

The Council of Biomedical Graduate Students

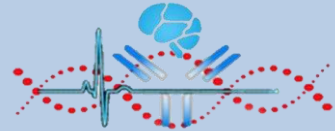
2018 Graduate Research Forum



COLLEGE OF MEDICINE
AND LIFE SCIENCES

THE UNIVERSITY OF TOLEDO

BIOMEDICAL SCIENCES
GRADUATE PROGRAM



THE UNIVERSITY OF
TOLEDO

Sponsored by:



**GRADUATE STUDENT
ASSOCIATION**



Graduate Research Forum

March 15th-16th, 2018



Keynote Speaker

Thomas Hornyak

(M.D, Ph.D)

Chair, Department of Dermatology
Associate Professor of Dermatology and
Biochemistry and Molecular Biology
University of Maryland School of Medicine

Keynote Speech “Genomic Approaches to Discovery in Melanocyte Biology and Melanoma”

Thursday March 15th

Location: Interprofessional Immersive Simulation Center within the CCE building

- Poster Presentations: 10am-12pm, Atrium
- Oral Presentations: 1pm-4pm, Room 1200 (Theater)

Friday March 16th

- Lunch with Keynote Speaker: 12pm-1pm, CCE 0111
- Keynote Reception: 3:15pm-3:45pm HEB Lobby
- **Keynote Speech: 4pm-5pm HEB 110**

Contact the Council of Biomedical Graduate Students (CBGS) with any questions at CouncilGraduateStudents@utoledo.edu



THE UNIVERSITY OF TOLEDO

BIOMEDICAL SCIENCES
GRADUATE PROGRAM



THE UNIVERSITY OF
TOLEDO



**GRADUATE STUDENT
ASSOCIATION**

2018 Graduate Research Forum

March 15th-16th

**Interprofessional Immersive Simulation Center (IISC)
Health Science Campus**

Thursday, March 15th

<i>Time</i>	<i>Event</i>	<i>Location</i>
10:00 am – 12:00 pm	Preliminary Poster Session	IISC Atrium
12:00 pm – 1:00 pm	Lunch	IISC 1217
1:00 pm – 4:00 pm	Preliminary Oral Sessions	IISC 1200

Friday, March 16th

<i>Time</i>	<i>Event</i>	<i>Location</i>
9:00 am – 10:15 am	Final Poster Session <i>(closed to public)</i>	IISC 1200
10:30 am – 11:30 pm	Final Oral Session <i>(closed to public)</i>	IISC 1200
12:00 pm – 1:00 pm	Lunch with Dr. Thomas Hornyak <i>(RSVP'd participants only)</i>	CCE 0111
3:15 pm – 3:45 pm	Keynote Reception	HEB Lobby
4:00 pm – 5:00 pm	Keynote Lecture	HEB 110

Keynote Lecture

***"Genomic Approaches to Discovery in Melanocyte Biology and
Melanoma"***

2018 Keynote Speaker



Thomas Hornyak, M.D, Ph.D

Chair, Department of Dermatology

Associate Professor of Dermatology and Biochemistry and Molecular Biology
University of Maryland School of Medicine

Dr. Hornyak's lab focuses on defining subpopulations of melanocyte progenitor cells and their contribution to the development of melanocytic neoplasms.

The Council of Biomedical Graduate Students

The University of Toledo Council of Biomedical Graduate Students consists of officers and representatives from the College of Medicine and Life Sciences and the College of Pharmacy and Pharmaceutical Sciences at the University of Toledo. This includes the Biomedical Science Graduate Program and related graduate programs in Pharmacy, Medicinal & Biological Chemistry, and from the Center of Excellence in Biomarker Research & Individualized Medicine (BRIM) at the Health Science Campus.

The overall purpose of the Council is to facilitate discussion amongst graduate students pertaining to any issue that may affect graduate life; to represent graduate student interests before the UT faculty, GSA (our main campus counterparts), and administration; and to organize events and activities beneficial to graduate student life.

We meet regularly, at least once per month, to discuss any current issues that need to be addressed and to plan and organize upcoming events. **The meetings are open to all graduate students** to encourage discussion of ideas and concerns pertaining to graduate student life. However, only elected members of the Council may vote during the meetings.

Annual events organized by the CBGS include:

- **Graduate Student Picnic** - A summer social event for new and current students
- **Career Forum** - Held in autumn to help guide students for career decisions
- **Graduate Research Forum** - Held in late winter to allow students to showcase their research and get helpful advice from faculty and fellow students

Visit us at <http://www.utoledo.edu/med/grad/biomedical/cbgs/>

The CBGS would like to thank all the volunteers, judges, and the UT Graduate Student Association (GSA). This year's forum was FULLY sponsored by GSA. The forum could not happen without all of you.

THANK YOU!



**GRADUATE STUDENT
ASSOCIATION**

Poster Presentations

GROUP A	Cara DeAngelis	P1	GROUP E	Apurva Lad	P21
	Amal A El Daibani	P2		Irum Syed	P22
	Amanda Blaker	P3		Chrysan Mohammed	P23
	Caroline Lambert	P4		Josh Breidenbach	P24
	Mohammed Rasool	P5			
GROUP B	Claire Meikle	P6	<u>Poster Contest Procedure</u> - See presentation guidelines on page 8. <u>Preliminary Session</u>- Each group will be judged by three faculty members and one finalist will be selected from each group. <u>Final Session</u>- Finalists will present to: - Dr. Thomas Hornyak, 2018 GRF Keynote Speaker - Dr. Randall Ruch, Associate Professor of Cancer Biology - Dr. Marcia McInerny, Distinguished University Professor - The top three presenters will be awarded: \$300 for first place \$200 for second place \$100 for third place		
	Dan Craig	P7			
	Sangita Sridharan	P8			
	Saroj Chakraborty	P9			
	Daniyah Almarghalani	P10			
GROUP C	Briana Zellner	P11			
	Usman Ashraf	P12			
	Sarah Galla	P13			
	Yashna Walia	P14			
	Samkeliso Mpendulo Dlamini	P15			
GROUP D	Shungang Zhang	P16			
	Jagaree Das	P17			
	Vassili Bletsos	P18			
	Fatimah Khalaf	P19			
	Ahsan Bairam	P20			

Oral Presentations

Oral Contest Procedure

GROUP 1	Yuqi Zhang	01
1:00-2:00 pm	Iyad Manaserh	02
	Amit Chougule	03
	Jessica Saul-MacBeth	04

- See presentation guidelines on page 8.
- Preliminary Session- Each group will be judged by three faculty members and one finalist will be selected from each group.

GROUP 2	Tupa Basuroy	05
2:00-3:00 pm	Cory Howard	06
	Cara Peter	07
	Kelsey Murphy	08

- Final Session- Finalists will present to:
 - Dr. Thomas Hornyak, 2018 GRF Keynote Speaker
 - Dr. Randall Ruch, Associate Professor of Cancer Biology
 - Dr. Marcia McInerny, Distinguished University Professor

GROUP 3	Darren Gordon	09
3:00-4:00 pm	Jin Chen	010
	Augustus Tilley	011
	Saad Moledina	012

- The top three presenters will be awarded:
 - \$300 for first place
 - \$200 for second place
 - \$100 for third place

Guidelines for Poster and Oral Presentations

POSTER PRESENTATIONS

1. Each poster presentation will be given a maximum of 15 minutes, including time for questions. Presenters are advised to limit their explanation of posters to 10 minutes (max 12 minutes) to allow time for questions.
2. Poster boards (size - 3 feet high and 4 feet wide) will be provided. Whether or not you have a professionally printed poster will not affect your scores in any way. However, please make sure all text, figures and diagrams are clear and legible. If you wish to show data in the form of videos, please inform CBGS members one day before the forum via email. The students are responsible for bringing their own devices (laptop, ipad etc) to show their videos (and making sure it is adequately charged). Although the council will try to help the student as far as possible, the council is not responsible for the electronic devices (i.e. losing battery etc.).
3. Each presenter will be given a number for their poster. All presenters are requested to put up their posters on their respective poster board (presenters will be informed of their poster numbers prior to the forum).
4. Judging Criteria: The judging will be based on knowledge of the subject, explanation of background and significance, organization of poster, clarity of presentation, conclusions supported by data, ability to finish presentation in allotted time, ability to answer questions and overall presentation.

