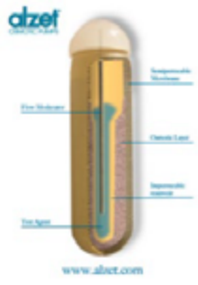
About 30 million people in the U.S. suffer from mood and anxiety disorders and, as a result, shoulder enormous personal and economic burdens. Unfortunately, many of those who live with such disorders do not adequately respond to currently available medications, so developing new drugs and treatment strategies for such disorders is imperative. Inflammation in the brain (neuroinflammation) plays an important role in these brain disorders. However, there are relatively few medications on the market that target neuroinflammation. We previously discovered that a drug (β-FNA) inhibits activation in specialized brain cells called astrocytes that are instrumental in neuroinflammation. We then performed studies using mice and demonstrated that a single injection of the drug protected not just against neuroinflammation, but also reduced the sickness behavior that accompanied exposure to a bacterial product. The current proposal will significantly advance the therapeutic relevance of this drug as a neuroprotective agent, particularly in the context of *extreme social stress or "bullying"*. This project will involve subjecting a mouse to a larger and aggressive strain of mouse for 2h/day for 6 consecutive days; a protocol called Repeated Social Defeat (RSD). This form of social stress reliably produces both neuroinflammation and anxiety-like behavior in mice. We will test the hypothesis that our drug can reduce or prevent stress-induced neuroinflammation and anxiety. We first will examine whether drug treatment *during* the social stress prevents neuroinflammation and/or the behavioral deficits that follow "bullying". We then will examine whether the drug prevents or reduces the duration of neuroinflammation and/or behavioral deficits when it is administered *after* the social stress. Overall, this project will significantly advance our understanding of the novel anti-inflammatory and neuroprotective actions of this drug, particularly, in terms of brain disorders associated with extreme social stress. Ultimately, the results of this project may lead to improved treatment options for addressing mood and anxiety disorders.

Recent Findings

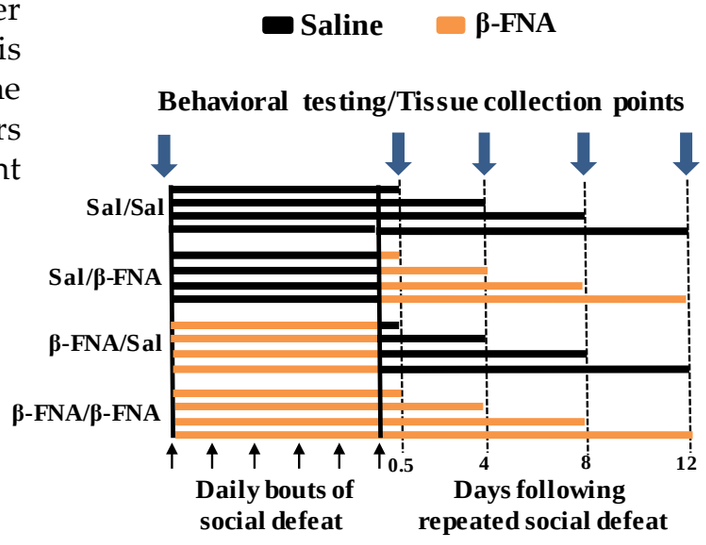
- A single bout of social defeat induced stress and peripheral inflammation in C57BL/6J males (but, not in females)
- A single bout of social defeat reduced IL-1β in the brain of C57BL/6J females (but, not in males)
- In males, repeated social defeat resulted in reduced social interaction with CD-1 mice ("bully strain"), but not with other C57BL/6J mice



Repeated Social Defeat ("bullying") protocol. Three experimental mice have a larger, aggressive mouse (of a different strain) placed in their cage for 2h/day for 6 consecutive days.



Osmotic mini-pump that provides continuous delivery of the drug (β-FNA). The mini-pump is surgically implanted under the skin.



Summary of the experimental design. The timeline emphasizes when the drug is delivered relative to RSD.

Brain Rehabilitation for People with Opioid and/or Meth Use Disorder

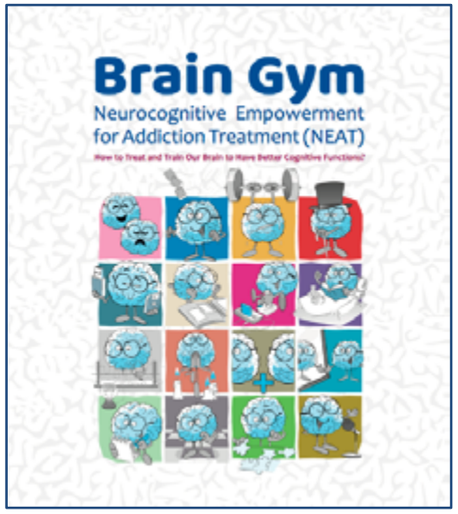
Neurocognitive Empowerment for Addiction Treatment (NEAT): A Randomized Controlled Trial for Opioid and/or Meth Addiction

Hamed Ekhtiari, MD, PhD (PI), Robin Aupperle, PhD (CI), Laureate Institute for Brain Research HR18-139 Neurobiology

Project Narrative

Addiction to opioids and methamphetamine (meth) is among the costliest and deadliest of mental health disorders in the United States. The US in general and the State of Oklahoma in particular face a crisis in opioid addiction. Opioids (including prescription opioids, heroin, and fentanyl) were involved in more than 42,000 deaths in 2016 in the US, more than any year on record and accounting for more deaths than road accidents and gun violence combined and the rate of overdose/death due to meth is being tripled during last 5 years. Chronic addiction is not only associated with increased mental health symptoms, such as anxiety and depression, but also with brain (neural and cognitive) deficits. Cognitive deficits in memory, attention, decision-making, and cognitive control disturb normal daily functioning and attempts for abstinence. Current treatment programs for opioid addiction are mainly focused on abstinence, with the assumption that these neural and cognitive deficits will subsequently heal. However, these deficits are found to persist even after a long-term abstinence and are thought to contribute to relapse, decreases quality of life, and lack of reintegration into society. However, there has been a relative lack of research focused on identifying interventions targeting cognitive deficits in the context of addiction.

The aim of this study is to characterize clinical efficacy for an intervention targeting neural and cognitive deficits associated with opioid and/or meth addiction by enhancing awareness and use of cognitive skills in the context of substance use recovery. This aim will be accomplished by randomizing 80 subjects with opioid and/or meth addiction who are already enrolled in substance use treatment in the state of Oklahoma to also complete a novel "Neurocognitive Empowerment for Addiction Treatment" (NEAT) program. NEAT will be novel in (a) its use of cartoons, brain awareness games and real-life scenarios to ensure it is interactive and engaging, (b) the focus on the role of neurocognitive deficits in recovery from substance use and co- occurring mental health symptomatology, and (c) its incorporation of neuroscientific findings specific to substance use to the training and exercise strategies. Subjects will be followed for six months to explore the efficacy of NEAT compared to the control intervention on substance use and mental health symptoms, objective cognitive performance, and functional and structural brain changes using magnetic resonance imaging (MRI). This research study has the potential to identify a novel intervention that can be integrated into substance use treatment programs in Oklahoma and nation-wide to more effectively support recovery from addiction.

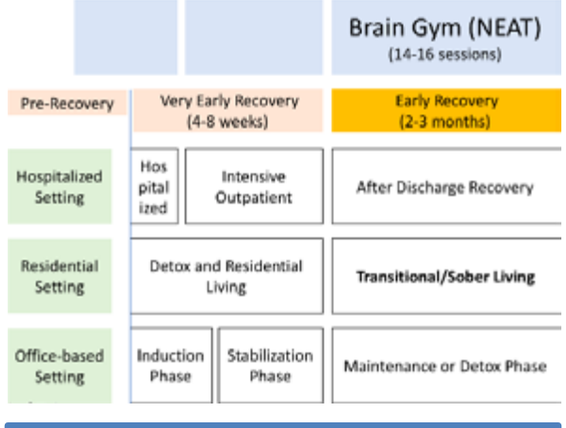


Brain Gym (NEAT) workbook cover. We use cartoons as a tool to communicate with patients.

Brain Gym (NEAT) cognitive architecture in 16 sessions. Cognitive modules are added gradually to each other from simpler to more complex ones

Recent Accomplishments

- Started the randomized clinical trial (RCT) phase of the study.
- Completed the RCT intervention for the first two groups (n=23) and started the third group (n=11) in the Women in Recovery (WIR) center
- Finalized the NEAT protocol, workbook, and therapist manual based on the pilot results.
- Scheduled the 3 month follow up for the first intervention group.



Brain Gym (NEAT) as an adjuvant in the treatment as usual setting for addiction recovery.

A Protein in the Eye that plays an Important Role in Protecting the Eye from Disease

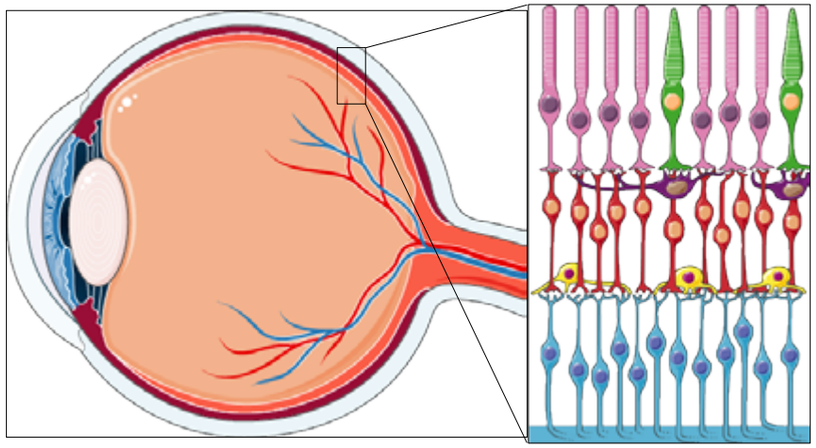
The Role of TRAF3 in Retinal Function and Inflammation

PI: Jami Gurley, PhD/Mentor: Michael Elliott, PhD/Dean McGee Eye Institute; University of Oklahoma Health Sciences Center

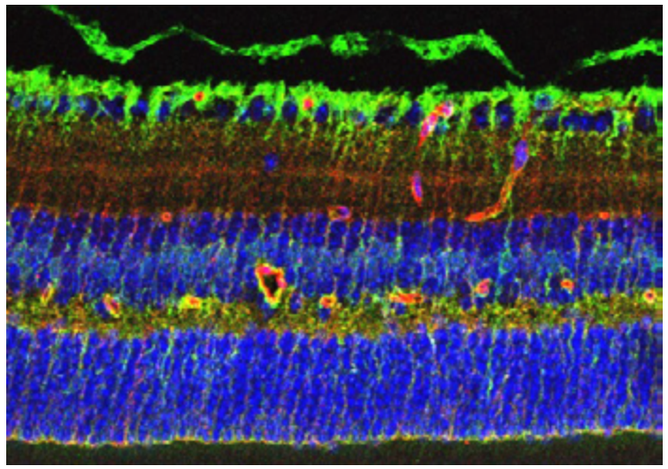
OCAST Project: HF18-008 **Research Area: Neurobiology**

Project Narrative

Short-term inflammation is the body's normal response to fighting infection. These destructive process are carried out by the immune system and are intended to fight foreign invaders. However, *long-term* (**chronic**) inflammation occurs with certain diseases and conditions and can damage healthy tissue like the **retina**. The retina is the tissue in the back of the eye that processes light and allows us to see the world around us. Because the retina resides in a unique location in the body, it is somewhat separated from the rest of the body's immune system. Thus, it is important for us to first understand how immune processes are different in the retina in order to develop better treatments for retina-related diseases that lead to vision loss. We think that a protein called "TRAF3" plays an important role in immune processes in the retina.



A picture showing the location of the retina, which is the delicate structure at the back of the eye that processes light into visual images.



A microscope image taken of the retina. We can label different molecules to "stain" the retina so that we can see how the retina is effected.

Recent Accomplishments

- We have developed a genetic mouse model that will test what happens to the retina when we remove TRAF3. This helps us understand the role TRAF3 plays in the retina.
- We have found that the TRAF3 protein is present at very high levels in the retina.
- We have found that the TRAF3 protein does play a role in immunity in the retina.
- We are continuing our work to understand the specific role (mechanism) TRAF3 plays in retinal immunity.

Understanding Chronic Pain: Is it All in Your Head?

Central Epigenetic Reprogramming of Amygdala Receptor Expression in Stress-Induced Chronic Pain

Beverley Greenwood-Van Meerveld, Ph.D. **HR18-040-1** **Neuroscience**

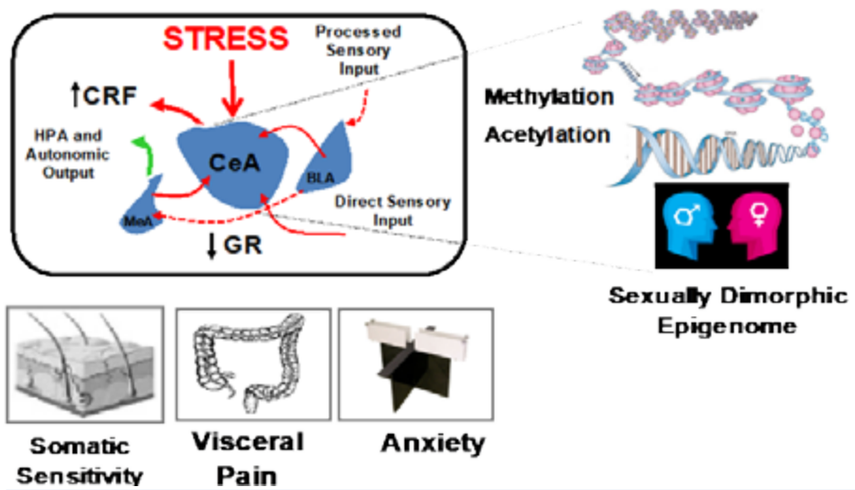
Project Highlights

Chronic pain, especially abdominal pain, is poorly managed and is a major cause of productivity loss in working Oklahomans. Stress is known to trigger or worsen abdominal pain. Our lab has previously shown that the amygdala (CeA), an important brain region for the processing of emotions, plays a pivotal role in chronic stress induced pain. Our recent preliminary data shows that short-term exposure to environmental enrichment (EE) reverses stress-induced abdominal pain in rats. However, **we need a better understanding of the mechanisms that underlie EE in order to translate our findings into new therapeutic options.** Therefore, we want to answer three important questions.

- 1) What are the molecular mechanisms within the CeA responsible for chronic pain?
- 2) Is this mechanism different in the CeA of females and does this explain why females are more susceptible to stress-induced abdominal pain?
- 3) Can EE be employed to target the underlying mechanism in the CeA to reverse chronic pain?

Taken together our studies will **determine whether key brain pathways and molecular mechanisms can be targeted to treat stress-induced chronic pain and to raise awareness of potential sex differences in the mechanisms underlying stress-induced chronic abdominal pain.**

Chronic abdominal pain is common in females and often worsened during episodes of stress.



Exposure to psychological stress produces persistent and sexually dimorphic epigenetic alterations in amygdala activity leading to chronic pain.

Recent Accomplishments

- We evaluated and optimized the short-term and persistent efficacy of EE on stress-induced visceral pain in both males and females.
- Experiments addressing the effect of EE on stress-related hormones and receptors in the emotional brain are in progress.
- We recently started to investigate whether the effects of psychological stress involve epigenetic mechanisms in the amygdala.

Cognitive behavioral therapy is a new approach to treat stress-induced abdominal pain in women.