ORAL PRESENTATIONS

1. Each oral talk will be given a maximum of 15 minutes including time for questions. Presenters are advised to limit their presentations to 10 minutes (max 12 minutes) to allow time for questions.
2. There is no restriction for the number of slides per presentation. Please make sure all text, figures and diagrams on the slides are clear and legible.
3. All presenters are requested to hand over their presentations (in a USB drive) to their respective session moderators at least 15 minutes prior to the start of the oral presentation sessions. (Presenters will be informed of their sessions prior to the forum.)
4. Judging Criteria: Judging will be based on knowledge of the subject, organization and clarity of the talk, conclusions supported by data, ability to finish the talk in allotted time, ability to answer questions, and overall presentation.

Poster Presentation Abstracts

P1: Characterization of the phage shock protein response in *Vibrio cholerae*

Cara M. DeAngelis and Jyl S. Matson

Department of Medical Microbiology and Immunology

Cholera is a severe intestinal infection characterized by voluminous, watery diarrhea that can be fatal within hours. It is caused by the marine bacterium *Vibrio cholerae* of serogroups O1 and O139. While rare in the United States and other industrialized nations, cholera is endemic in more than 50 countries. In both its aquatic and intestinal life cycles, *V. cholerae* will encounter various stressful conditions, such as fluctuating pH, bacteriophage predation, and exposure to antimicrobial peptides that may negatively affect the integrity of the inner membrane. The phage shock protein (Psp) system is a stress response pathway that senses and responds to such insults. The Psp system is conserved in many clinically relevant Gram-negative bacteria, however, has remained completely uncharacterized in *V. cholerae*. Therefore, our goal is to identify inducers, verify genetic regulation, and determine stress relief mechanisms of the Psp response in *V. cholerae*. To identify inducers of the Psp system in *V. cholerae*, we constructed a transcriptional fusion strain where the *psp* promoter was cloned upstream of the endogenous *lacZ* gene. We also created clean deletions of the core *psp* genes: *pspA*, *pspB*, *pspC* and *pspF*, to aid in understanding their regulatory and functional roles. In addition, we subjected the abovementioned mutant strains to different growth conditions and survival assays to further determine what role the Psp system plays in *V. cholerae*.

Grant Support: R01 and start-up funds

P2: Impacts of Genetic Polymorphisms on the Sulfation of Diethylstilbestrol and Afimoxifene by Human Cytosolic Sulfotransferase SULT1E1

Amal A. El Daibani and Ming-Cheh Liu.

Department of Pharmacology and Experimental Therapeutics

Previous studies have demonstrated that sulfation, as mediated by the estrogen-sulfating cytosolic sulfotransferase (SULT) SULT1E1, is involved in the metabolism of diethylstilbestrol and afimoxifene in humans. It is an interesting question whether the genetic polymorphisms of SULT1E1, the gene that encodes the SULT1E1 enzyme, may have a significant impact on the therapeutic as well as adverse effects of these two drugs. In this study, five missense coding single nucleotide polymorphisms (SNPs) of the SULT1E1 gene were selected for investigating the sulfating activity of the resulting SULT1E1 allozymes toward diethylstilbestrol and afimoxifene. Corresponding cDNAs were generated by site-directed mutagenesis and recombinant SULT1E1 allozymes were bacterially expressed and purified by affinity chromatography. Purified allozymes of SULT1E1 were shown to display widely different sulfating activities toward diethylstilbestrol and afimoxifene. Kinetic analysis showed further distinct K_m (reflecting substrate affinity) and V_{max} (reflecting catalytic activity) of the five SULT1E1 allozymes with diethylstilbestrol as a substrate. Taken together, these findings highlighted the significant and sometimes dramatic differences in the drug-sulfating activities of the SULT1E1 allozymes, which may have implications in the metabolism of diethylstilbestrol and afimoxifene in individuals with different SULT1E1 genotypes. Such information may in the future be useful for designing personalized regimens of diethylstilbestrol and afimoxifene.

Grant Support: University of Toledo

P3: Inflammation Mediates Enhanced Neurotoxicity after Co-exposure to Alcohol and Methamphetamine

Amanda L. Blaker and Bryan K. Yamamoto

Department of Neurosciences

Methamphetamine (Meth) and alcohol abuse are common comorbid conditions but little is known about the neurochemical consequences of their co-abuse. Since each drug alone possesses inflammatory properties that contribute to their individual neurotoxic effects, we hypothesized that serial exposure to ethanol and Meth would produce greater neurotoxicity than after either drug alone. Male Sprague-Dawley rats were allowed to drink 10% ethanol, every other day for one month. One day after the last ethanol exposure, Meth (10mg/kg x 4 injections) or saline injections were administered. The results show that rats increased intake and preference for ethanol over 4 weeks and had elevated lipopolysaccharide in serum and brain as well as cyclooxygenase-2 in the brain (*p<0.05). One week after Meth, striatal dopamine and serotonin concentrations were depleted by 95% and 75%, respectively, in ethanol-drinking rats. The substantial dopamine depletions produced by Meth after ethanol drinking were greater than after Meth in water-drinking rats and were paralleled by a decrease in tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta (*p<0.05) and a decrease in motor function measured via rotarod test (*p<0.05). Neurotransmitters were not depleted in rats that drank ethanol but not challenged with Meth. To examine the role of inflammation, administration of a cyclooxygenase inhibitor during ethanol drinking blocked the enhanced neurotransmitter depletions after Meth and suggests that inflammation during ethanol drinking mediates the enhanced neurotoxicity after Meth. Future studies will investigate the interaction between glutamate and inflammation in the synergistic effects produced by the serial exposure to ethanol and Meth.

Grant Support: NIH 1R01DA042737

P4: Identification and description of Burkholderia pseudomallei proteins that bind host complement-regulatory proteins via in silico and in vitro analysis

Caroline Lambert, Laura Nejedlik, Irum Syed, R. Mark Wooten

Department of Medical Microbiology and Immunology

Burkholderia pseudomallei (Bp) is a motile gram-negative bacillus; an environmental saprophyte and can persist intracellularly. Bp is the causative agent of melioidosis, a febrile disease which commonly presents as sepsis. Our current emphasis is exploring how Bp evades the innate immune response via binding to alternative pathway regulatory protein factor H (fH), preventing complement deposition on Bp's surface. FH-binding proteins (fHbps) exist in a microbe's outer membrane (OM) to facilitate exposure and binding to fH.

Several microbial species possess fHbps including, Y. enterocolitica, via YadA, N. meningitidis via fHbp and H. influenza via P5. We hypothesize that Bp possesses one or more fHbps. Western blot analysis in our lab using OM protein preparations demonstrated fH binding to whole cell Bp and B. thailandensis, an environmental avirulent strain of Burkholderia. In Burkholderia, BpaC appears to have a role in resistance to serum-mediated killing. This suggests its role as a potential fHbp.

BLAST database searches identified BpaC in Bp 1026b as possessing meaningful homology to Y. enterocolitica YadA. Homology and ab initio-based modeling predicted BpaC to possess four structural domains. Topological algorithms speculate domain two as being entirely extracellularly exposed. Protein binding energy prediction server PRODIGY determined binding affinity between BpaC domain two and fH domains 19-20 to be stronger than the interaction between known fHbp OspE in B. burgdorferi and fH domains 19-20.

These findings support bpaC interaction with fH to prevent complement deposition via the alternative pathway. In vivo methods to confirm this interaction are on-going.

Grant Support: National Institute of Health

P5: Genetic polymorphism effect of the human SULT1A1 on the sulfation of some nonopioid and opioid analgesics

Mohammed I. Rasool, Prof. Ming-Cheh Liu

Department of Pharmacology and Experimental Therapeutics

Nonopioid and opioid analgesics have been widely and successfully used for long/short term clinical management of a diverse array of pathophysiological conditions either as over the counter or prescribed medications. Among the known human sulfotransferase (SULT) enzymes, SULT1A1 is believed to be the most important SULT in xenobiotic metabolism due to its broad substrate range. Previous studies indicated that SULT1A1 enzyme was involved in the sulfation of acetaminophen, O-DMN, and tapentadol. The purpose of this study was to investigate the effects of single nucleotide polymorphisms (SNPs) in human SULT1A1 genes on the sulfating activity of SULT1A1 allozymes towards those analgesics. A systematic data search was performed to determine the nonsynonymous missense SNPs in human SULT1A1 genes. Nine recombinant SULT1A1 allozymes were generated, expressed, and purified. Enzymatic characterization of the purified SULT1A1 allozymes exhibited, compared with the wild-type enzyme, significant and dramatic differences in their catalytic activity with the tested substrates. The data from this investigation might yield additional insights into the functional consequences of amino acid substitutions that would help in anticipating the analgesic action, side effects, and toxicity profiles of those analgesics when used for individuals with different SULT1A1 genotypes.

Grant Support: This work was supported in part by a grant from National Institutes of Health (Grant # R03HD071146).

P6: Effect of platelet-leukocyte aggregates on platelet and T cell activity in lung cancer patients

Claire Meikle, Joseph Jeffries, Adam Meisler, Erin Crawford, James Willey, Randall Worth

Department of Medical Microbiology and Immunology

Cancer patients are known to be at high risk for thrombosis, a leading cause of death among cancer patients. Platelet-leukocyte aggregates (PLAs) are associated with increased thrombosis risk, but it remains unclear how PLA formation may influence immune function in cancer. We tested the hypothesis that platelets interact with T cells in cancer-specific ways, leading to changes in platelet and T cell function. Whole blood samples from lung cancer patients and healthy volunteers were labeled with specific antibodies, fixed, then analyzed using flow cytometry to detect platelet-T cell aggregates and surface markers of activation. Lung cancer patients had significantly more platelet-T cell aggregates than healthy controls (36.89% of total T cells vs. 18.15%, respectively). There was a modest increase in activation of unbound platelets in lung cancer patients compared to healthy controls (mean fluorescence intensity of P-selectin 18.28 and 11.18 units, respectively), whereas T cell-bound platelets were significantly more activated in lung cancer patients compared to healthy controls (MFI of 138.4U and 35.15U, respectively). Ultimately we seek to elucidate the mechanism and downstream effects of platelet-T cell aggregate formation on immune surveillance and thrombosis in lung cancer patients. As immunotherapy becomes more commonly used to treat cancer, an understanding of the role of platelets in mediating T cell behavior and platelet sensitivity will be critical to optimizing T cell therapy efficiency while reducing thrombosis risk in lung cancer patients.

P7: Inter-Laboratory Harmonization of Next Generation Sequencing Somatic Mutation Assays for Cancer Response Prediction

Daniel J. Craig, Thomas M. Blomquist, Erin L. Crawford, James C. Willey

Department of Cancer Biology

Next generation sequencing (NGS) analysis to identify treatment-specific molecular targets associated with significantly higher cancer response (Precision Therapy) is advancing rapidly. However, inter-laboratory reproducibility in reporting of mutation fraction is sub-optimal, in part due to uncertainty resulting from variation in specimen quality and technical artifacts introduced throughout the analysis. In preliminary studies with PCR-amplicon NGS libraries, each diagnostic specimen was mixed with an internal standard (IS) mixture (ISM) of competitive spike-ins prior to library preparation. This approach enabled reliable measurement of 95% confidence limits around each somatic mutation measurement, controlled for variation in specimen adequacy, and enabled reliable measurement of actionable mutation fraction as low as 0.01%. In order to expand application across all NGS platforms we prepared an ISM according to a novel design suitable to measure tumor somatic mutations (analytes) in 24 genes by either PCR-amplicon or hybrid capture libraries for NGS analysis. Through collaboration with the FDA-led SEquencing Quality Control Project Phase 2 (SEQC2) we developed highly characterized cell line mixtures to assess cross-site reproducibility and mutation detection accuracy of multiple targeted NGS platforms. In pilot studies, mixture of SEQC2-24 ISM with specimens did not interfere with analysis by existing workflow for PCR-amplicon (ThermoFisher) or hybrid capture (Roche) library NGS and was associated with low imprecision (coefficient of variation <10%). It is expected that results obtained through completion of SEQC2 will support the conclusion that use of SEQC-24 ISM and related pipeline will enable reliable measurement of somatic mutation fraction to a level significantly lower than that currently in practice.

Grant Support: George Isaac Cancer Research Fund

P8: Targeted therapies against Breast cancer stem-like cells

Sangita Sridharan and Dayanidhi Raman

Department of Cancer Biology

Breast cancer represents one of the major reasons for fatalities worldwide, claiming a staggering 15% of all the cancer-related deaths in women. It is often not the primary tumor itself, but recurrence and metastasis leading to high mortality rates due to breast cancer. Recurrence can often be attributed to the presence of residual cancer stem-like cells (CSCs) that persist even after the chemotherapeutic regimen. Also termed as tumor initiating cells, these cancer stem-like cells contribute significantly to the tumor landscape owing to their unparalleled ability to recapitulate the heterogeneous bulk tumor and relative quiescence, thereby endowing them with the ability of being refractory to chemo/radio-therapeutics. This residual population forms a major reason why monotherapy fails invariably in the clinics and patients relapse. Therefore, combinatorial approaches co-targeting the bulk tumor and CSCs will be necessary. Towards that effect, we have been successful in isolating and characterizing distinct stem cell populations that are positive for CSC markers - aldehyde dehydrogenase (ALDH+) and CD44+/CD24- population. The ALDH+ CSCs formed mammospheres in anchorage-independent conditions. Currently, we are generating paclitaxel-resistant cell line to challenge with novel chemical inhibitors. This will be employed in a chemical screen to identify novel drugs/inhibitors that could co-target bulk tumor cells, naïve and the drug-resistant CSCs. The promising compounds would be then translationally tested in pre-clinical studies.