Protein Finds A New Way to Protect Brain Cells from Ischemic Stroke

Unconventional Neuroprotective Actions of sAATF in Ischemic Brain Injury

PI: Qing Guo, University of Oklahoma Health Sciences Center

OCAST Project: HR16-074

Research Area: Neurobiology

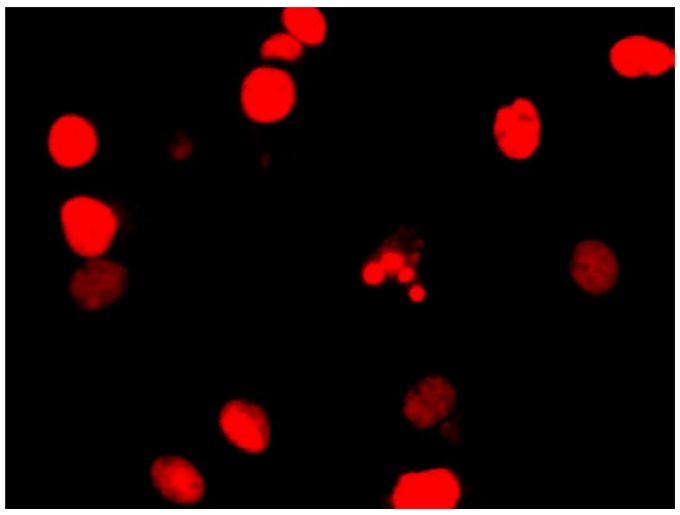
Project Narrative

Apoptosis antagonizing transcription factor (AATF) is commonly found inside the cell. However, we have unexpectedly noted that a significant amount of AATF was actively secreted extracellularly by brain cells under acute ischemic conditions. Secreted AATF (sAATF) confers potent neuroprotective actions. This extrinsic protective mechanism of sAATF is highly unusual because sAATF lacks a classical N-terminal signal peptide for extracellular secretion. The proposed work will use multidisciplinary approaches to investigate the mechanisms of AATF secretion and sAATF-mediated neuroprotection in models of ischemic stroke. The long-term goal is to provide sAATF as a new and long-lasting therapeutic agent for neuronal injury and to reduce the health care costs associated ischemic stroke.

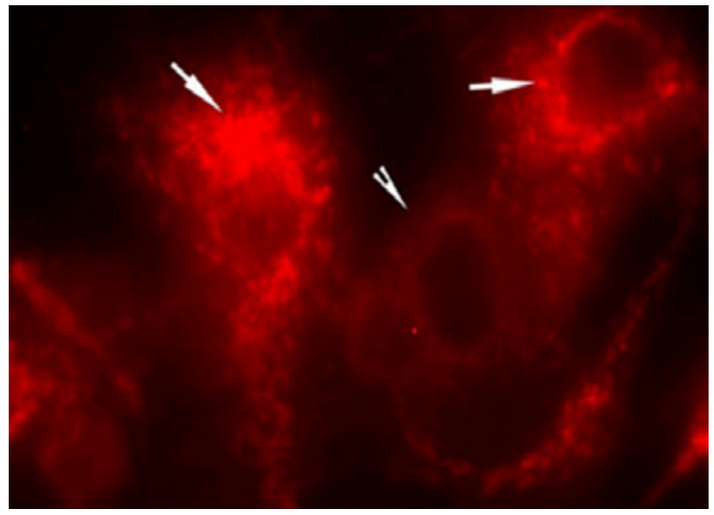
Recent Accomplishments

- TAT-SAP12, a small AATF peptide conjugated with a protein transduction domain, can be safely delivered across the blood-brain-barrier to the brain parenchyma after intraperitoneal injections.
- SAP-12 effectively blocks RIPK3/MLKL-mediated neuronal necroptosis after ischemic stroke in vivo.

Researchers in Guo lab examine markers of brain cell death and survival in ischemic stroke.



AATF transfected cells were largely rescued from apoptotic cell death induced by ischemia/reperfusion.



Fluorescently labeled siRNAs were introduced into the cells for gene expression studies.

Using menthol to study how the nervous system distinguishes bad from good

Taste and oral sensory processing in the brain

PI: Christian Lemon, University of Oklahoma OCAST Project: HR16-108 Research Area: Neurobiology

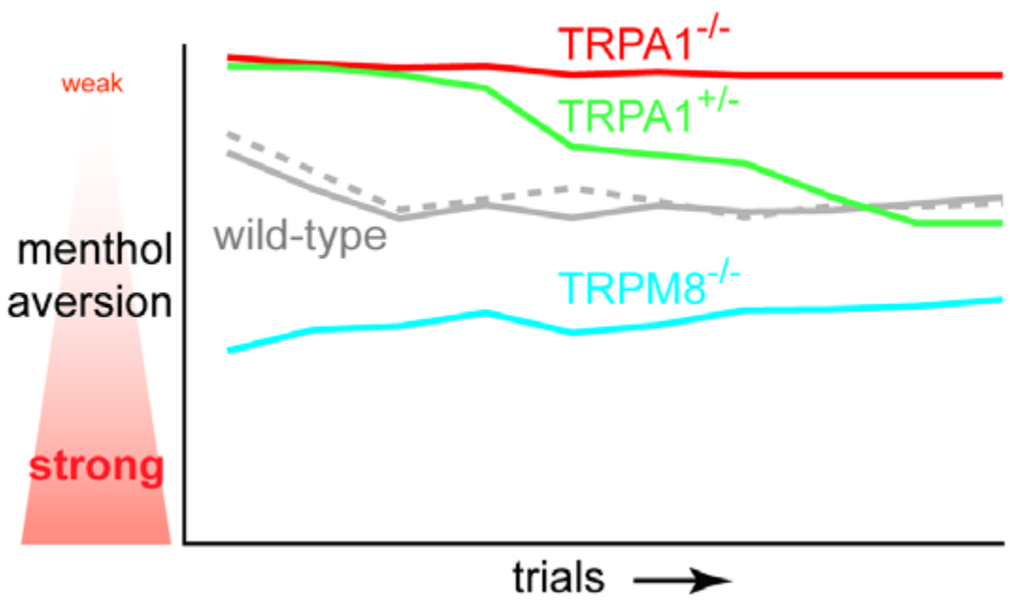
Project Narrative

Noxious stimuli and pain are sensed by somatosensory neural pathways that perform multiple functions, including detection of touch and thermal sensations. Yet how somatosensory neurons distinguish noxious from innocuous stimuli is not fully understood. Here, we developed a novel behavioral method in mice to study biological mechanisms mediating oral sensitivity to the somatosensory stimulus menthol, which elicits pleasant "coolness" at low but irritation and aversion at high concentrations. Using genetically engineered mice, we discovered that while menthol stimulates multiple types of somatosensory neurons, only a subset contributes to its aversive feature. These data add to our understanding of mechanisms of nociceptive signaling in the nervous system. Delineating this process will be important for the development of novel therapeutic approaches to mitigate pain.

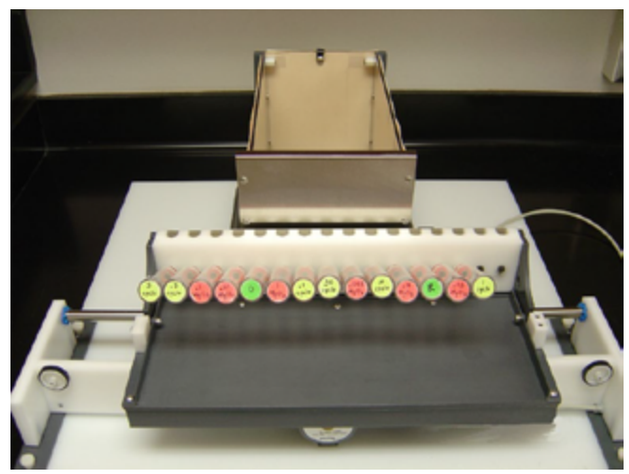
Menthol crystals and a mint candy

Recent accomplishments

- We have successfully developed an assay for measuring sensory-guided behaviors in mice to menthol – a "minty" flavoring agent and somatosensory stimulus that has both pleasant and aversive features.
- Using these assays and genetic manipulations, we have identified neural circuits mediating menthol aversion.
- Our results will support further studies on mechanisms of somatosensory and nociceptive signaling in the brain.



Genetic manipulation of molecules on neurons that respond to menthol and the impact on menthol aversion



Equipment for measuring sensory-licking responses to menthol-flavored solutions in mice

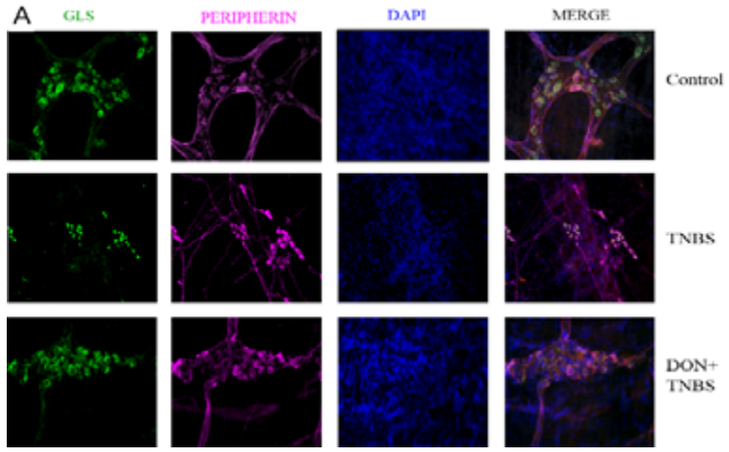
Glutamate Initiates Peripheral Neuroimmune Mechanisms In Colitis

The role of glutamatergic sensory neurons in the initiation of and response to colitis

Kenneth E. Miller, Oklahoma State University CHS **OCAST Project: HR16-003** **Neurobiology**

Project Narrative

Colitis is inflammation of the bowel and is listed as an inflammatory bowel disease. The nervous system's connection with the bowel is an important component of colitis, but our understanding is limited about nerves that initiate and maintain colitis or types of sensory nerve cells that are changed during colitis. We have determined that all sensory nerve cells use glutamate to communicate and release glutamate into tissue. We have shown that glutamate release in injured tissue is an important part of inflammation and that sensory nerve cells go through changes in glutamate related proteins during inflammation. Our central idea is: Glutamate release from sensory nerves contributes to the initiation and maintenance of colitis. We have three aims using a chemical induced model of colitis in rats during colon inflammation. Our aims include: 1. Glutamate starts immune mechanisms in colitis. 2. Colitis affects sensory nerve cells. 3. Nerve growth factor contributes to colon inflammation and alters nerve cells during colitis. Molecular and protein techniques will be used to explore these aims. The results from our studies will give insight about inflammation in the colon and how nerve cells are changed. These results can be important for the development of novel therapies for patients with colitis pain by regulating glutamate in the colon.



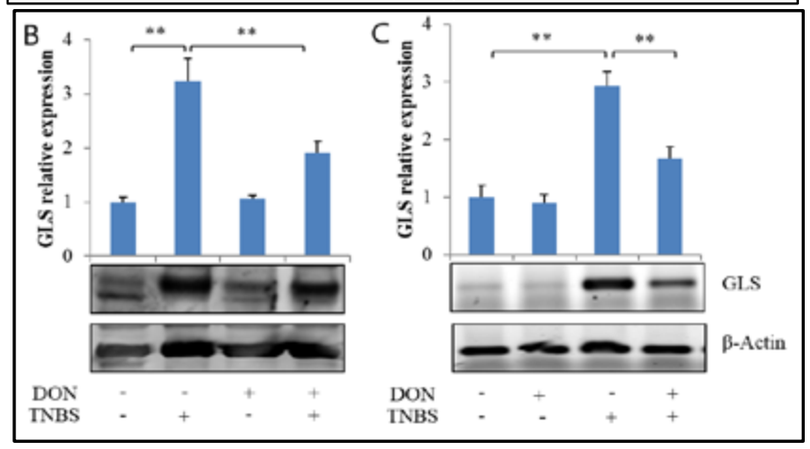
Myenteric ganglia stained with GLS (green), Peripherin, a neuronal marker (magenta) and DAPI for nucleus (blue). TNBS treatment disrupted myenteric ganglia and reduced the number of neurons per ganglia. DON pretreatment decreased TNBS-induced destruction of myenteric ganglia and the number of neurons compared to TNBS treatment.

Experimental Design

- Sprague-Dawley rats (6-8wks old) were given intracolonic infusion of 25% ethanol (control) or 5% 2,4,6-trinitrobenzene sulfonic acid (TNBS) in 25% ethanol to induce colitis.
- Some of the rats were pretreated (overnight) and co-treated with 6-diazo-5oxo-L-norleucine (DON), glutaminase (GLS) inhibitor.
- After 24hr, rats were euthanized by CO2 asphyxiation and colons were extracted for analysis.

Recent Accomplishments

- Colitis increases GLS expression in colon mucosal cells.
- DON reduces colon inflammation and GLS expression.
- Glutamatergic myenteric ganglia are disrupted by colitis while DON restores structural and cellular damage.
- This work indicates that GLS inhibition can be used for treating visceral pain and restoring neuronal plasticity in IBS patients.



B, GLS protein expression was decreased in DON pretreated colon compared to TNBS colitis. C, GLS mRNA levels were decreased in DON treated animals compared to TNBS treated animals. Beta actin was used for loading control in WB and used as an internal control in PCR.

Changes in clotting cells after concussion may lead to increased risk for stroke many years after the injury

Thrombotic and inflammatory mechanisms in traumatic brain injury

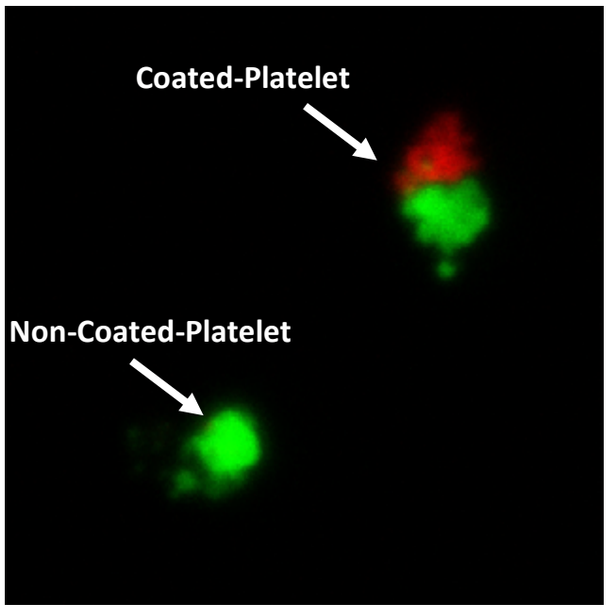
PI: Calin Prodan, MD, OUHSC

OCAST Project: HR19-111

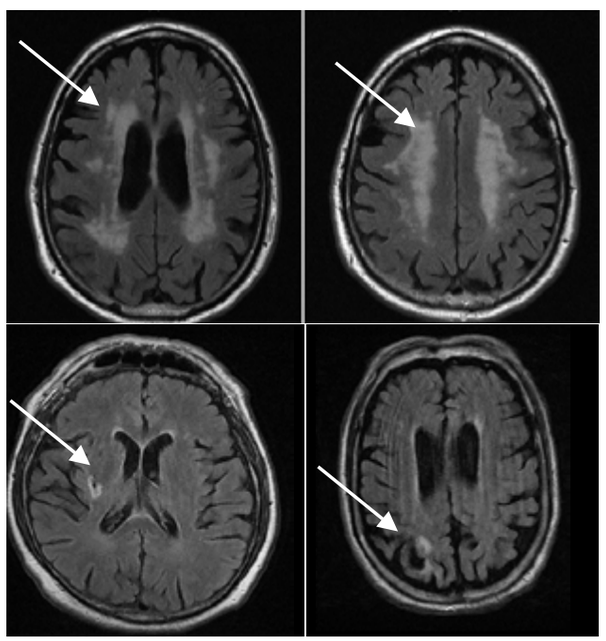
Research Area: Neurobiology

Project Highlights

Traumatic brain injury occurs in almost 2 million people in the United States each year; most of these cases are mild traumatic brain injuries (concussions). Although mild, these injuries are linked to a long-term increase in the risk for stroke later in life. We have found that patients with repeated concussions suffered during military service have increased levels of clotting cells (coated-platelets) many years after the last injury, and these levels were linked to increased inflammation. Among concussion patients, those with the highest levels of coated-platelets were most likely to have silent vascular changes (silent strokes) seen only on brain MRI scans. The proposed work will investigate how previous concussions lead to increased inflammation, higher levels of clotting cells and silent brain changes that predate stroke and its devastating effects. The results of the proposed work may lead to potentially new therapies to prevent stroke and help us understand how best to protect the brain in those at risk for concussion.