Grant Support: University of Toledo startup funds, OCRA grant, R21 -Dr.Dayanidhi Raman

P9: A polymorphic variant of Secreted Phosphoprotein 2 as a quantitative trait nucleotide linked to the heritability of blood pressure and bone mineral density in a gender dependent manner

Saroj Chakraborty, Ying Nie, Xi Cheng, Blair Mell, Sarah Galla, Peter J Czernik, Beata Lecka-Czernik and Bina Joe

Department of Physiology and Pharmacology

Hypertension is a complex polygenic disease caused by a combination of genetic and environmental factors. Rat models serve as tools to dissect and prioritize genetic factors as candidate genes causing hypertension. One such candidate gene prioritized through systematic linkage and substitution mapping is Secreted Phosphoprotein 2 (Spp2). A single non-synonymous G/T polymorphism between the Dahl Salt-Sensitive (S) rats and Spontaneously Hypertensive Rats (SHR) at the Spp2 locus was hypothesized to cause a reduction in blood pressure (BP) and bone mineral density (BMD) observed in the S.SHR congenic strain spanning the Spp2 locus. To test this hypothesis, a novel rat model was generated using the CRISPR/Cas9 precision-engineering technology, whereby the 'G' allele at the Spp2 locus of the S rat was replaced by the 'T' allele of the SHR rat. Protein modeling prediction by SWISSPROT indicated a significantly altered protein structure of the Spp2 protein in Spp2 knock-in model. Radiotelemetry and micro-CT was done with Spp2 knock-in rats using S rat as control. Systolic BP of the Spp2 knock-in male rats was significantly lower compared to that of the non-founder S rats. However, systolic BP of the Spp2 knock-in female rats was significantly higher compared to that of the non-founder S rats. In addition, the bone volume by total volume ratio was significantly lower in female Spp2 knock-in rats. These data provide conclusive evidence for a single nucleotide polymorphism within the Spp2 gene as a quantitative trait nucleotide (QTN) responsible for the inheritance of blood pressure and bone mineral density.

Grant Support: Funding for this work to BJ from the NHLBI/NIH (HL020176) is gratefully acknowledged

P10: Molecular Cloning, Expression, and Characterization of A Novel Zebrafish Cytosolic Sulfotransferase, SULT5A1

Daniyah Almarghalani, Ming-Cheh Liu

Department of Pharmacology & Experimental Therapeutics

Sulfotransferase enzymes (SULTs) are responsible for phase II detoxification of xenobiotics as well as regulation of many endogenous compounds, including thyroid/ steroid hormones, bile acids, and catecholamine neurotransmitters. In recent years, zebrafish is emerging as an important animal model for drug metabolism research. This study is part of an overall effort to establish the zebrafish as a model for studying drug sulfation. By searching the GenBank database, the last remaining zebrafish sequence encoding a putative SULT, designated SULT5A1, was identified. Zebrafish SULT5A1 was subsequently cloned, expressed, purified, and characterized. Substrate specificity of zebrafish SULT5A1 was analyzed using a panel of more than 147 xenobiotics, endogenous compound, bile acids, and commercially available bile alcohols. SULT5A1 showed strong sulfating activity toward bile acid and bile alcohol compounds, including 5 α -cyprinol, 5 β -cyprinol, 5 β -scymnol, 5 β -cholestantriol, PZ, and 5 α -lithocholic acid. It also exhibited significant activity toward endogenous compound, including DHEA and pregnenolone. However, SULT5A1 showed no activity toward xenobiotics. pH iv dependence and kinetic studies were performed using zebrafish SULT5A1 with 5 α -cyprinol, 5 α -petromyzonol (PZ), DHEA, and pregnenolone as substrates.

Grant Support: The University of Toledo

P11: Francisella tularensis Cell Wall Remodeling and Virulence

B. Zellner and J.F. Huntley

Department of Medical Microbiology and Immunology

Francisella tularensis (Ft), the causative agent of tularemia, is one of the most dangerous bacterial pathogens known. Due to its low infectious dose, ease of aerosolization, and high morbidity and mortality, it has been designated as a Tier 1 Select Agent. Despite decades of research, few Ft virulence factors have been identified or characterized. Characterization of novel bacterial virulence factors is important to understand the molecular mechanisms by which Ft causes disease. We previously demonstrated that Ft encodes a disulfide bond formation protein ortholog, DsbA, which is required for virulence in macrophages and mice. Using a molecular trapping approach, we identified >50 DsbA substrates, approximately half of which are annotated as hypothetical proteins. In the current study, we selected one of these previously uncharacterized DsbA substrates, FTL1678, for detailed analysis. Using bioinformatic tools, FTL1678 was found to contain a putative LD-carboxypeptidase domain, indicating a potential role in peptidoglycan remodeling. An isogenic mutant of FTL1678 was completely attenuated in a mouse pulmonary infection model, with decreased lung colonization and inability to disseminate to livers or spleens. Importantly, immunization with this mutant provided significant protection against pulmonary challenge with the fully-virulent Ft strain SchuS4 (BSL3). Membrane integrity testing revealed increased susceptibility of the mutant to several antibiotics and detergents. In addition, electron microscopy analysis of the mutant showed increased cell wall thickness. Current studies are comparing peptidoglycan structure between wild-type Ft and the mutant using mass-spectrometry and examining defects in macrophage intracellular trafficking/replication by the mutant.

Grant Support: This work was supported by NIH-NIAID Grant R01 AI093351 and bridge funding from the University of Toledo College of Medicine and Life Sciences to JFH.

P12: ROLE OF COUP-TFII, A NUCLEAR RECEPTOR FAMILY MEMBER PROTEIN IN GLUCOSE HOMOEOSTASIS AND BLOOD PRESSURE REGULATION IN DAHL SS RATS

Usman Ashraf and Sivarajan Kumarasamy

Department of Physiology and Pharmacology

Consumption of diets that are rich in salt and/or fats develops cardiovascular complications such as hypertension, metabolic syndrome, and associated diseases. Through genome wide association studies, a locus with significant association for hypertension was found in human chr15 containing the gene chicken ovalbumin upstream promotor transcription factor 2 (COUP-TFII). Targeted disruption of Coup-TFII locus in Dahl Salt Sensitive (SS) rats improves their BP and renal function compared to wild-type rats under high salt diet fed condition. In-vitro, Coup-TfII expression levels are modulated in response to glucose levels in H4IIE rat hepatoma cells. Under high glucose condition Coup-TfII expression was decreased ($p \leq 0.05$); whereas in low glucose condition Coup-TfII expression was increased ($p \leq 0.05$). Further, Coup-TfII gene expression was up-regulated in SS rat liver during fasting as compared the re-fed condition ($p \leq 0.05$). Furthermore, the Coup-TfII mutant rat demonstrated a low fasting blood glucose and superior glucose response, along with an increase in insulin sensitivity measured by glucose and insulin tolerance test ($p \leq 0.05$). mRNA expression analysis in livers of SS and Coup-TfII mutant rats revealed key enzymes involved in both beta oxidation and gluconeogenesis were down regulated in Coup-TfII mutant rats compared to SS rats ($p \leq 0.05$). Previously, we have shown that the SS rat when maintained on a high calorie diet (60% Kcal) significantly increases the BP compared to rats fed with low calorie (10% Kcal) diet. Overall, these findings suggest that further understanding of Coup-TFII function in the event of diet induced changes in glucose homeostasis and BP will advance our knowledge in cardiovascular diseases.

Grant Support: American Heart Association 16SDG27700030

P13: Differential Roles of Antibiotics on Gut Microbiota in Rat Genetic Models of Hypertension

Sarah Galla, Saroj Chakraborty, Xi Cheng, Ji-Youn Yeo, Blair Mell, Helen Zhang, Bina Joe

Department of Physiology and Pharmacology

Alterations in gut microbiota are associated with pathologies ranging from cancer to neurological, immunological, cardiovascular and renal disorders. Studies in animal models and humans have demonstrated a link between gut microbiota and hypertension, a leading risk factor for cardiovascular and renal disorders. Reshaping microbiota through transplantation or by intake of antimicrobial agents, such as salt and minocycline, are reported to modulate blood pressure (BP) beneficially or adversely, depending on the genetic makeup of the host. This suggests that broad-spectrum antibiotics, prescribed to eliminate bacterial infections, may influence the extent of hypertension depending on host genome. To test this hypothesis, three antibiotics of different classes, neomycin, minocycline, and vancomycin, were administered to two genetic models of hypertension, the Dahl Salt-Sensitive (S) rat and the Spontaneously Hypertensive Rat (SHR), both of which develop hypertension, but for disparate genetic reasons. Regardless of the class, oral administration of antibiotics caused an elevation in systolic BP in the S rat, while minocycline and vancomycin, but not neomycin, caused a reduction in systolic BP in the SHR. Interestingly, these changes were accompanied by alterations in the gut microbiota and disparate levels of pro-inflammatory markers depending on the antibiotic and the strain. Our results demonstrate that alterations in BP occur and vary in response to oral antibiotics and that host-microbial interactions contribute to the observed differences of individual BP responses to oral antibiotics. Our study shows that hypertensive subjects, depending on their genomes and microbiomes, could have altered BP responses to antibiotic usage.

Grant Support: Funding for this work to BJ from the NHLBI/NIH (HL020176) is gratefully acknowledged.

P14: Effect of Nitric Oxide Expression on Chaperone Protein Hsp90 and its implication in Breast Cancer

Yashna Walia, Dr. Saori Furuta

Department of Cancer Biology

Breast cancer is one of the leading cause of cancer related death among women worldwide. Facing yet slow improvement in breast cancer mortality but a steady increase in the number of incidence, it is vital to advance our understanding of the cause of breast cancer. Our project involves determination of the link between reduction of Nitric Oxide level and the activation of Hsp90, a major chaperone protein, targeted for cancer treatment, in the breast tissue during malignant progression. Our lab has previously found that normal mammary glands produce the basal level of NO, whereas, its inhibition led to the formation of premalignant lesions. In my preliminary findings, it was also seen that under low Nitric Oxide level there was a dramatic activation of Hsp90. We thereby, formed an hypothesis that Nitric Oxide downregulates the activation of Hsp90 via S-nitrosylation, which is a major post-translational modification regulated by Nitric Oxide. The primary goal of my project is divided into three main categories: 1) To identify whether Hsp90 is S-nitrosylated in the breast tissue under normal physiological conditions, 2) whether and how S-nitrosylation regulates Hsp90 activity and 3) To identify how the loss of S -nitrosylation of Hsp90 contributes to malignancy. We will particularly focus on the involvement of activation of the Her2 oncogene, one of the major client of Hsp90, in formation of precancerous lesions upon depletion of Nitric Oxide. Through this project, I expect that the proposed research will fundamentally advance our insight into the cause of breast cancer.

Grant Support: UT Startup Fund and ORCA

P15: Synthesis and Biological Evaluation of HDAC Inhibitors with an Imidazole-Based Metal-Binding Group

Samkeliso M Dlamini,¹ Ayad Al-Hamashi,¹ Abdulateef Alqahtani,¹ Radhika A. Koranne, ² Maisha S. Rashid, ² William Taylor, ² and L. M. Viranga Tillekeratne ¹

1. Department of Medicinal and Biological Chemistry, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, OH 43606.

2. Department of Biological Sciences, College of Natural Sciences and Mathematics, University of Toledo, Toledo, OH 43606."

Cancer is the second leading cause of death in the United States of America and the entire world and the disease burden is exacerbated due to resistance to drugs that are currently in clinical use. Histone deacetylase (HDAC) enzymes are highly expressed in cancer cells and they are considered viable targets for drug intervention. Four HDAC inhibitors have been approved by the US FDA as anticancer drugs for clinical use, but these drugs have numerous side effects, at least partly due to their low HDAC isoform selectivity. Most of these drugs have hydroxamic acid group as the metal-binding group. Hydroxamates are promiscuous in their metal-binding ability and also have poor pharmacokinetic properties. In this study, we used molecular modeling studies to design and develop a new class of HDAC inhibitors incorporating a new imidazole-based metal-binding group. The compounds showed promising cell growth inhibitory activity comparable to or better than that of the clinically used HDAC inhibitor SAHA and a cell phenotype which is distinctly different from what was observed with SAHA. The design, synthesis and biological activity of these compounds will be presented.

P16: A Novel CD40-target Peptide Inhibits Pro-inflammatory Signaling in Renal Proximal Tubule Epithelial Cells

Shungang Zhang, MS, Andrew Kleinhenz, BS, Erin Crawford, MS, David Kennedy, Ph.D., Steven T. Haller, Ph.D

Department of Medicine

Introduction: We have demonstrated that activation of the CD40 receptor (an essential mediator of immunity and inflammation) significantly contributes to the development of renal injury both clinically in patients with renal disease and experimentally using our novel CD40 mutant model in which CD40 function is abolished. Activation of CD40 in renal proximal tubule epithelial cells induces local inflammation in the kidney contributing to the development of renal injury. We treated human proximal tubule epithelial cells (HK2 cells) with soluble CD40 ligand (sCD40L) to induce CD40 signaling as well as with a novel CD40-targeted peptide (designed to inhibit sCD40L from binding to CD40) to test the hypothesis that inhibition of CD40 signaling in proximal tubules significantly reduces the release of pro-inflammatory mediators.

Methods: HK2 cells were treated with sCD40L at a concentration of 100ng/ml for 24h to induce CD40 signaling. Cytokine secretion from HK2 cells was detected using an antibody-conjugated cytokine secretion assay. A CD40-targeted peptide (1ug/ml, 10ug/ml and 100ug/ml) was incubated in the presence and absence of sCD40L prior to treatment and the effects were evaluated by Western blot and real-time PCR assays.

Results: Treatment with sCD40L resulted in a five-fold increases in the pro-inflammatory mediator monocyte chemotactic protein-1 (MCP-1) ($P < 0.01$). Co-treatment with the CD40-target peptide significantly reduced MCP-1 expression by three-fold compared to sCD40L treatment alone ($P < 0.01$).

Conclusion: Inhibition of CD40 signaling using a novel CD40-targeted peptide significantly reduced proximal tubule expression of MCP-1 and may serve as a potential therapy for local inflammation in renal disease.