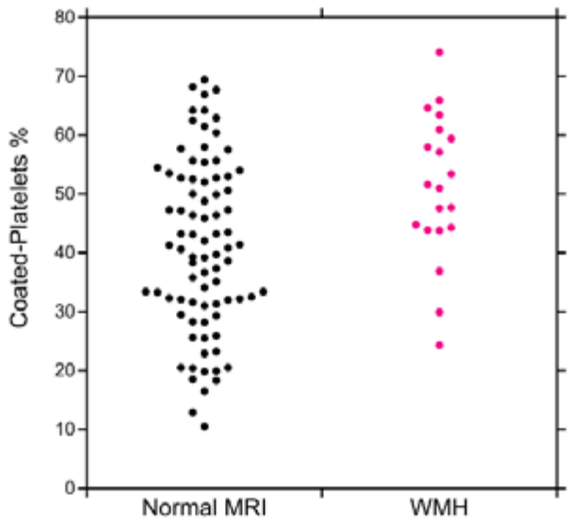
Confocal microscopy image of coated-platelets: green = platelet receptor, red = fibrinogen



Brain MRI scans with silent vascular changes

Recent Accomplishments

- We recently initiated the first year of our project, as a collaborative approach that involves the VA Medical Center, OUHSC and OU.
- We have developed specific research protocols that will allow our research team to access selected patients with documented concussions.
- We have finalized a protocol for obtaining repeat brain imaging with MRI scans in individuals with concussions and prior MRI scans.



Levels in patients with normal and abnormal MRI

PTSD and Chronic Pain: Inflammation Drives Pain Development

Post-traumatic stress disorder and Co-morbid chronic pain: Evidence that TNF initiates a sequelae involving Nociceptin/Orphanin FQ (N/OFQ)

Kelly Standifer, University of Oklahoma College of Pharmacy, OUHSC HR17-041 Neurobiology

PTSD produces long-lasting pain and increased pain sensitivity in 35% of patients. The severity of pain correlates with the severity of PTSD symptoms, making treatment of pain and PTSD symptoms more difficult and less effective. A common theme linking PTSD and these painful conditions is nervous system inflammation and altered mood. This is a significant health and economic problem for victims of natural disasters and violence as well as the many returning veterans, we were the first group to specifically study the problem of pain associated with PTSD experimentally. The Single Prolonged Stress (SPS) model of traumatic stress produces conditions and symptoms in rats similar to those reported in humans including anxiety, sleep disturbances, hyperarousal, depression and increased pain sensitivity.

Pain sensitivity, inflammation and mood are all modulated by a brain peptide, N/OFQ. Pain and anxiety-like behaviors associated with PTSD are reversed by blockade of the actions of N/OFQ. N/OFQ is increased by TNF in animal models of pain. We found that serum TNF levels increase within 4 hr of traumatic stress. Allodynia (or pain in response to a stimulus that is not normally painful, such as touch) appears 24-72 hr. Inhibiting TNF synthesis prevents increased serum TNF, allodynia and N/OFQ up-regulation. However, the ability of TNF to activate this process and increase N/OFQ has not been tested directly. We hypothesize that increased serum TNF in response to traumatic stress activates pain sensors in the body to produce pain, mediates anxiety-like symptoms and increases N/OFQ. Investigating the mechanisms by which TNF regulates N/OFQ following PTSD will provide critical information for development of new therapeutic agents for pain and other conditions in which N/OFQ is involved (Parkinson's Disease, depression, anxiety, traumatic brain injury, addiction).

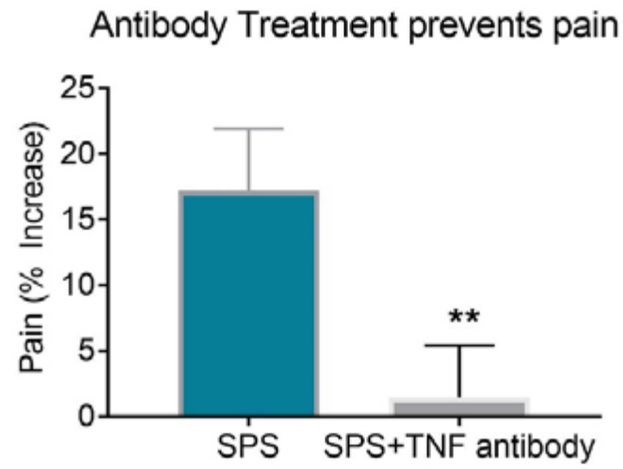
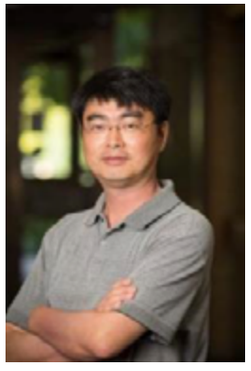


Fig. 1. Anti-TNF antibody injected on day of SPS prevented development of traumatic stress-induced allodynia. N=6 per group.

Recent Accomplishments

- Treatment with an antibody against TNF shortly after exposure to trauma prevents development of pain, anxiety-like symptoms and elevated N/OFQ.
- TNF injection (iv) alone produces acute, transient, pain, but no changes in anxiety or N/OFQ.
- TNF+trauma exacerbates and hastens appearance of pain.



This work was performed by Dr. Yong Zhang

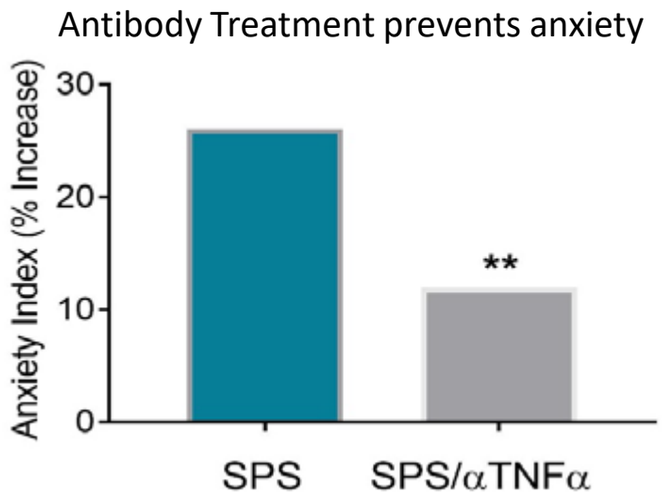


Fig. 2. Anti-TNF antibody injected on day of SPS prevented development of traumatic stress-induced anxiety behaviors. N=6 per group.

Understanding brain-based causes of attention-deficit/hyperactivity disorder (ADHD): Memory input method matters.

Neurocognitive Deficits Underlie ADHD

PI: R. Matt Alderson, Oklahoma State University **OCAST Project: HR17-051-3** **Research Area: Psychology**

Project Highlights

Attention-deficit/hyperactivity disorder (ADHD) is a complex, chronic, and potentially debilitating disorder of brain, behavior, and development that affects 3-5% of school-age children at an annual U.S. cost of illness of over $36 billion. Current interventions, including behavioral therapy and stimulant medication (e.g., Ritalin), are effective at reducing immediate difficulties but do not produce lasting benefits after they are discontinued. Recent research has sought to discover underlying neurological causes ADHD symptoms (i.e., inattention, hyperactivity, and impulsivity), with hopes that identifying such causal mechanisms will ultimately lead to improved treatment approaches and less dependence on stimulant medication. ADHD-related deficits of working memory – the ability to temporarily store and manipulate information in one's mind – have garnered attention as a promising target for research and intervention. This project study seeks to examine a specific aspect of working memory (i.e., the "episodic buffer") that has not yet been well-studied, but holds considerable promise to inform future intervention and educational techniques.

Example trial from memory task: Children must identify if the probe (the red swatch in this example) is the same color, shape, or color and shape.

Clinical psychology doctoral student conducting statistical analyses of preliminary data for publication and research presentations.

Clinical psychology doctoral students preparing the memory task experiment for the next child participant.

Recent Accomplishments

- Year 2 of the study focused on recruitment of participants, data collection, and data entry. These goals were all met.
- Preliminary analyses were conducted on a subset of the data, resulting in 4 papers (3 published, 1 under review) and 5 presentations at national conferences (i.e., the annual meetings of the American Psychological Association, the Society for Research in Child Development, and the Association for Behavioral and Cognitive Therapies).
- Additional data collection and preliminary analyses are ongoing, consistent with proposed goals of the project.

Dads and the Development of Infants in Oklahoma

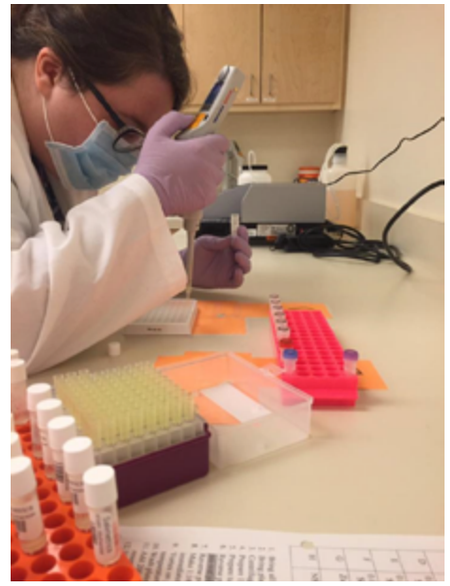
Family Hormonal Profiles of Resilience: Defining Fathers' Roles in Infant Biosocial Development

PI: Jennifer Byrd-Craven, Oklahoma State University **OCAST Project: HR17-003** **Research Area: Psychology/Public Health**

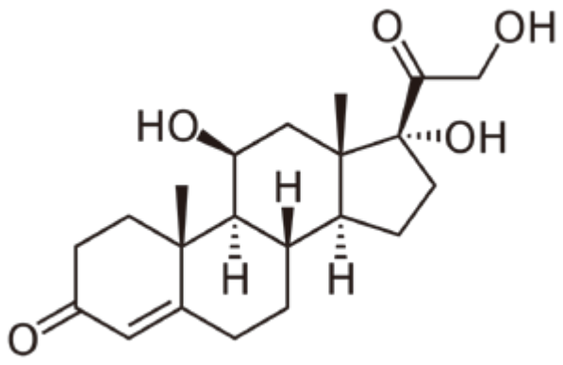
Project Narrative

Fathers play an important role in infant development, yet the nature of their support varies widely across individuals and contexts. Fathers contribute directly to their children through caregiving and skill development, and contribute indirectly through support of the infant's mother. Mothers who receive more social support have infants with better health outcomes than mothers without this support. Past research, however, fails to distinguish the effects of social support from different family members on infant outcomes. We know even less about families faced with economic and social adversities. One emerging indicator of infant cognitive, emotional, and health outcomes is mother-infant stress response system coordination. Greater similarity in stress system activity between mothers and infants is related to responsive caregiving and positive infant learning outcomes. Fathers may contribute to infant and child development by promoting mother-infant bonds, as indicated by stress system coordination, and reduced inflammation. In addition to stress hormones, a number of other hormones are involved in parenting behavior specifically, such as testosterone and progesterone.

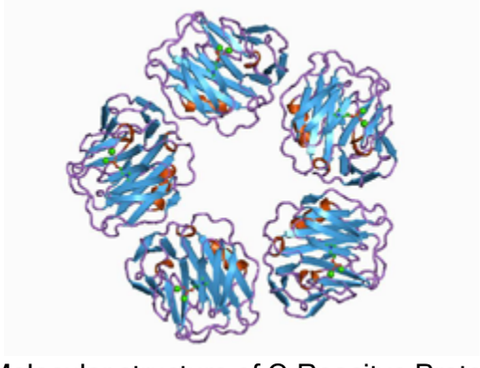
Currently, we know little about fathers' pattern of hormonal changes, or hormonal profile, in response to caregiving activities, or how it affects the pattern of changes within the family. This longitudinal study will identify patterns of father involvement associated with positive and negative outcomes for mother-infant dyads during the first 18 months of life. We will recruit families with and without fathers present from across central Oklahoma, and will focus recruitment on disadvantaged populations. We will observe families' interactions when infants are 4, 12, and 18 months old. Before and after these interactions, we will collect saliva for stress hormones, markers of inflammation, and hormones related to parenting. We will also assess infant health and development monthly. This will allow us to determine if the hormonal profile of the family is related to parent-infant interactions and infant health and development. With a more complete understanding of fathers' contributions to mother-infant stress system coordination, findings may inform intervention programs focusing on optimizing support for the mothers and their infants across a diversity of family contexts.



Nikki Clauss, graduate research assistant, processes saliva samples for cortisol levels



Molecular structure of cortisol, released in response to stress, and one of the primary hormones of focus



Molecular structure of C-Reacitve Protein (CRP), a marker of inflammation

Recent Accomplishments

- **We have completed sample recruitment and have begun analyzing data from 4-month olds**
- **Preliminary results show that fathers' testosterone responses are related to mother-infant cortisol coordination. Further, preliminary results suggest that mother's social support is associated with lower infant cortisol responses.**

Insomnia, Post-Trauma Nightmares, and Suicide Risk

CBT-I versus ERRT: Impact on Sleep, Nightmares, and Suicidal Ideation

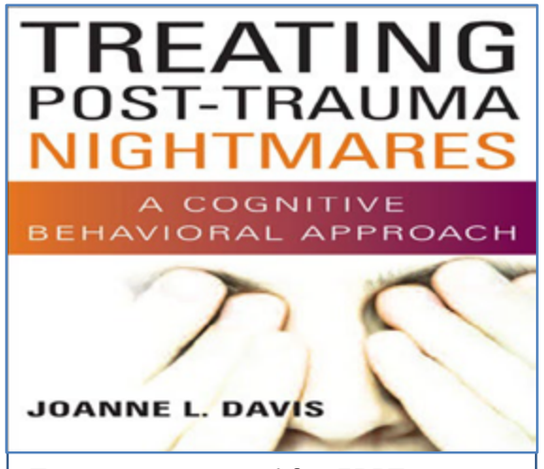
PI: Dr. Joanne L. Davis, University of Tulsa **OCAST Project: HR17-087** **Research Area: Nutrition/Psychology/Public Health**

Project Narrative

Oklahoma has the 13th highest suicide rate in the country and suicide is the 7th leading cause of death in the state. Research finds an association between suicidality and sleep disturbances and nightmares. While many studies have found that sleep disturbances in general are related to suicidality, there appears to be a unique relationship between the experience of nightmares and suicidality. Currently there are two evidence based sleep treatments that have shown to be effective in improving nightmares, sleep quantity and quality, and related psychopathology: Cognitive Behavioral Therapy for Insomnia (CBT-I) and Exposure, Relaxation, and Rescripting Therapy (ERRT). We are interested in examining whether treating insomnia and nightmares may lead to a reduction of suicidal ideation.

TiTaN
THE UNIVERSITY OF TULSA INSTITUTE OF TRAUMA, ADVERSITY, & INJUSTICE

TITAN is the organization providing the space for this research trial.



Treatment manual for ERRT

Recent Accomplishments

- Extensive recruitment efforts have been made in the Tulsa area.
- Nine participants have been randomized to receive either CBT-I or ERRT treatment. Five of them have completed treatment.
- Preliminary results have found that both of these treatments reduce the severity of suicidal ideation by 40%.

SLEEP STRONG

The collective phrase used to describe the ongoing nightmare research conducted at The University of Tulsa.

Brain activity can tell researchers what leads to chronic anxiety.

Neural Mechanisms of Chronic Worry

PI: DeMond Grant, Oklahoma State University

OCAST Project: HR16-023

Research Area: Psychology

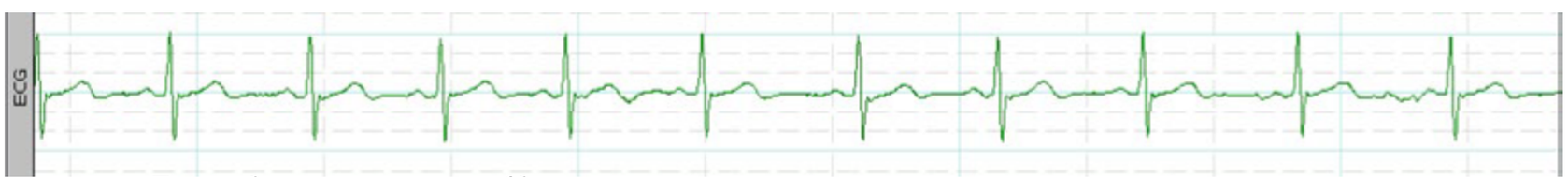
Lay Abstract

Anxiety is a normal emotional reaction, characterized by physical sensations (increased heart rate) and negative thoughts. However, for some individuals worry can become chronic and uncontrollable. Worry interferes with our daily activities, such as taking a test, solving problems, and falling asleep. People who experience excessive worry and anxiety also are at increased risk for medical problems, such as heart attacks and gastrointestinal problems. Therefore, research is needed to help us understand what leads to this chronic and prevalent health condition.