Grant Support: University of Toledo Medical Research Society Awards

P17: Elucidation of the cellular function of TRIM79 reveals distinct mechanisms of tick-borne flavivirus restriction

Jagaree Das, Brian H. Youseff, John B. Preslold, R. Travis Taylor

Department of Medical Microbiology and Immunology

Vector-borne flaviviruses, like West Nile, dengue, and tick-borne flaviviruses (TBFVs), infect millions worldwide, causing severe encephalitis or hemorrhagic fever with no specific antiviral treatment and limited vaccines available. To identify new therapeutic options, we previously identified a TBFV-specific mediator of the interferon response called TRIM79. TRIM79 binds to the viral polymerase NS5, critical for viral replication inside the host, and targets it for lysosomal degradation. TRIM proteins are potent antiviral mediators, in addition to regulators of innate immunity pathways, though their normal function inside the cell is largely unknown. TRIMs contain a ubiquitin ligase domain and function through direct ubiquitination of substrate proteins. To gain insight into the mechanism used by TRIM79 to inhibit TBFV infections, we sought to identify its normal cellular role. In silico modeling suggested an interaction with TRAF6, an adapter enzyme for TLR and $IL1-\beta$ signaling. Additionally, work from our lab revealed TRAF6 as co-factor for the viral protease, and necessary for optimal virus replication. We confirmed a physical interaction between TRAF6 and TRIM79 by confocal microscopy and co-immunoprecipitation assays. To see whether TRIM79 impacts TRAF6 function, we used luciferase assays to evaluate TRAF6-dependent promoter activation in the presence or absence of TRIM79. We found that TRIM79 inhibited TRAF6 activity in a dose-dependent fashion, suggesting that TRIM79 is a new negative regulator of TLR signaling, and thus may also inhibit TBFVs by two distinct mechanisms. Future studies will determine the role of TRAF6 on TRIM79 restriction of TBFVs.

Grant Support: 1K22AI099020

P18: The Role of CD40 Signaling in Chronic Renal Allograft Rejection in a Hypertensive Rat Model

Vassili S. Bletsos, Joshua D. Breidenbach, Steven T. Haller, Stanislaw M. Stepkowski

Department of Medical Microbiology and Immunology

Background: While playing a fundamental role in B and T lymphocyte interaction along with macrophage activation, CD40 is also expressed in kidney proximal tubule epithelial cells. We have explored the role of CD40 signaling in kidney proximal tubule epithelial cells on renal interstitial fibrosis (IF) in models of hypertension (HTN); and renal chronic allograft rejection (CAR). We used spontaneously hypertensive salt-sensitive Dahl (Dahl-S) and CD40 mutant (Dahl-SCD40mut) rats in which CD40 function is abolished. As previously reported, Dahl-SCD40mut showed significantly less fibrosis and improved kidney function parameters over Dahl-S rats without differences in blood pressure, suggesting that CD40 regulates IF in HTN. We hypothesize that CD40 signaling regulates IF in both HTN and CAR. Methods/Results: Dahl-S and Dahl-SCD40mut rat kidneys were examined for renal IF at 64 days of age or at 90 days post renal transplantation to normotensive Brown Norway (BN) allogeneic recipients. Normotensive BN recipients of renal allografts treated for 30 days with tacrolimus (to block acute rejection) displayed significantly reduced IF in kidneys from Dahl-SCD40mut donors compared to Dahl-S donors as assessed by Masson's trichrome staining ($P < 0.01$). Dahl-SCD40mut kidney transplants also had reduced collagen 1A1, 3A1 as well as MCP-1 and PAI-1 expression compared to Dahl-S kidney grafts ($p < 0.05$ for all). Conclusion: Our data suggest that CD40 signaling significantly contributes to the development of interstitial fibrosis in chronic allograft rejection. We propose that direct inhibition of CD40 signaling in kidneys may have a dramatic impact on IF in chronic allograft rejection.

Grant Support: Internal Funds

P19: Cardiostonic steroid signaling through Na/K-ATPase- α -1 and Src kinase enhance functional interactions between immune cells and endo/epithelial cells

Fatimah K. Khalaf MBChB, Amal Mohamed BS, Andrew L. Kleinhenz BS, Erin L. Crawford MS, Jiang Tian PhD, Zijian Xie PhD, Deepak Malhotra MD PhD, Steven T. Haller PhD, and David J. Kennedy PhD

Department of Medicine

Introduction: Cardiostonic steroids (CTS) are Na/K-ATPase α -1 isoform (NKA α -1) ligands that are increased in volume expanded states associated with renal diseases, such as chronic kidney disease. We have found that CTS mediate pro-inflammatory response upon binding and signaling through the NKA α -1. Immune cell adhesion is a critical step in the inflammatory response, however it is unknown whether CTS play a role in driving this important process.

Objective: We tested the hypothesis that CTS signaling through NKA α -1 and Src kinase enhances immune adhesion to epithelium that advance inflammation.

Methods/Results: We found that in THP-1 monocytes the CTS telocinobufagin (TCB, 10 nM, 24 hours) enhanced the expression of the β 2 integrin family members CD11b/CD18 ($p < 0.05$) which are important in cellular adhesion and cell cell interactions. Additionally, TCB induced the expression of intercellular adhesion molecules I-CAM and V-CAM (both $p < 0.05$) in a human endothelial cell line. Next, we found that TCB induced increases in the adhesion of monocytes to endothelial cells compared to vehicle control ($p < 0.05$). Next, we tested the effect of TCB on macrophage adhesion in 2 stable cell lines derived from LLC-PK1 renal proximal tubular cells which had either normal 90% NKA α -1 knock-down. Further, pretreatment of wild type cells with a specific peptide inhibitor of the NKA α -1-Src kinase pathway. Additionally, we found that rats injected with TCB showed a significant increase in the accumulation of immune cells in the peritoneal cavity compared to vehicle treated animals ($p < 0.05$). Finally, we infused TCB into mice. Peritoneal macrophages collected from mice expressed increased adhesion markers. Also adhesion markers induced by TCB were decreased in NKA-Het mice while they were increased in Tg vs WT ($p < 0.05$).

Conclusion: These findings suggest that CTS potentiate immune cell activation and adhesion to endo/epithelium through an Na/K-ATPase- α -1/Src dependent mechanism.

Grant Support: American Heart Association (National) Scientist Development Grant Award Number: 14SDG1865001C

P20: Impact of SULT1A3/SULT1A4 Genetic Polymorphisms on the Sulfation of Catecholamines and Serotonin by SULT1A3 Allozymes

Ahsan F. Bairam, Ming-Cheh Liu

Department of Pharmacology and Experimental Therapeutics

Previous studies have demonstrated the involvement of sulfoconjugation in the metabolism of catecholamines and serotonin. The current study aimed to clarify the effects of single nucleotide polymorphisms (SNPs) of human SULT1A3 and SULT1A4 genes on the enzymatic characteristics of the sulfation of dopamine, epinephrine, norepinephrine and serotonin by SULT1A3 allozymes. Following a comprehensive search of different SULT1A3 and SULT1A4 genotypes, twelve non-synonymous (missense) coding SNPs of SULT1A3/SULT1A4 were identified. cDNAs encoding the corresponding SULT1A3 allozymes, packaged in pGEX-2T vector were generated by site-directed mutagenesis. SULT1A3 allozymes were expressed, and purified. Purified SULT1A3 allozymes exhibited differential sulfating activity toward catecholamines and serotonin tested. Kinetic analyses demonstrated further differential substrate affinity and catalytic efficiency of the SULT1A3 allozymes. Collectively, these findings provide useful information relevant to the differential metabolism of dopamine, epinephrine, norepinephrine and serotonin through sulfoconjugation in individuals having different SULT1A3/SULT1A4 genotypes.

Grant Support: This work was supported in part by a grant from National Institutes of Health (Grant # R03HD071146).