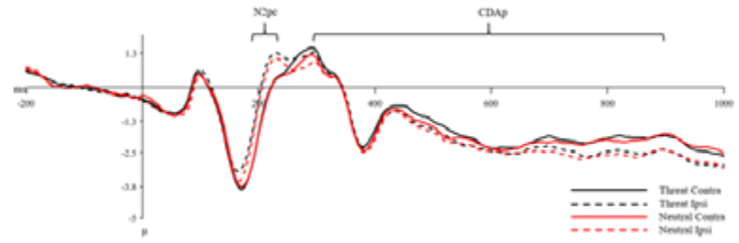
Although we know the consequences of excessive anxiety, no research has found what causes worry. This study examined how anxiety sensations affect our brain, leading to uncontrollable worry. We are measuring brain activity using EEG to directly assess what triggers negative emotional reactions. Because worry increases the likelihood of developing both psychological and medical conditions, this research will be highly important to millions of people in the United States.

Recent Accomplishments

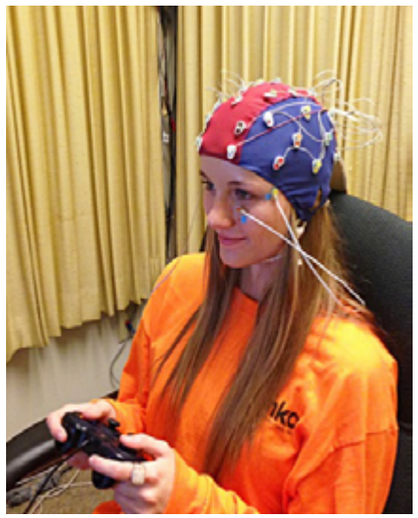
- One manuscript evaluates how worry affects indicators of threat such as anxiety sensations
- Results indicated that chronic worriers have difficulty suppressing threatening stimuli, even when unrelated to ongoing tasks



Example measurement of heart rate



Preliminary Error-Related Negativity waveform indicating increased processing of errors



Natalie Braden, a research assistant, wearing an EEG cap

Quality of Life and Digital Hearing Aids

Health-related Quality of Life Benefits from Advanced Digital Technology Hearing Aids

Carole E Johnson, PhD, AuD, PI **Grant# HR 16-118**

Hearing Evaluation, Rehabilitation, and Outcomes (HERO) Laboratory; Department of Communication Sciences and Disorders; College of Allied Health; University of Oklahoma Health Sciences Center, Oklahoma City, OK 73117

Lab personnel: AM Jilla, AuD (Lab Manager); JH Park, BS; JJ Huddleston, C Wilkerson, M Donnell, & K Rock

Consultants: H Abrams, PhD; JL Danhauer, PhD

Project Narrative

Only ~1 person in 5 with sensorineural hearing loss (SNHL) pursues amplification, possibly due to the high cost of advanced digital technology (ADT) hearing aids (HAs). The NIDCD-NIH is striving to increase accessibility and affordability of hearing healthcare for patients with mild to moderate SNHL. Research needs to focus on patient populations, hearing technology, and service-delivery models. We are conducting a 3-yr randomized clinical trial (RCT) (baseline vs. 2 mo) in addition to a longitudinal, cross-sectional study (baseline, 2 mo, 6 mo, and 1 yr follow-up) on the health-related quality of life (HRQoL) benefits of entry-level ADT HAs dispensed to adults with low incomes primarily with mild to moderate SNHL. Our primary clinical endpoint is the World Health Organization Disability Schedule II (WHO-DAS 2.0, 2012) and the secondary measure is Hearing Handicap Inventory for the Elderly (HHIE: Ventry & Weinstein, 1982). 86 participants (43 control; 43 treatment group) are needed for 80% power (effect size of 0.62) to detect a difference in change of the WHO-DAS 2.0 total score between participants randomized to either treatment (HAs) or waiting-list control groups. Those in the waiting-list control group are fit with ADT HAs after the RCT component. All participants are being followed for 1 yr with outcomes measured at 2, 6, and 12 mo after the hearing aid fitting. We have recruited nearly all of our participants through mo 10 of project yr 3. So far, the results of our project indicate that entry-level ADT HAs improves the HRQoL of unserved and underserved citizens with SNHL within the state of Oklahoma.

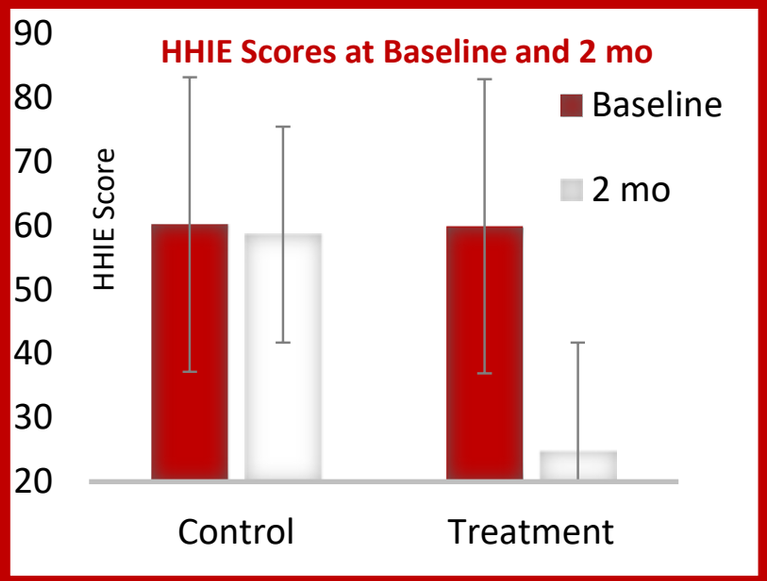


Figure 1. The treatment group fit with ADT HAs showed a significant reduction in participation restriction (improvement in HRQoL) on the HHIE measured whereas those in the waiting-list control group did not

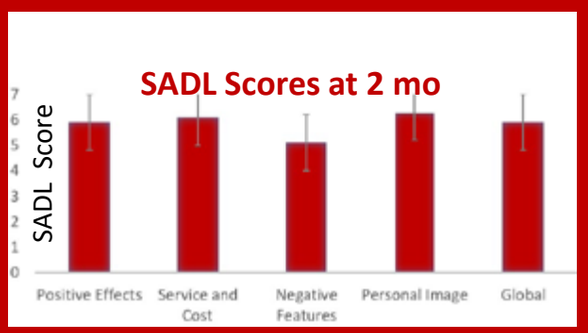


Figure 2. HA wearers were satisfied with their devices at 2 mo post-fitting on the Satisfaction with Amplification in Daily Life scale (SADL) (1 = low; 7 = high)

Untreated SNHL results in a reduction of HRQoL

Recent Accomplishments

- Presented preliminary results at the American Academy of Audiology Meeting in Nashville, TN
- Won best poster for Excellence in Amplification and Assistive Devices

Determining if a firefighter is fit-for-duty

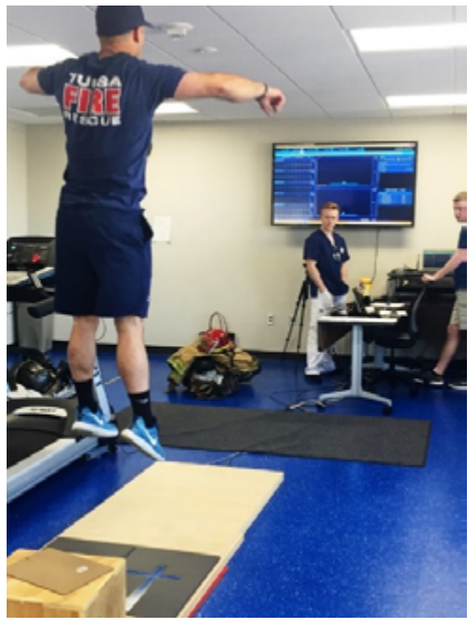
Fit-for-duty: An Examination of the Efficacy of the Physical Abilities Test in Determining Physical Readiness

PI: Roger Kollock, The University of Tulsa **OCAST Project: HR18-054** **Research Area: Nutrition/Psychology/Public Health**

Project Highlights

Data from the U.S. Bureau of Labor Statistics indicates that firefighters have a nonfatal casualty rate of approximately 520 and 664 per 10,000 full-time local and state firefighters, respectively. Reports also indicate fatality rate of approximately 35 fatalities for every 100,000 full-time firefighters. To help minimize the risk of fatal and nonfatal casualties, local municipalities often require firefighters complete an annual physical abilities test (PAT) to determine if they are fit-for-duty. The PAT used by the Tulsa Fire Department is a modified version of the nationally recognized candidate physical abilities test (CPAT). The PAT as conducted by the Tulsa Fire Department consists of seven separate events performed in sequence. These events include: stair climb, hose drag, search maze, rescue, forcible entry, ladder raise and extension, ceiling breach and pull. The original CPAT was developed to help fire departments identify pools of trainable candidates. However, to date there is no evidence suggesting that passing the CPAT or modified PAT is a valid indicator of physical readiness and or an indicator of casualty risk in incumbent firefighters. Thus, a "need" exists to explore the relationship of the PAT to scientific based measures of physical fitness. Evidence obtained from this project will help support the ongoing measures to enhance physical readiness evaluation methods, reduce the overall casualty rate, and reduce the number of worker compensation claims for the City of Tulsa and provide valuable insight to surrounding municipalities.

Firefighter performing a WFI stepmill test of aerobic capacity



Firefighter performing a vertical jump

Recent Accomplishments

- We have completed testing on the 1st cohort of participants (N=19)
- We have recently presented our preliminary findings at the National Athletic Trainers' Association 70th Annual Meeting and Clinical Symposium, Las Vegas, NV, June 2019
- 3 scientific abstracts were accepted for poster presentation at the Central States American College of Sports Medicine (ACSM) Annual Conference, Broken Arrow, OK, October, 24-25, 2019

Firefighter performing a single leg landing test to assess dynamic balance

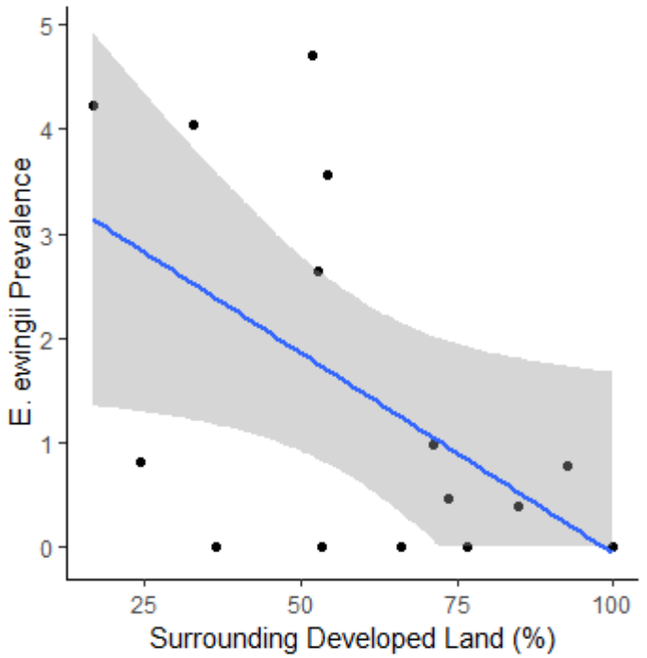
What's my risk of getting a tick-borne disease in an urban area?

Local and landscape-scale drivers of tick distribution and tick-borne pathogen prevalence in rapidly expanding urban and suburban areas

PI: Bruce Noden, Oklahoma State University **OCAST Project**: HR16-038 **Research Area**: Public Health

Project Narrative

Most of earth's population lives in urban areas, and an urban lifestyle changes how different species, including humans, insects, and wildlife, thrive and develop. These changes often lead to increases in infectious diseases, including diseases transmitted by arthropods (ticks, mosquitoes, fleas). No study has focused on how urban environments affect tick populations in a major US city. Our results indicate there is a risk of encountering ticks and pathogens across the entire urbanization gradient from exurban areas to the urban core, although some tick species and pathogens (*Dermacentor variabilis* and *Ehrlichia ewingii*) may be less common in intensely urbanized areas. Our results also suggest that birds are important dispersers of ticks within urban landscapes and that summer resident birds and migrant birds during more sedentary periods of their annual cycle may be particularly vulnerable to tick infestations. The information from this study will increase awareness about ticks and tick-borne diseases in U.S. cities, help public health officials to educate urban residents about avoiding tick exposure, and focus resources on the urban 'hot spots' where the risk of transmitting tick-borne disease is especially high.

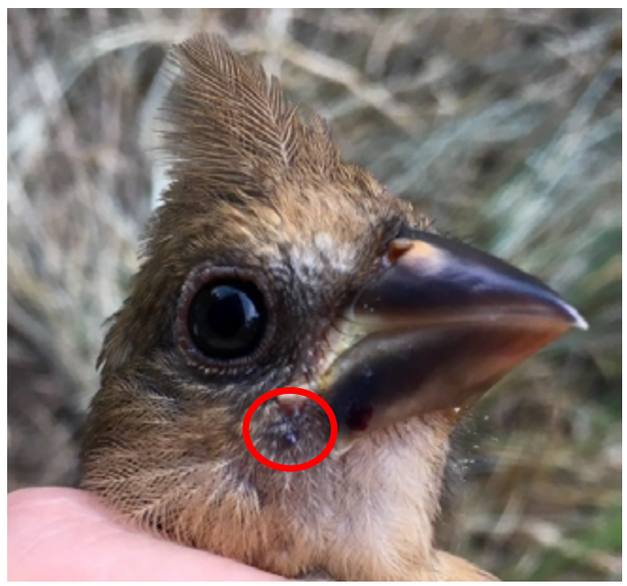


A linear regression (with 95% CI). of *Ehrlichia ewingii* prevalence at all sites.

American dog tick (top) and Gulf Coast tick (bottom), two of the three tick species collected

Recent Accomplishments for Completed Study

- In 2017 and 2018, we collected 10,299 ticks of 3 species in 16 different Oklahoma City parks.
- We collected 322 ticks off 495 individual birds of 21 different species.
- We found *Ehrlichia chaffeensis*, *E. ewingii*, Panola Mountain Ehrlichia, *Borrelia lonestari*, *Rickettsia parkei*, *R. rhipicephali*, '*Candidatus* R. andeanae' in ticks collected, but no Tularemia spp.
- We presented our preliminary findings at both national and regional conferences.