P21: Impact of pre-existing liver disease on microcystin hepatotoxicity

Apurva Lad, Adam Spegele, Andrew Kleinhenz, Fatimah K. Khalaf, Shungang Zhang, Robin Su, Deepak Malhotra, Steven Haller, David Kennedy

Department of Medicine, Department of Medical Microbiology and Immunology

Microcystins are a class of hepatotoxic cyclic heptapeptides produced by cyanobacteria that pose a serious and growing global public health risk. The health based criteria for safe exposure limits to microcystins has been extrapolated to humans from toxicology studies performed only in healthy animal models. However, the effect of these hepatotoxins in at-risk settings such as pre-existing liver disease is unknown. We tested the hypothesis that the No Observed Adverse Effect Level (NOAEL) of microcystin, as established in healthy animals, would cause significant hepatic injury in a murine model of Non-alcoholic Fatty Liver Disease (NAFLD). To study this, we gavaged male Leprdb/J mice, a genetic model of NAFLD, with 50µg/kg or 100µg/kg microcystin-LR or vehicle every 48 hours for 4 weeks (n=12-16 mice/group). Quantitative histopathological analysis of the stained sections indicated that there was a significant increase in microvesicular steatosis in a dose-dependent manner (p<0.01). Moreover, we observed a trend in decreased survival with control group showing 100% survival whereas the 50µg/kg and 100µg/kg group showing 93% and 85% survival respectively. Next, we investigated the changes in genetic markers of major drug-induced hepatopathology using quantitative PCR and observed that treatment with MC-LR yielded significant increases in genetic markers of cholestasis, steatosis, non-genotoxic hepatocarcinogenicity, necrosis, and generalized hepatotoxicity (p<0.05). Furthermore, treatment with microcystin-LR also significantly elevated hepatic antioxidant enzyme gene expression levels, as well as genes involved in reactive oxygen species metabolism and oxygen transporters (p<0.05). Our results suggest that the NOAEL of MC-LR results in significant hepatic injury in NAFLD.

Grant Support: Ohio Department of Higher Education - State of Ohio Sea Grant Lake Erie Research Initiative Award Number: N-124973-01

P22: The Identification of a Factor H-Binding Protein in Burkholderia

I. A. Syed, L. S. Nejedlik, C. L. Lambert, M. E. Woodman, M. Mulye, and R. M. Wooten

Department of Medical Microbiology and Immunology

Background: Melioidosis is caused by the encapsulated Gram-negative organism *Burkholderia pseudomallei* (Bp). Due to its low LD₅₀, high infectivity, and antibiotic resistance, Bp is considered a Tier 1 select agent and there is great interest in characterizing virulence factors that may be targets for novel therapeutic agents. Our lab has previously shown that unopsonized Bp is not efficiently cleared by macrophages or neutrophils.

Hypothesis: We hypothesize that Bp has inherent resistance to complement deposition, mediated by expression of outer membrane proteins (OMPs) that bind complement factor H (fH) to avoid clearance by host immune mechanisms.

Methods and Results: While Bp and Bt were efficiently cleared by neutrophils if they possessed a critical threshold of C3 deposition, normal human serum (NHS)-opsonized Bp displayed less C3 deposition than Bt. Upon far-Western analysis of Bp and Bt OMPs for fH binding, it was determined a protein capable of binding fH is ~37kD. Mass spectrometric analysis of the 37kD proteins extracted from Bp and Bt OMP preps produced 300+ hits, and further bioinformatics analysis produced a short list of putative candidates.

Conclusions: Bp is more resistant to C3 deposition than Bt, but can be efficiently cleared/killed by neutrophils if critical C3 levels are achieved. Bp and Bt express at least one protein on their outer membrane capable of binding fH.

Moving Forward: We are currently cloning and expressing the best candidates to determine their relative abilities to bind fH. We are also constructing gene-knockouts for these candidates in Bp to assess functional effects.

Grant Support: The National Institute of Health

P23: Paraoxonase Regulation of Cardiotonic Steroids in Chronic Kidney Disease

Chrysan J. Mohammed BS1, Subhanwita Ghosh MS1, Bruce S. Levison PhD2, Yanmei Xi MA1, Pamela S. Brewster MS1, Andrew L. Kleinhenz BS1, Erin L. Crawford MS1, Deepak Malhotra MD PhD1, Richard W. James PhD3, Philip A. Kalra MD4, Steven T. Haller PhD1, David J. Kennedy PhD1

1Department of Medicine, 2Department of Physiology and Pharmacology, University of Toledo College of Medicine and Life Sciences, Toledo, Ohio, USA; 3Department of Internal Medicine, Geneva University Hospital, Geneva, Switzerland; 4Salford Royal Hospital, Salford, United Kingdom.

The 2-pyrone ring structure of cardiotonic steroids (CTS) is critical for their binding to the Na⁺/K⁺-ATPase and initiation of pro-fibrotic signaling in chronic kidney disease (CKD). Paraoxonases (PONs) are enzymes capable of hydrolyzing compounds similar to the 2-pyrone rings in CTS, however the native physiologic substrate(s) of PON's are unknown.

Hypothesis: 2-pyrone containing CTS are substrates for PON hydrolytic activity and this specific activity is decreased in CKD.

Methods/ Results: We examined the ability of the CTS to compete with a chemically similar specific fluorogenic substrate of PON's (7-hydroxycoumarin). Purified RR and QQ genotype of PON-1 was reacted with 7-hydroxycoumarin in the presence and absence of CTS. PON-1 hydrolytic activity toward 7-hydroxycoumarin was significantly reduced in the presence of CTS for both genotypes. To confirm that this reduction was related to hydrolysis of CTS, a specific LC-MS assay was developed to measure the 2-pyrone active form of CTS. Incubation of CTS with PON-1 overexpressing cells showed a significant decrease in the 2-pyrone form of CTS at 24 hours. We measured circulating PON-1 protein (ELISA) and 2-pyrone-like activity in diabetic nephropathy CKD patients vs non-CKD controls. We found that while circulating PON-1 protein levels was not significantly increased across CKD stages, circulating PON-1 2-pyrone-like activity was significantly decreased across all CKD stages vs non-CKD controls.

Conclusion: CTS may be physiologic substrates for PON's and participate in a novel regulatory mechanism via hydrolysis of the CTS 2-pyrone ring. Furthermore, circulating PON-1 appears to have diminished 2-pyrone-like activity in the setting of CKD.

Grant Support: NIH-NHLBI Award Number: 1RO1HL137004

P24: Development of a novel model for chronic renal allograft nephropathy

Joshua D. Breidenbach, Vassili S. Bletsos, Steven T. Haller, Stanislaw M. Stepkowski

Department of Medical Microbiology and Immunology

Background: While the development of immunosuppressive drugs has removed many of the boundaries in the use of kidney transplantation, there continue to be challenges. The most notable is chronic allograft nephropathy (CAN). Renal CAN is characterized by interstitial fibrosis (IF) and tubular atrophy (TA). These changes (referred to as IFTA) occur in 40% of kidney allografts after 3-6 months, and 65% after 2 years post-transplantation. An additional risk is hypertension in the renal transplant recipient which ranges from 50% to 80% in adult recipients and from 47% to 82% in pediatric recipients. This presents a need for an animal model that can incorporate both the alloimmune response and hypertension. A transplanted animal must be mismatched with its donor and treated with the correct level of immunosuppression so that the traditional pathological signs of CAN develop. We hypothesize that a Brown Norway (BN) rat transplanted with a Dahl-S kidney and treated with the right regime of tacrolimus will provide for a novel model of CAN. Methods: BN rats were transplanted with a Dahl-S kidney after unilateral-nephrectomy. The recipient received 1.5 mg/kg/day injections of Tacrolimus from post-operative day (POD) 0-14, 1.5 mg/kg 3 times a week from POD 15-30, and then again from POD 52 to organ harvest on POD 90. Results: BN recipients of Dahl-S kidney grafts developed IFTA shown by histological analysis using both H&E and Masson's trichrome stains. The development of this model will open the door to future studies of CAN and hypertension in renal transplantation.