A tick (circled) found on the bill of a Northern Cardinal

The Oklahoma Study of Native American Pain Risk, Part 2 (OK-SNAP II)

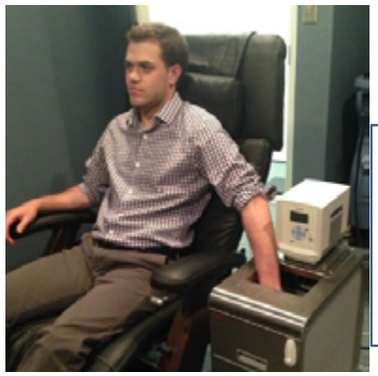
Does Glucose Dysmetabolism Contribute to Native American Pain Disparities?: A Pilot Study

PI: Jamie Rhudy, PhD, The University of Tulsa **OCAST Project: HR18-039** **Research Area: Psychology**

Project Narrative

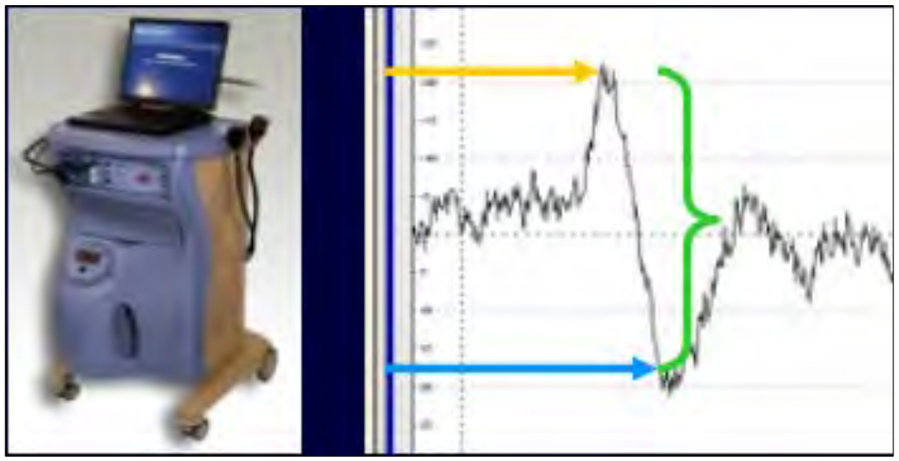
Native Americans (NAs) have a higher prevalence of chronic pain than any other U.S. racial/ethnic group, yet there have been few attempts to understand the mechanisms contributing to this disparity. Diabetes also disproportionately affects this population. One of the many complications of diabetes is neuropathy (nerve damage) that affects peripheral and central nerves associated with pain and its regulation. Some of these neurotoxic effects can even occur in response to subclinical glucose dysmetabolism (eg, prediabetes). Consistent with this, our preliminary data on healthy, pain-free, non-diabetic participants found a relationship between diabetes risk factors and pain promoting processes. Specifically, we found that participants with ≥3 diabetes risk factors had: 1) deficits in peripheral small fibers that carry pain signals, 2) enhanced central sensitization (spinal hyperexcitability), 3) impaired descending pain inhibition (inability to dampen pain), and 4) decreased pain tolerance (enhanced pain sensitivity). Most in this high risk group were NAs suggesting this pain risk pathway may be particularly problematic for this population. We hypothesize these effects stem from individual differences in glucose dysmetabolism, but this can only be inferred because glycemic status (eg, HbA1c) was not measured. Thus, the relationship between glucose dysmetabolism and pain risk in NAs needs to be directly studied in persons with verified variation in glycemic status. The aim of this study is to determine the influence of glucose dysmetabolism on markers of pain risk in NAs. Testing will use state-of-the-art quantitative sensory testing methods that assess all levels of the nervous system (peripheral fibers, central sensitization, pain inhibitory processes, pain perception). This research is expected to impact minority health disparities in several ways: 1) identify mechanisms that contribute to NA pain disparities, 2) provide evidence that NAs have a unique pain risk pathway (eg, hyperglycemia) that warrants a tailored intervention approach, and 3) lay the groundwork for interventions that can be implemented prior to diabetes onset when they may be more effective in eliminating neurotoxicity and pain risk.

A sensor is applied to the back of the leg to record a marker of central sensitization (spinal cord hyperexcitability).



Pain perception is assessed from a painfully cold circulating water bath

Pain inhibitory processes are tested using a “pain inhibits pain” paradigm



Peripheral A-delta fibers are being assessed from contact heat evoked potentials evoked from the distal leg

Accomplishments:

- The project began Aug 1, 2018
- Data collection started March 2019
- To date, out of 145 interested individuals, 18 were eligible for screening and 10 individuals have completed testing

Increasing mothers' connection to their babies to help them to be healthier

Enhancing Maternal-Fetal Bonding to Promote Healthy Pregnancies and Reduce Adverse Perinatal Outcomes

PI: Karina M. Shreffler, Oklahoma State University ***OCAST Project: HR19-129*** ***Research Area: Health Research***

Project Highlights

The study focuses on rapidly increasing maternal-fetal bonding, a mediator of the relationship between unintended pregnancy and maternal health behaviors during pregnancy, through a recently piloted two-week intervention, BLOOM (**B**abies and **M**oms, connected by **L**ove, **O**penness, and **O**pportunity). Using a randomized clinical trial design in a longitudinal, multi-ethnic cohort study of 160 girls and women (ages 15-29), participants are randomly assigned into one of four groups for the intervention. The intervention examines the effectiveness of attachment-focused text exercises and fetal Doppler monitors for enhancing maternal prenatal attachment.

Impacts of prior interventions to reduce unintended pregnancy and its associated adverse birth outcomes have been modest; therefore reducing the negative association between unintended pregnancy and adverse outcomes through enhancing maternal prenatal attachment makes this project highly significant. This project will be the first to target maternal-fetal bonding through the use of inexpensive fetal Doppler monitors, making it highly innovative. The positive impacts of this work include information that will be used to reduce the impact of unintended pregnancy for adverse infant health outcomes.

PI Shreffler trains a GRA on enrollment and survey procedures

Research lab members swab a baby's cheek.

Recent Accomplishments

- Survey planning complete and programming of assessment 1 complete and currently undergoing review.
- GRAs have undergone human subjects, recruitment, informed consent, and participant payment management trainings.

BOOM: **B**abies and **M**oms connected by **L**ove, **O**penness, and **O**pportunity

Understanding Difficulties with Regulating Emotions

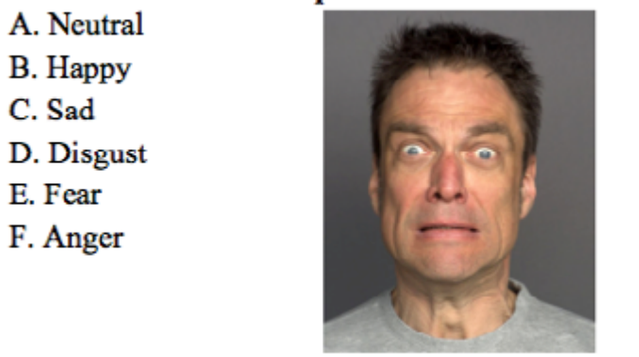
Identifying a Direct Path to Emotion Dysregulation in Borderline Personality

PI: Stephanie N. Mullins-Sweatt, Oklahoma State University **OCAST Project Number: HR-18-079** **Research Area: Psychology**

Project Narrative

Emotion dysregulation (ED) in individuals with borderline personality traits is relevant to public health because ED is directly related to significant and serious negative health outcomes, such as suicide, substance misuse, and risky sexual behavior. Understanding ED is necessary in order to inform effective interventions targeting these negative outcomes. While there is a marked association between ED and detrimental consequences, the biopsychosocial mechanisms by which components of ED interact to produce negative outcomes and psychiatric disorders are unclear. Given that ED is a core feature of borderline personality disorder (BPD), delineation of the pathway from ED to negative outcomes within BPD is necessary in order to inform efficacious interventions aimed at regulating affect within BPD. The long-term goal of this study (Components of Regulating Emotion; CORE) is to identify the route wherein ED produces significant negative health outcomes, in order to inform targeted treatments. The first required step to identify the pathways of this model is to test the direct route from emotion sensitivity to heightened negative and unstable affect. CORE will provide evidence for the path from emotion sensitivity to unstable affect within ED. Identification of this path will inform subsequent hypotheses isolating related components of the ED model within individuals with borderline personality traits. This contribution is significant, as it will begin to address a specific path of ED that has yet to be tested, with an ultimate goal of understanding the mechanisms that translate ED into significant negative outcomes. This long-term research goal can then contribute to the identification and implementation of interventions targeting the detrimental process of ED. This research will broadly impact the field of psychopathology by gaining a precise understanding of the pathway to ED.

Facial morphing sensitivity task examples



Identification of prototypic emotions example

Recent Accomplishments

- The focus of Year 1 was identification of graduate student research assistants, development and programming of the Emotion Discrimination Task, recruitment of 41 participants, data collection, and data entry. These goals were all met.
- We successfully recruited 42 individuals in Year 1. Forty-one individuals completed Sessions 1 and 2 as well as the EMA and EDT portions of the study.
- Participant recruitment is ongoing, consistent with the proposed timeline of the project.

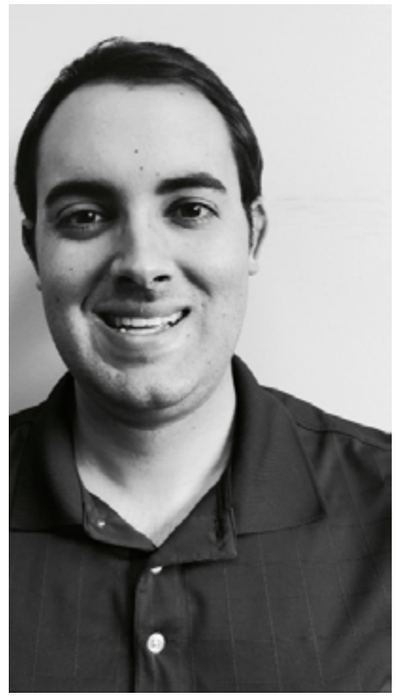
Addiction studies are not reproducible and many fail in the early stages

An Attempt to Reproduce Systematic Reviews in Addiction Science

Matt Vassar, Oklahoma State University CHS HR18-119-2 Psychology

Project Highlights

Systematic reviews are a highly regarded methodology because they tend to be systematic and reproducible. Nevertheless, actual attempts to replicate systematic reviews remain sparse. Koffel and Rethlefsen evaluated the reproducibility of systematic review search strategies and found that only 22% of systematic reviews provided a reproducible search strategy for a single database and only 13% provided a reproducible strategy for all databases (Koffel & Rethlefsen, 2016). Furthermore, the reporting of search elements differed greatly by discipline. The findings from this study provide a valuable foundation for additional work on systematic review reproducibility; however, this study was limited to evaluating reproducibility based on reported search items identified as important in the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) statement. The process of conducting systematic review searches likely contributes additional factors that influence their reproducibility, and they would remain unknown from descriptive accounts alone. Furthermore, the inclusion of a single year in this study limits our understanding of reproducibility over time. Page et al evaluated the reproducibility of meta-analyses from published systematic reviews and determined that 66% reported the data necessary to reproduce the meta-analytic effect estimates and subsequent subgroup or sensitivity analyses (Page et al., 2017). Koffel and Rethlefsen and Page et al. limited their evaluations to specific components of the systematic review process, and neither provided a comprehensive assessment of systematic review reproducibility.



Recent Accomplishments

We have completed data collection for 10 systematic reviews in addiction science.

We will be writing a manuscript to submit for publication based on our findings.

Project Team: Chase Meyer, Andrew Ross, DO, and Cole Wayant.

Investigating the Relationship between Environmental Exposures and Cancer in Oklahoma

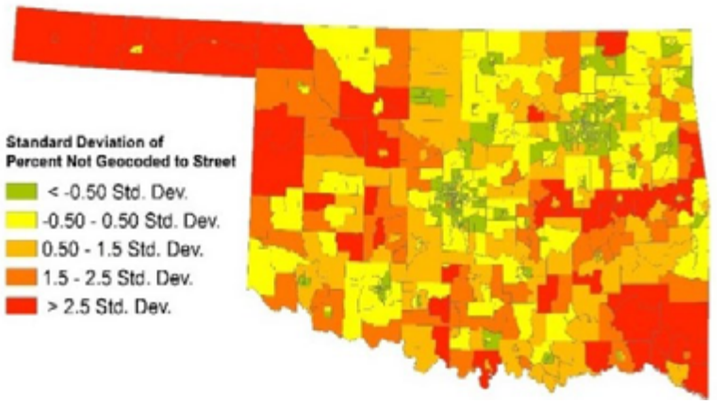
Improving Geocoding of Cancer Registry Data and Development of a Spatiotemporal Database of Environmental Exposures

Michael C. Wimberly, U. of Oklahoma

OCAST Project Number: HR16-048 Research Area: Public Health

Project Highlights

- Oklahoma has the 11th highest age-adjusted cancer mortality rate in the US.
- It is important to have accurate address data to understand potential environmental and behavioral risk factors for cancer.
- Development of an environmental database provides a single location for multiple types of environmental contaminants to facilitate health research.
- By better understanding the distribution of cancer in Oklahoma, we can work with policy makers to enhance prevention and screening areas in high-risk locations and populations.



Distribution of Oklahoma Central Cancer Registry cancer cases not geocoded to the street level

Theme	Total
Administrative	4
Air	15
Industrial	3
Land	7
Physical Characteristics	31
Water	53
Total	**113**

Data items by theme in the environmental exposure database[1]

Recent Accomplishments

- Completed an environmental exposure database for Oklahoma[1]
- Completed geocoding Oklahoma Central Cancer Registry and University of Oklahoma Central Cancer Registry Geocoding (manuscript in preparation)
- Next steps: obtain data on residential history and conduct descriptive analyses

Among records not previously geocoded to the street level, we improved geocoding of Oklahoma Central Cancer Registry records by 40%

1. Dilekli N, Gopalani SV, Campbell JE, Janitz AE. A geospatial environmental concentrations database of Oklahoma, United States. In Press at Data in Brief, August 2019.

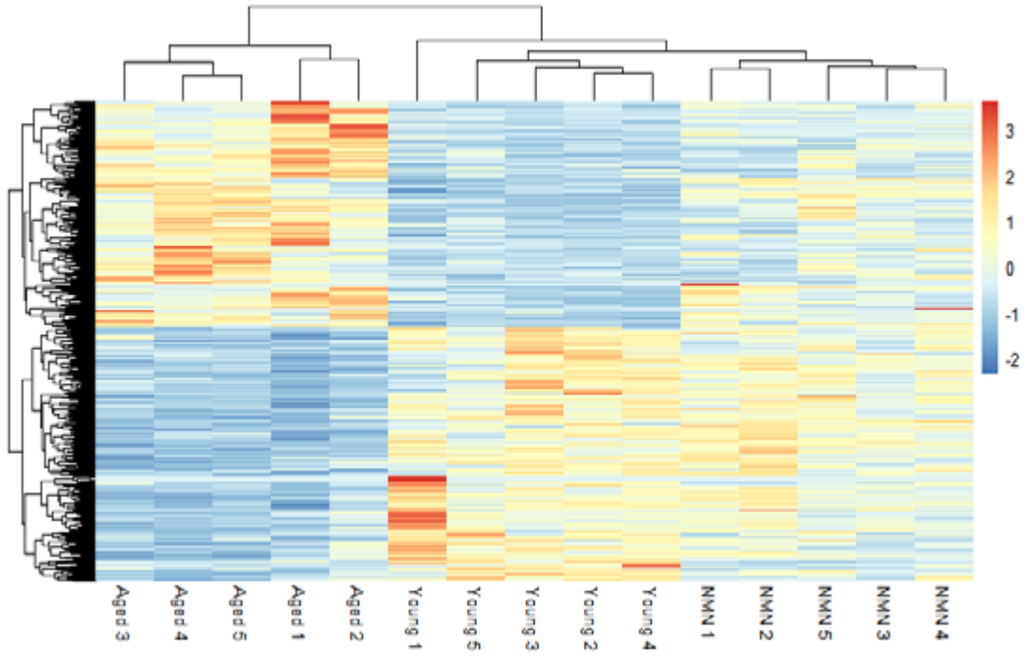
NOVEL MECHANISM OF AGE-RELATED CEREBROMICROVASCULAR DYSFUNCTION

PI: Anna Csiszar, MD, PhD
University of Oklahoma Health Sciences Center

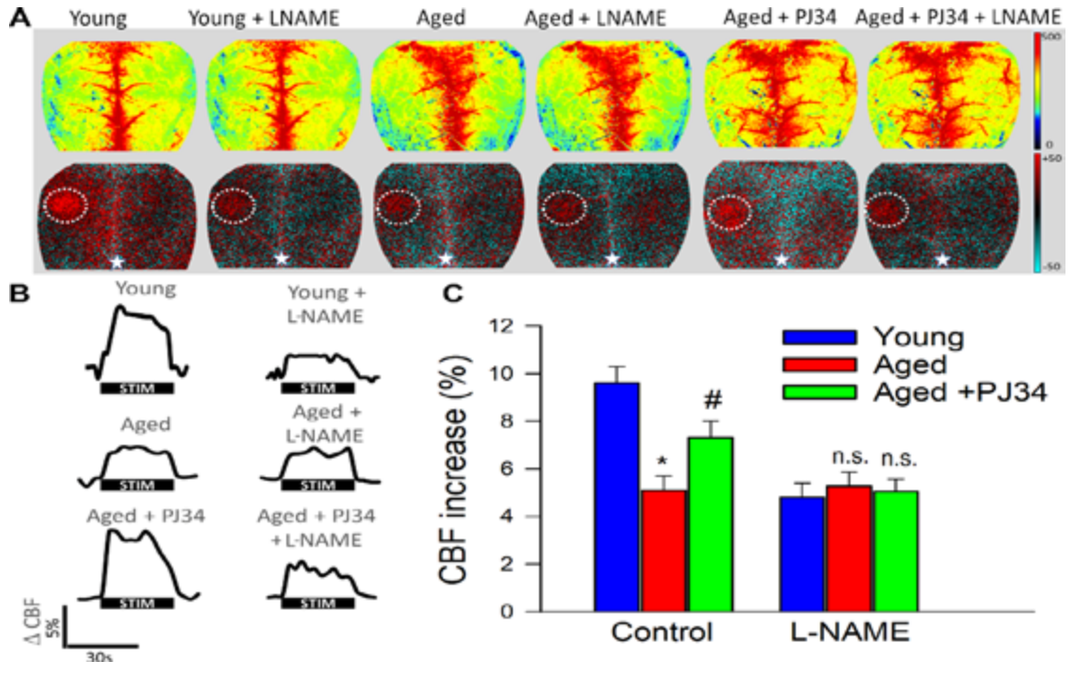
OCAST Project: HR18-092-2
Research Area: Physiology/Pharmacology

Project Narrative

Moment-to-moment adjustment of cerebral blood flow (CBF) is essential for normal neuronal function in the brain. It is regulated via neurovascular coupling (NVC), which maintains the continuous supply of oxygen and nutrients to the neurons, however it becomes progressively impaired during aging, increasing the risk for vascular cognitive impairment. Although vascular causes of age-related cognitive decline are likely preventable. We recently demonstrated that rescue of endothelium-dependent NVC responses mitigates cognitive impairment in aged mice. NAD+ is a rate-limiting co-substrate for anti-aging enzyme SIRT1, which is a key regulator of cellular redox homeostasis. With age cellular NAD+ availability decreases. There is increasing evidence that age-related overactivation of the poly(ADP-ribose) polymerase (PARP-1), a critical NAD+ utilizing enzyme, contributes significantly to reduction of cellular NAD+ levels. Further, in aged mice enhancing NAD+ biosynthesis by treatment with nicotinamide mononucleotide (NMN; a NAD+ intermediate) or decreasing NAD+ utilization by inhibiting PARP-1 reverses age-related dysfunction. The potential protective effects of NMN and/or PARP-1 inhibition on the cerebral microvasculature have not been investigated. The objective of the present proposal is to elucidate the mechanistic effects of age-related decline in cellular NAD+ and to develop novel therapeutic approaches for prevention/treatment of vascular cognitive impairment in the elderly.



Heatmap generated from RNA-SEQ data, showing the 596 genes differentially expressed in aged mice compared to young. Beneficial effects of NMN treatment are proven by decreased inflammatory gene signature. Normalized to Z-score



Treatment with the PARP-1 inhibitor PJ-34 improves NO mediation of neurovascular coupling responses in aged mice.

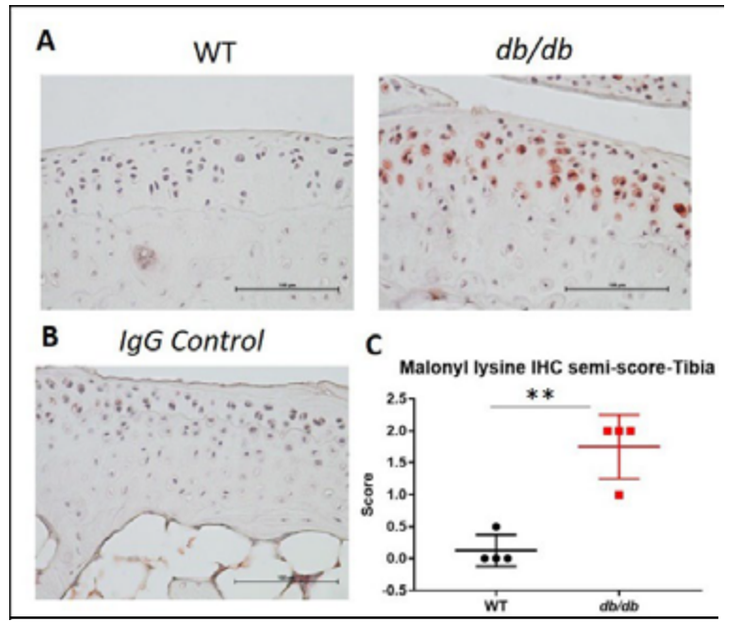
Evidence that obesity causes metabolic changes in cartilage that increase the risk of developing osteoarthritis

Role of diabetes-induced lysine malonylation in chondrocyte metabolism and osteoarthritis

PI: Timothy M Griffin, Oklahoma Medical Research Foundation OCAST Project: HF18-022 Research Area: Physiology/Pharmacology

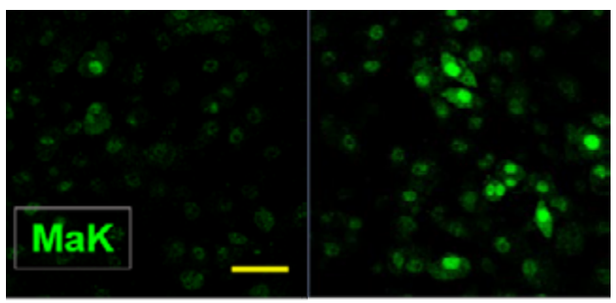
Project Highlights

Osteoarthritis (OA) affects joints throughout the body, especially the knee with the loss of articular cartilage as a central feature. Obesity speeds up the loss of cartilage, which makes it even more difficult to exercise and lose weight. It used to be thought that obesity caused OA by increasing physical wear and tear of cartilage. However, obesity also increases hand OA, and exercise protects rather than damages joints. An alternative explanation is that obesity-related metabolic diseases, such as diabetes, increase OA risk. Diabetes changes how cells make energy. The effect of diabetes on cartilage cell metabolism is not known, but recent findings show that cells from OA cartilage do not make energy as efficiently as those in healthy cartilage. The goal of this project is to test a new theory of cell metabolic damage called "carbon stress", which describes how over-nutrition causes metabolic byproducts to accumulate in cells, bind to proteins, and modify protein activity. We have shown that a specific type of metabolic byproduct, malonyl-lysine, accumulates in the cartilage cells of obese, diabetic mice. In addition, the enzyme that removes this byproduct, Sirt5, is produced at lower levels in OA cartilage. Therefore, we hypothesize that diabetic metabolic conditions and reduced Sirt5 increase the accumulation of malonyl-lysine in cartilage cells, which reduces energy production and speeds up cartilage loss. We are testing how diabetic metabolic conditions cause malonyl-lysine byproducts to accumulate and alter cellular energy production in cartilage. We will also test how the loss of Sirt5 alters cartilage cell function and increases the risk of developing OA. This research is important because it may lead to new metabolic treatments that preserve cartilage cell function and slow the progression of OA.



Lysine malonylation accumulates in cells in cartilage of diabetic mice. Immunohistochemical staining for malonyl lysine on tibias of 6 month male WT (A, left) and *db/db* (diabetic, A, right) mice shows a strong increase in protein lysine malonylation in diabetic cartilage. IgG negative control (B). Semi-quantification of malonyl lysine staining (C). 0=no staining; 1=light staining; 2=strong staining. **p<0.01, n=4.

Control Glucose^{hi} Insulin^{hi}



Cartilage cells grown in the lab under diabetes-like condition (high glucose and high insulin) caused malonyl-lysine (MaK, shown in green) to accumulate in chondrocytes.

Recent Accomplishments

- Malonyl-lysine, a metabolic byproduct that impairs cellular metabolism by binding to proteins, is increased in the cartilage of diabetic mice.
- The enzyme that removes protein-bound malonyl-lysine, called Sirt5, is decreased in OA cartilage.
- High glucose and high insulin diabetic conditions caused the accumulation of malonyl-lysine in chondrocytes.

Griffin Lab: Dr. Griffin is 2nd from right, and Dr. Shouan Zhu, the post-doctoral fellow leading this project, is 1st from right.

Diabetes causes heart proteins to be abnormally modified

A Novel Mechanism of Diabetic Cardiomyopathy

PI: Kenneth Humphries, OMRF ***OCAST Project:*** **HR17-094** **Research Area: Physiology**

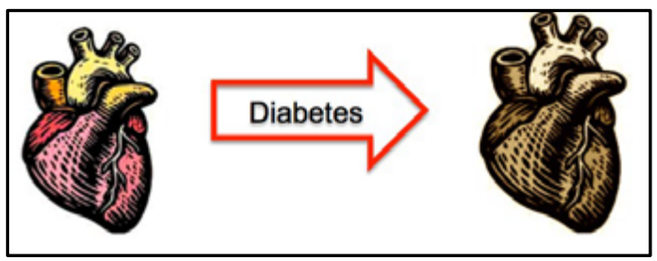
Project Narrative

Diabetes increases the risk and occurrence of heart disease and heart failure. It's critical to understand why this happens so that it can be prevented or treated. This research project is examining how diabetes leads to the increase in modified proteins in the mitochondria of heart cells. Mitochondria are the powerhouses of cells that convert nutrients into energy. Our research has shown that when specific proteins are modified, it changes how mitochondria function and this can impair normal metabolism. Thus, we're seeking to identify how improperly modified proteins may contribute to diabetic heart disease. Maintaining proper mitochondrial function, by decreasing these protein modifications, may be a therapeutic option.

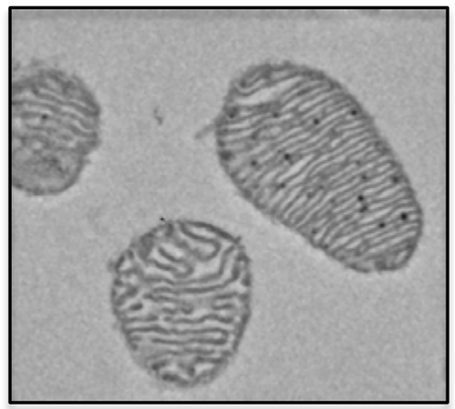
Dr. Kenneth Humphries, head of the research project.

Recent Accomplishments

- We have a newly published paper reporting discoveries of how heart mitochondria respond to changes in diet (Newhardt et al. *Journal of Biological Chemistry,* 2019).
- We have undertaken exciting new studies using a new mouse model that has increased modifications of mitochondrial proteins specifically in the heart.



Diabetes can damage the heart, leading to an increased risk of heart disease and heart failure. It is critical to understand how this damage occurs so that better treatment options can be developed.



Mitochondria, the powerhouse of the cell, are isolated from hearts of control and diabetic mice. The function of mitochondria and the occurrence of protein modifications are determined. The above image is an electron micrograph of mitochondria isolated from a control mouse.

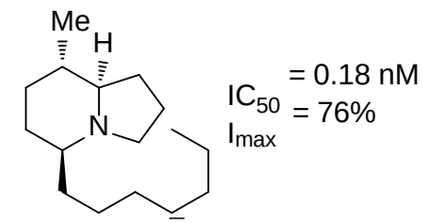
Chemical probes for developing effective antismoking agents

DISCOVERY OF INDOLIZIDINE (-)-237D ANALOGS AS SELECTIVE α6 NICOTINIC RECEPTOR ANTAGONISTS*

PI: Syed Raziullah Hussaini, The University of Tulsa OCAST Project: HR18-049 Research Area: Physiology/Pharmacology

Project Narrative

Annually, cigarette use causes 7,500 Oklahoma deaths. Better anti-smoking agents are needed. Current smoking cessation agents bind to many nicotinic acetylcholine receptor (nAChR) subtypes, which causes unwanted side effects. Compounds that target only α6* nAChRs could be more effective and may cause fewer side effects. Our goal is to discover analogs of (-)-237D that selectively inhibit α6* nAChRs. 237D has poor physicochemical properties. Its pharmacokinetic profile is unknown. We want to design novel analogs of 237D that have physiochemical properties suitable for in vivo studies while targeting only α6* nAChRs. Such analogs could serve as probes of α6* nAChR function in both in vitro and in vivo. These probes could determine the role of α6* nAChRs in nicotine addiction. To achieve this task, we are conducting computational, chemical, and pharmacological studies. Computational chemistry is being used to select 237D derivatives with optimal physiochemical properties. Molecular modeling of these analogs will help identify analogs that most tightly bind with α6* nAChRs. We will synthesize selected analogs via a metal-catalyzed coupling reaction. These analogs will be tested for their selectivity and potency towards α6* nAChRs using pharmacological assays. Lead analogs will be used as probes to determine the role of α6* nAChRs in addiction-relevant behavioral models and may serve as new smoking cessation agents targeting α6* nAChRs. The proposed work is a collaborative project involving Drs. Hussaini (TU), Mooers (OU Health Sciences), and Wallace (OSU Center for Health Sciences) research teams.



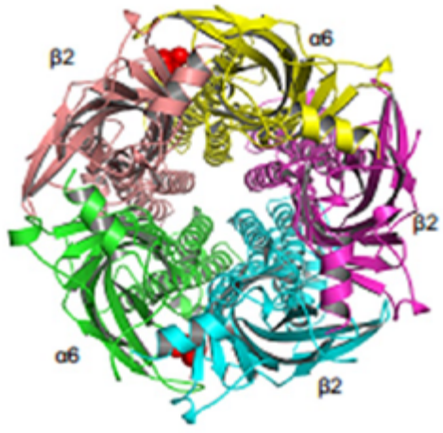
Indolizidine ( )-237D

Recent Accomplishments

Since August 2018

- We have generated > 2,000 structures of analogs that have suitable physiochemical parameters for in vivo studies.
- We finalized a metal-catalyzed coupling reaction between donor/acceptor-substituted diazo compounds, which resulted in the following publication.
  - ACS Omega 2019, 4, 269–280.
- We have built a homology model for α6β2 and presented this work in a poster presentation at the Annual Oklahoma Structural Biology Symposium.

Adama Kuta, a graduate student from monitoring the progress of the metal-catalyzed coupling reaction



Computer model of α6β2 nicotinic receptor showing nicotine (modeled as red van) binding at the α-β interfaces

Do genetics influence the metabolism and physiological effects of caffeine?

The role of genetic polymorphisms on the pharmacokinetics and pharmacodynamics of caffeine: implications for cardiometabolic function

PI: Nathaniel Jenkins, Ph.D.
Oklahoma State University

HR19-028

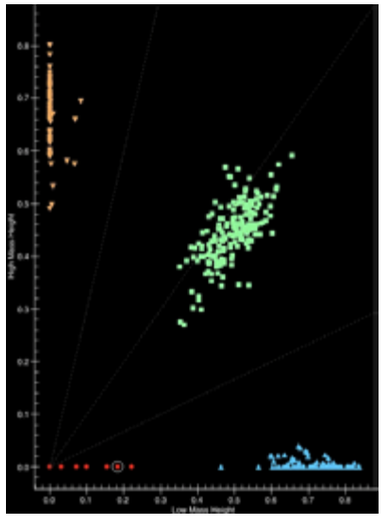
Physiology/Pharmacology

Project Highlights

Eighty to 90% of adults worldwide report habitual consumption of caffeine-containing beverages. Both heart rate and blood pressure are under regulation by the sympathetic nervous system (SNS), which plays a vital role in cardiac contractility, blood pressure, renin release, renal vascular resistance, sodium reabsorption, and blood flow. Caffeine is a potent SNS stimulant and is therefore a dietary factor that has been implicated as a risk factor for cardiovascular disease (CVD). Further, caffeine has been shown to influence post-meal metabolism, which is significant because most westerners spend the large majority of their day in a post-meal (i.e., fed) state.

While we know that genetic factors play a role in CVD, it is probable that inherited predisposition to CVD is related to polymorphic variants that affect risk only after exposure to specific factors. Consequently, the identification, characterization, and use of genetic modifiers is critical to more fully understand the true biological effects of dietary factors such as caffeine, and its role in CVD. Recent studies have specifically implicated genetic differences in the enzyme responsible for metabolizing caffeine, cytochrome P450 (CYP1A2), and in the adenosine A_{2A} (ADORA2A) receptor as loci that may influence caffeine's metabolism and physiological effects. Given the growing popularity of 'energy' drinks and other new food products containing caffeine, there is a significant need for improved understanding of the physiological role of caffeine in the body and identification of individuals who may be particularly susceptible to caffeine's cardiovascular effects.

This study is seeking to determine the influence of these two genetic variants on caffeine's metabolism and cardiometabolic effects (1) while fasting and (2) while fed.



An example cluster plot of logarithm height of the low- versus high-mass allele peaks, from which the participants are genotyped

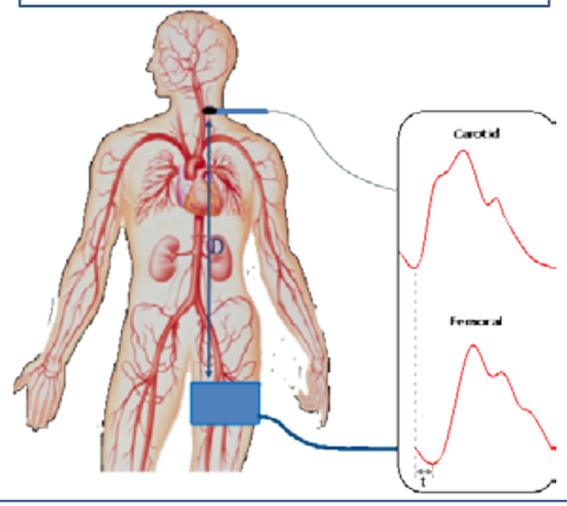
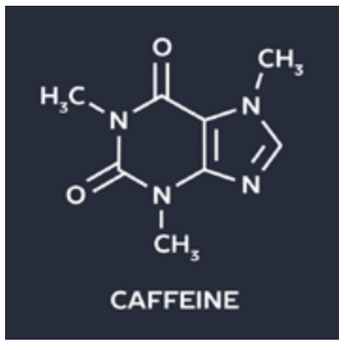


Illustration of carotid-femoral pulse wave velocity (cfPWV), which is estimated as the distance (D) between sampling sites and the time delay (t) between arterial waveforms.



Recent Accomplishments

- This project was recently awarded (8/1/2019) and a new fund number was established internally on 10/4/2019
- IRB has been submitted and approved for Phase I
- Currently preparing to begin recruitment and hope to start data collection in January 2020

Podocyte Protective Effect of Neuropilin-1

PI: Jian-xing Ma, University of Oklahoma Health Sciences Center

OCAST Project: HR16-041 Research Area: Physiology/Pharmacology

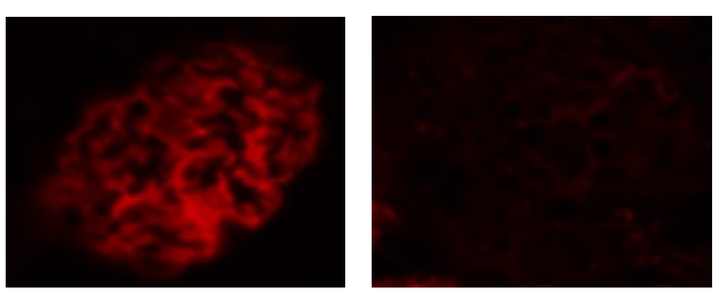
Project Highlights

This project aims to identify a new causative factor for diabetic kidney complication, namely diabetic nephropathy. Diabetic nephropathy is a common complication of diabetes and a major cause of kidney failure and lacks effective drug treatments. We have recently found that the protein Neuropilin-1 is decreased in the kidneys of both type 1 and type 2 diabetic animal models. This project will further study the function of this protein as a protective factor in the kidney and study if the decreased levels of this protein in diabetes are responsible for diabetic kidney complication.

Recent Progress

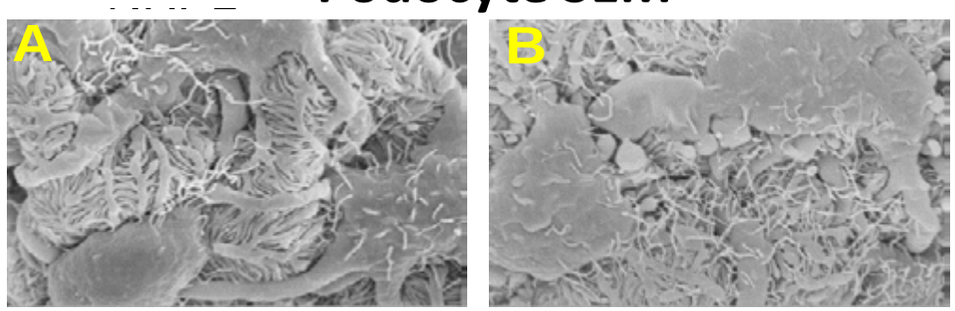
- Neuropilin-1 deficiency induce proteinuria, a symptom of diabetic complication.
- Neuropilin-1 deficiency impair kidney function.
- Neuropilin-1 deficiency exacerbates diabetes-induced kidney fibrosis.

Podocin Immunostaining



WT with DM NRP! KO with DM

Podocyte SEM



WT NRP-1 KO

Magnetic heating of nanoparticles and metal implants clear painful bone infections

Magnetic hyperthermia combined antimicrobial targeting of bone pathogens

PI: Ashish Ranjan, Oklahoma State University OCAST Project# HR17-060 Research area: Osteomyelitis

Osteomyelitis is a serious infection of bone that can lead to adverse outcomes including a need for extensive surgical debridement and limb amputation. Systemic treatment of osteomyelitis requires long duration administration of a combination of antimicrobials with limited specificity, incurs adverse side effects and can cause drug resistance and recurrence. To improve osteomyelitis treatment and enable non-invasive treatment, this project will test an innovative combination of superparamagnetic thermally-sensitive liposomes (sLTSL) that can be triggered to release antimicrobial payloads locally in bones using a portable alternating magnetic field (AMF) hyperthermia devise. Success of this cutting-edge translational research will provide an innovative technology to non-invasively deliver into bone higher antimicrobial drug payloads, minimize need of surgical debridement and amputation, and strategically enhance bacterial sensitivity to antimicrobial therapies by addition of precise mild localized warming.

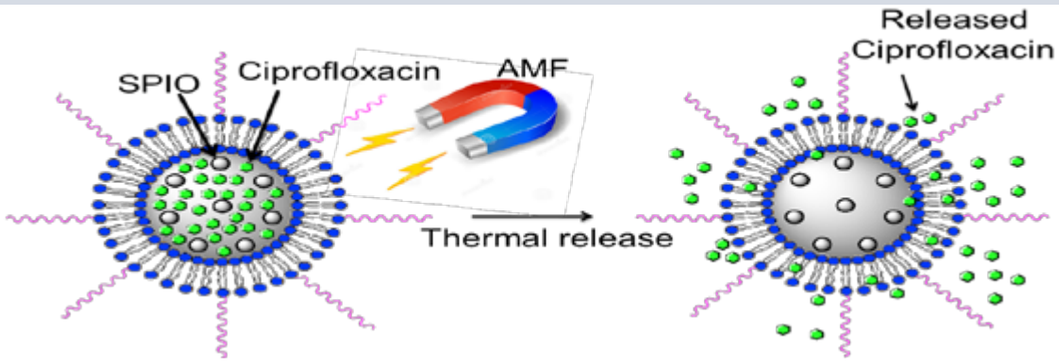
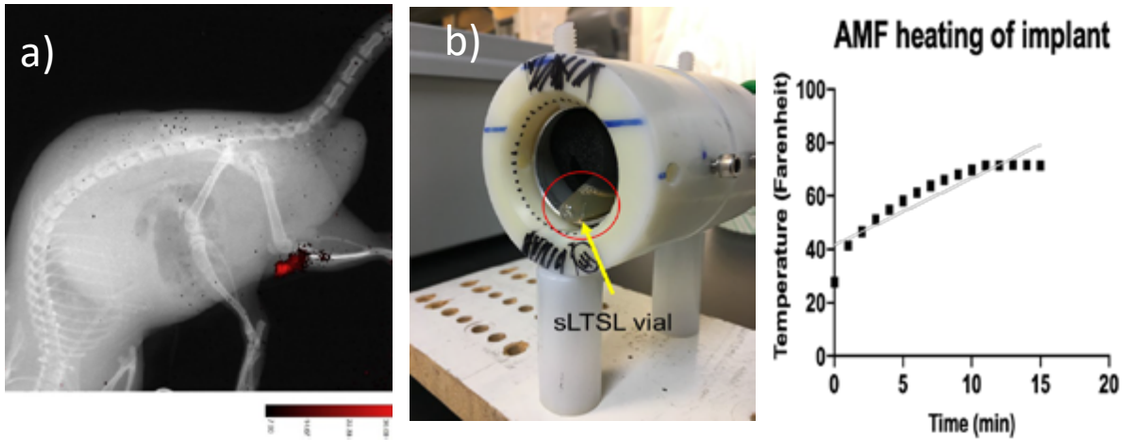


Fig. 1. sLTSLs release antimicrobials in a targeted manner in combination with AMF hyperthermia

Key Accomplishments (2018-2019)

- *Establishment of osteomyelitis in rodent models*
- *Localization of sLTSLs with Halbach array*
- *Real-time non-invasive heating of SPION and metallic implants with AMF*



a) An osteomyelitis (red) generated in a rat model in our laboratory; b-c) Novel halbach array magnet allowing heating of SPIONs and metallic implants with AMF (Co-I: Piao)

Role of an inflammatory cell death pathway in age-associated inflammation

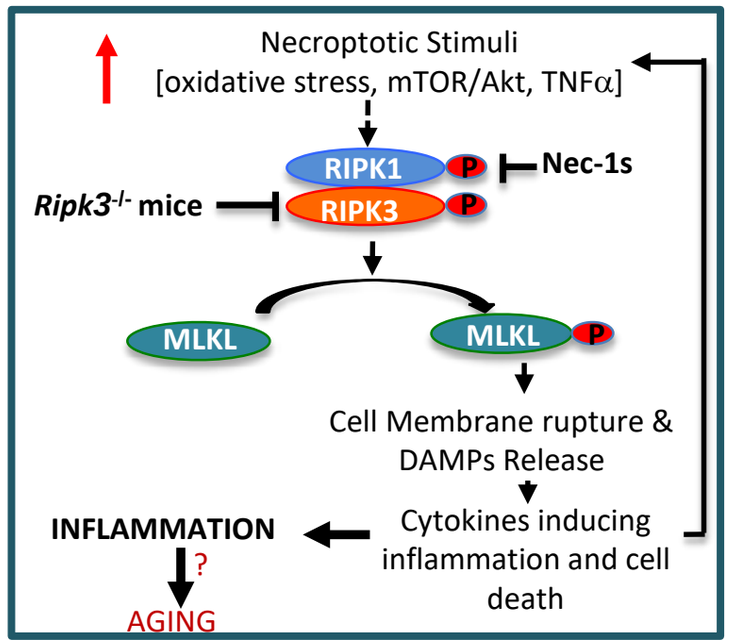
Testing the Role of Inflammation in Aging and Age-related Diseases

PI: Deepa Sathyaseelan, OUHSC **OCAST Project Number: HR18-053** **Research Area: Physiology/Pharmacology**

Project Narrative

A common feature of aging and age-related diseases (e.g. type 2 diabetes, cardiovascular diseases, cancer, Alzheimer's disease etc) is low-level chronic inflammation, termed sterile inflammation (indicating inflammation in the absence of detectable pathogens) or inflammaging. Inflammaging is an important risk factor for both morbidity and mortality in older people and is characterized by high levels of pro-inflammatory cytokines (molecules that causes inflammation). Even though the association between inflammation, aging and age-associated diseases are known, whether inflammaging is causing aging and age-associated diseases, and pathway(s) that mediate inflammaging are not known.

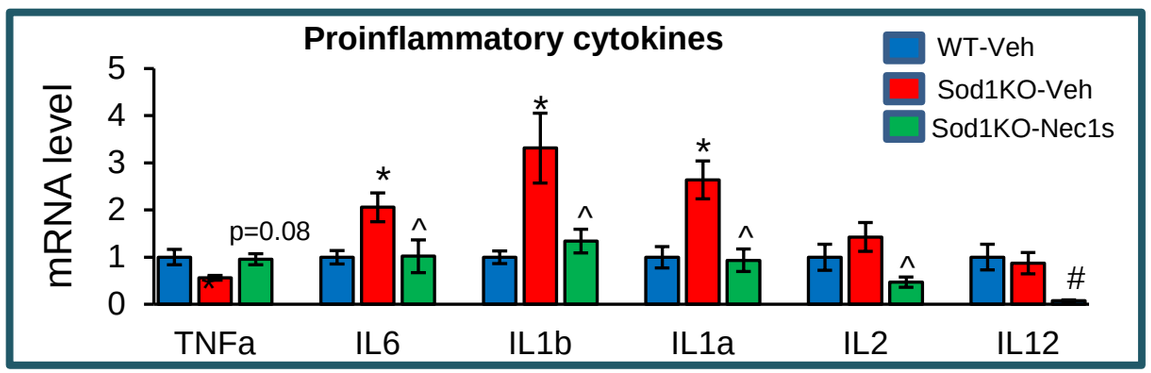
'Necroptosis' is a newly identified form of cell death that causes inflammation, however, role of necroptosis in inflammaging is unexplored. Using a mouse model of accelerated aging [mice deficient in an anti-oxidant enzyme, Cu/Zn-superoxide dismutase knockout mice (*Sod1KO* mice)], this research project will examine the role of 'necroptosis' in inflammation and aging phenotype in *Sod1KO* mice. The study will help us to identify whether necroptosis is a key pathway in inflammaging. This observation could be translationally important because pharmacological agents that inhibit necroptosis are available.



Proposed mechanism of necroptosis-mediated inflammation.

Recent Accomplishments

- Blocking necroptosis in *Sod1KO* mice using necroptosis inhibitor, Necrostatitin-1s reduced necroptosis and transcript levels of proinflammatory cytokines in the brain, spinal cord and liver of *Sod1KO* mice.
- Standardizing conditions for immunostaining to identify the cell types undergoing necroptosis in brain and liver of *Sod1KO* mice.



Images showing transcript levels of proinflammatory cytokines in the liver of wild type and *Sod1KO* mice treated with vehicle or Nec1s

MECHANISMS FOR THE DELETERIOUS EFFECTS OF AMYLOID BETA 1-42 WITH AGE

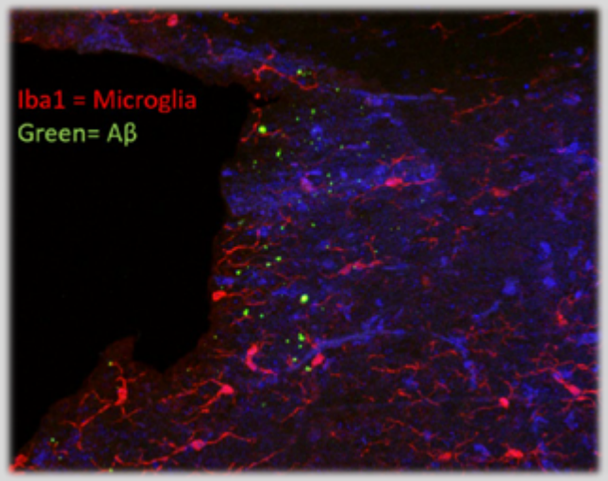
Susceptibility to Amyloid Oligomers in Response to Aging and Insulin/IGF-1 Resistance

William E. Sonntag, PhD, Sreemathi Logan, PhD , Alexander Yeganeh
University of Oklahoma Health Sciences Center

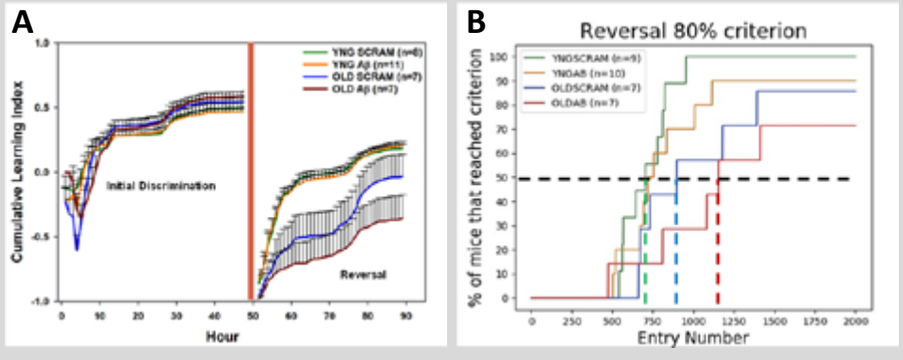
PI: William E. Sonntag **OCAST Project: HR18-120** **Research Area: Aging**

Project Highlights

Age is, by far, the single most important risk factor for cognitive decline, neurodegeneration and Alzheimer's Disease (AD). Before 60 years of age the incidence of cognitive decline and sporadic AD is rare; however, risk doubles every 5 years after 65 years of age and the age-group with the highest levels of AD are those older than 85. The etiology for the age-related increase in cognitive impairment and risk for neurodegenerative disease is unknown. Understanding the mechanisms contributing to the age-related component of the disease process and developing interventions is a promising, novel and prudent approach to delay Alzheimer's disease progression. To evaluate the increased sensitivity of the aging brain in the deleterious effects of Aβ, we developed a 28-day brain infusion model of $A\beta^{1-42}$ and validated its effects on cognitive function in young (6 m) and aged (24 m) mice and measure molecular changes by RNAseq. There are compelling data that the conserved Insulin/IGF signaling pathway is involved in the increased risk for neurodegenerative disease with age. Based on these results, we will further assess whether the loss of IGF-1 signaling in the brain with age makes it more susceptible to $A\beta^{1-42}$ and cognitive deficits.



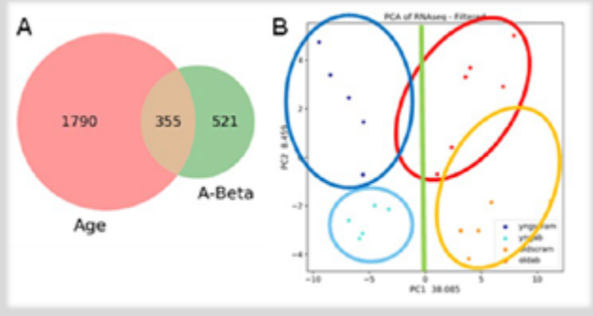
Aβ oligomer infiltration into the cortex infusion with activation of surrounding microglia (immune cells)



Spatial learning is impaired Aged mice with $A\beta^{1-42}$ infusion. **(A)** Learning Index showed a considerable decline in the aged mice which was further reduced with $A\beta^{1-42}$ treatment during the Reversal Phase. No differences were seen in the Initial Discrimination Phase between groups. **(B)** Entry numbers to criterion are increased with age and further increased with $A\beta^{1-42}$ treatment in the aged mice.

Recent Accomplishments

- We completed the evaluation and optimization of the ICV infusion studies in young and aged mice to assess sensitivity to insults such as $A\beta^{1-42}$.
- We are continuing to assess downstream targets identified (TNFalpha) for therapeutic interventions.
- We will also assess whether loss of IGF-1 confers sensitivity to cognitive dysfunction with age.



(A) Pie chart shows differentially expressed transcripts with Age and/or $A\beta^{1-42}$ treatment, which is represented by the PCA analysis **(B)** between groups

Irradiation-induced cognitive decline: role of endothelial senescence

PI: Priya Balasubramanian, PhD and Zoltan Ungvari MD, PhD

OCAST Project: HF19-028-1 Research Area: Physiology/Pharmacology

Project Narrative

Patients with metastatic brain tumors who are treated with whole brain irradiation (WBI) often experience progressive dementia as a result of this treatment. At the present time, no strategies exist to prevent radiation-induced brain injury and no additional treatments can reverse these effects. Increasing evidence shows that vascular factors have a critical role in cognitive decline by altering cerebral blood flow and thus the availability of oxygen and nutrients for active neurons. Adjustment of cerebral blood flow is required for normal neuronal function, and our data show that it becomes progressively impaired after WBI, increasing the risk for vascular cognitive impairment. This is in part caused by the dysfunction of the endothelium. Our research studies mechanisms of WBI-induced endothelial dysfunction and dysregulation of cerebral blood flow. Our hypothesis is that that WBI, through causing damage to the DNA and thus "reprogramming" endothelial cells (inducing a cellular response termed senescence), impairs the production of vasodilator nitric oxide preventing the increase of the cerebral blood flow when needed. Our expected outcomes will be an integrated understanding of the mechanisms that underlie the impairment of cerebral blood flow regulation post-WBI.

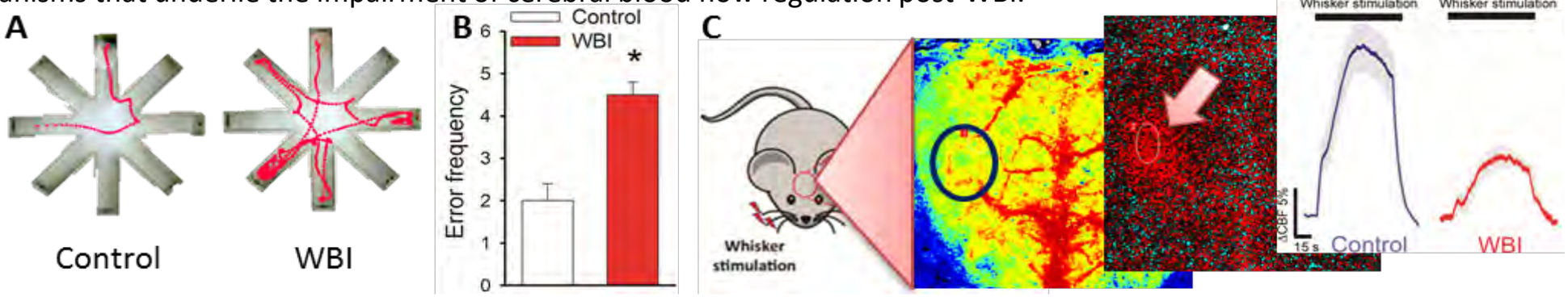
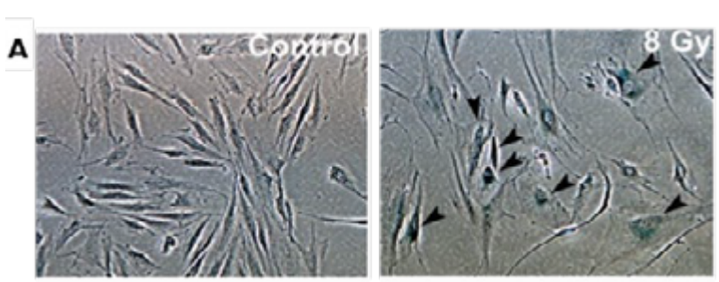
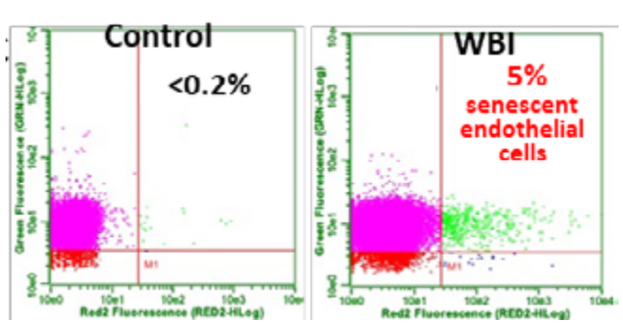


Figure 1.: WBI-induced cognitive decline is associated with impaired NVC. A) Representative paths to solve the RAWM maze. B) WBI significantly impairs cognitive performance (increased number of entries to incorrect arms). C) Measuring NVC using laser speckle contrast imaging. Right: Representative traces of CBF responses measured above the whisker barrel cortex during contralateral whisker stimulation.





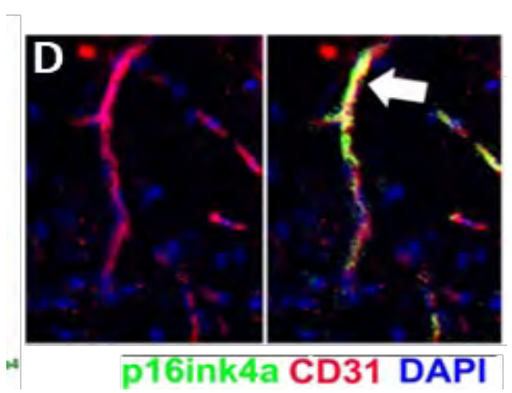


Figure 2.: Irradiation induces senescence in cerebromicrovascular endothelial cells both in vitro and in vivo.

Hunt For A New Drug For The Treatment Of Diabetes

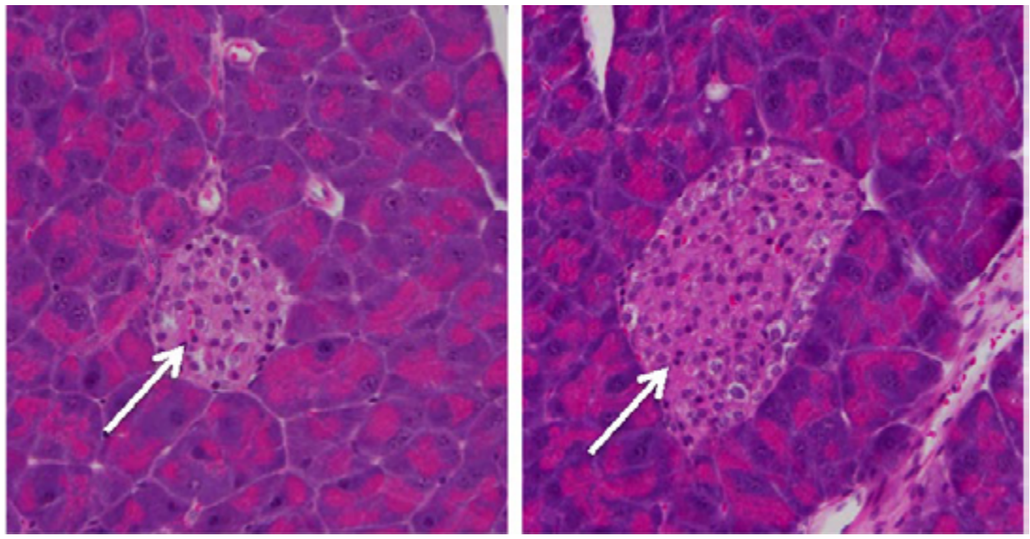
Pancreatic beta cell protection of natural product K50 and its mechanism of action

PI: Weidong Wang, OUHSC **OCAST Project: HR17-097** **Research Area: Physiology /Pharmacology**

Project narrative

Type 2 Diabetes affects more than 300 million individuals globally. Endoplasmic reticulum stress-mediated pancreatic β cell failure and death is a primary determinant in the pathogenesis of type 2 diabetes. These indicate the therapeutic potential for novel drugs that block stress-caused β cell apoptosis. However, there are no drugs on the market that prevent β cell death. We have used a high throughput screening approach to identify small molecules that protect β cells from ER stress-induced death. In this grant, we will study the β cell protective effect of a natural product K50 and its mechanism of action. We discovered that K50 protects β cells from ER stress-induced dysfunction and death in vitro and in vivo. We further found that K50 inhibits ER stress-induced hyperactivation of one unfolded protein response pathway IRE1α. As IRE1α is a dual kinase and RNase protein, we will determine if and how K50 inhibits IRE1α activity using biochemical, cellular and in vivo approaches. We will also determine the in vivo efficacy of K50 in ER stress-related diabetic animal models. These findings may therefore serve as a new drug lead for development to treat diabetes.

Akita, saline **Akita, K50**



Arrows point to the areas of β cell islets. The area is significantly larger in K50-treated Akita-diabetic mice than in control-treated counterparts.

Recent accomplishments

- Identified more potent β cell-protective compounds
- K50 normalized hyperglycemia in diabetic animal models

Targeting blood vessels to combat obesity and metabolic syndrome

Endothelial regulation of high-fat diet-induced obesity

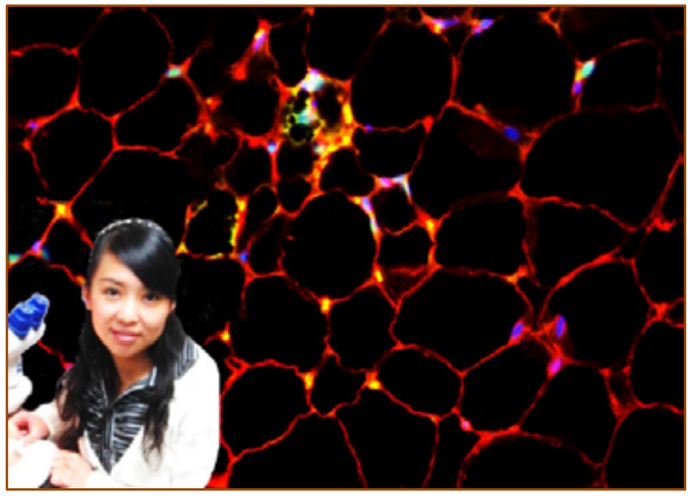
PI: Jian Xu, OU Health Sciences Center **OCAST Project: HR17-046** **Research Area: Physiology/Pharmacology**

Project Narrative

The World Health Organization predicts that obesity may soon become the most significant cause of poor health, in addition to malnutrition and infectious diseases. Half of the population in developed countries is overweight or obese. Over 15% of children in these countries are overweight or obese. Obesity-associated medical complications include type 2 diabetes mellitus and cancer, which account for at least 300,000 deaths per year in the U.S. Emerging evidence suggests that functional blood vessels are required in ameliorating obesity and the related metabolic complications; however, it remains largely unknown which target on blood vessels can be used to achieve this goal. This proposal is to test a blood vessel-based approach that may lead to better treatment of obesity and the associated insulin resistance. The proposal stemmed from a novel mouse model in which a potential target gene was deleted. Surprisingly, the gene deletion led to resistance to diets-induced obesity, attributable to a favorable and hormone-like peptide produced from blood vessel cells, a mechanism that has not been previously studied. The goal of the research is to determine how targeting the blood vessel protein improves metabolism in mouse models of obesity. Completion of this study would drive the future development of better therapeutics for obesity and metabolic syndrome.

Major Findings

- High-fat diet-feeding induces obesity but reduces a blood vessel protein in mouse fat tissues.
- Lack of this protein worsens obesity and insulin resistance in obese mice.
- Fat tissues of selected obese subjects show a reduction of the blood vessel protein that improves insulin resistance in obese mice.



Research associate Manna Li, MD, PhD from our lab was staining fat tissues to identify targets.

Sepsis is associated with higher risk of death in older adults and higher incidence of memory loss in survivors

Prevention of sepsis-induced multiple organ failure in old age

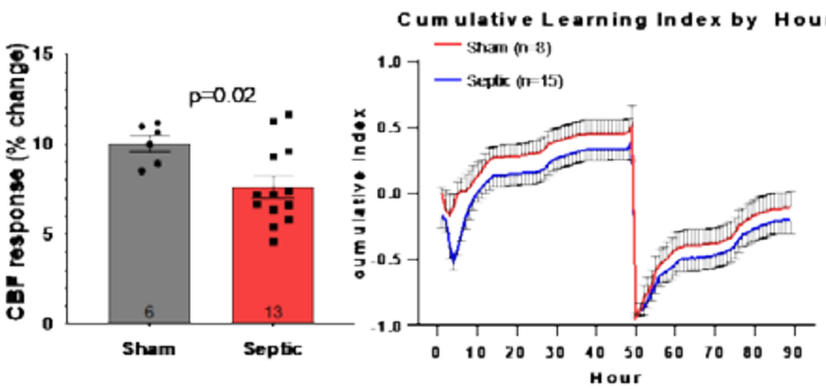
PI: Andriy Yabluchanskiy, MD, PhD
University of Oklahoma Health Sciences Center

OCAST Project: HR17-070-1

Research Area: Physiology/Pharmacology

Project Narrative

Sepsis is the tenth leading cause of death in patients over the age of 65, and the frequency of severe sepsis in older adults is 100 times higher comparing to younger persons. One of the major causes of death in sepsis is multiple organ failure, and recent evidence suggests that impaired vascular health is an important risk factor for its development. Compromised vascular health may also be responsible for loss of memory that develops in over 50% of sepsis survivors over 60 years of age, the magnitude of which is comparable to Alzheimer's disease. Our hypothesis is based on the knowledge that aging leads to an increased oxidative stress level in multiple cells in organs, including vascular cells, and that once these cells are compromised in an aging organism and subjected to a severe stress such as sepsis, their function rapidly deteriorates and may lead to multiple organ failure and death or severe impairments in survivors including such changes as memory loss and other.



Previously, we demonstrated that aging negatively affects the health of vascular cells in the brain and that these changes lead to an inadequate blood supply to match the demands of neuronal activity, and, subsequently, lead to progressive memory loss. To test whether sepsis further impairs these processes, we have used a mouse model of sepsis that is highly relevant to clinical scenario – we infected experimental animals with *listeria monocytogenes*, the most common bacteria to cause sepsis in humans. We found, that sepsis in aged animals (24 months old mice, equivalent to ~65 year old human) significantly impaired cerebral blood flow responses, which was associated with worse cognitive performance.

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We have also found that vascular health was compromised not only in the brain, but also in other larger arteries such as aorta, indicating that generalized vascular impairment may be a driving mechanisms for higher risk of multiple organ failure in aging.