Grant Support: Internal Departmental Funding

Oral Presentation Abstracts

O1: Role of mDia2 at Adherens Junctions in Epithelial Ovarian Cancer

Yuqi Zhang, Kathryn Eisenmann

Department of Cancer Biology

Epithelial ovarian cancer (EOC) cells disseminate within the peritoneal cavity, in part, via the peritoneal fluid as single cells, clusters, or spheroids. Initial single cell egress from a tumor involves the disruption of cell-cell adhesions as cells are shed from the primary tumor into the peritoneum. In epithelial cells, adherens junctions (AJs) are characterized by homotypic linkage of E-cadherins on the plasma membranes of adjacent cells. AJs are anchored to the intracellular actin cytoskeletal network through a complex involving E-cadherin, p120 catenin, β -catenin, and α E-catenin. However the specific players involved in the interaction between the junctional E-cadherin complex and the underlying junctional actin remains unclear. Recent evidence indicates that the actin-nucleating family of mammalian Diaphanous-related (mDia) formins plays a key role in epithelial cell AJ formation and maintenance. Binding of α E-catenin to linear F-actin inhibits association of the branched-actin nucleator Arp2/3, while favoring linear F-actin bundling. Our previous work showed that loss of mDia2 was associated with invasive egress from EOC spheroids. Current work indicates that mDia2 has a role at adherens junctions in EOC cells and human embryonic kidney (HEK) 293 cells through its association with α E-catenin and β -catenin. Both inhibition of mDia2 activity and depletion of mDia2 in EOC cells leads to disruption of cell-cell junctions, supporting the necessity of mDia2 for the stability of the junctional E-cadherin/catenin complex. Further experiments will be performed to determine the significance of the mDia2/catenin interaction in junctions maintenance, and thereby the effects of expression and activity of mDia2 in EOC metastasis.

Grant Support: Medical Research Society, Rita Sheely Endowment Fund

O2: Astrocyte Specific Insulin Receptor Deletion Contributes to Reproductive and Metabolic Dysregulation in Mice

Iyad H. Manaserh, Jennifer W. Hill

Department of Physiology and Pharmacology

Insulin resistance and obesity are associated with infertility and reduced GnRH release. It has been previously shown that male and female mice lacking insulin signaling in all cell types of the brain during development exhibit subfertility and develop diet-sensitive obesity, mild insulin resistance and elevated insulin and leptin plasma levels. To determine whether astrocyte insulin sensing plays an important role in the regulation of fertility, we generated mice lacking insulin receptors in astrocytes (GLIRKO mice). GLIRKO males and females showed a delay in balanopreputal separation or vaginal opening and first estrous, respectively. GLIRKO female mice also exhibited longer estrus cycle lengths and irregular estrous cyclicity. In addition, these mice exhibited decrease in pregnancy rate and litter size, and thus decreased fertility. Histological examination of testes and ovaries showed impaired spermatogenesis and ovarian follicle maturation. GLIRKO mice also show hypothalamic hypogonadism, but normal sexual behavior, which confirms that the observed subfertility phenotype is due to HPG axis dysregulation. GLIRKO male mice displayed higher body weight and growth, and significant differences in body composition at 7 months of age. These mice also showed insulin resistance at 2 months and both insulin and glucose intolerance at 7 months of age. Histological analysis showed altered liver morphology with higher fat droplets. GLIRKO mice displayed significant decrease in energy expenditure, and a striking decrease in body temperature during fed state and overnight fasting conditions. However, when fasted mice were exposed to 4C, they were able to mount a robust thermogenic response and rapidly increase body temperature. These observations support the idea that astrocytic population that integrate information about energy stores to regulate body temperature is different from those required to respond to a cold challenge. Overall, our findings suggest that impaired insulin sensing in astrocytes delays the initiation of puberty and affects adult reproductive function. They also suggest that that astrocytic insulin signaling regulate body weight, systematic glucose metabolism and regulate thermogenic responses to nutritional and cold challenges.

Grant Support: NIH R01 HD081792 grant to Dr. Jennifer W. Hill

O3: PPAR α negatively regulates sclerostin production in osteocytes

Amit Chougule, Lance Stechschulte, Beata Lecka-Czernik

Department of Physiology and Pharmacology

The nuclear receptor PPAR α is a major regulator of energy production and lipid metabolism, however its role in bone homeostasis has not been analyzed in details. We have showed that sclerostin protein, which inhibits Wnt signaling and has anti-osteoblastic activity, is under negative control of PPAR α . Loss of PPAR α results in larger bone cavity, thinner cortex, decreased bone formation, increased bone resorption, and increase in marrow fat volume (MFV). We analyzed endosteal osteoblasts (OB) and cortical osteocytes (OT) freshly isolated from femora of WT and PPAR α KO mice. OT from KO mice have increased expression of Sost gene encoding sclerostin, while as expected OB have decreased expression of Wnt signaling markers. Consistently, there is 2-fold increase in sclerostin protein levels in PPAR α KO femur. Conditioned media (CM) collected from primary cultures of PPAR α KO OT have significantly higher sclerostin levels as compared to CM from WT OT. In cocultures, KO CM increases expression of adipogenic markers in recipient WT marrow mesenchymal stem cells. ChIP assay confirmed that PPAR α binds to α PPRE in the Sost promoter in basal conditions and the binding is increased upon activating or decreased upon inactivating PPAR α through its ligands. Increased PPAR α binding correlates with decreased Sost promoter activity and mRNA expression. In summary, PPAR α acts as a negative regulator of sclerostin production in OT, the activity which is responsible for decreased bone formation and increased MFV in PPAR α KO mice. These findings, position PPAR α as a potential target to control sclerostin levels and increase bone formation.

Grant Support: National Institutes of Health DK105825

O4: A novel role for VCA0732, a protein important to antimicrobial stress responses in *Vibrio cholerae*

Jessica Saul-McBeth and Jyl Matson

Department of Medical Microbiology and Immunology

The genomic sequence of many *Vibrio cholerae* strains has been completed, however, the functions of many hypothetical proteins have not been determined. One way to confirm the function of hypothetical proteins is through transcriptomic analysis. Previously, we used RNA-seq to identify the transcriptome of *V. cholerae* in the presence and absence of the antimicrobial peptide (AMP) polymyxin B. One of the most interesting results concerns VCA0732, a hypothetical protein, whose expression was highly induced by polymyxin B and was dependent on an uncharacterized two-component system. VCA0732 is a homologue of YdeI in *Salmonella enterica* Typhimurium and YgiW in *Escherichia coli*. These proteins localize to the periplasm, are implicated to play a role in the general stress response, and are predicted to interact with AMPs. We found that Δ vca0732 is sensitive to both oxidative and pH stress, similar to *E. coli* Δ ygiW. Complementation with either VCA0732 or YgiW in both *E. coli* and *V. cholerae* restored survival to wildtype levels, indicating that the two proteins have similar function. To determine whether VCA0732 interacts with antimicrobial peptides, we tested the ability of VCA0732 to bind to different AMPs. We determined that VCA0732 binds both polymyxin B and LL-37. Additionally, we found that LL-37 can enter the periplasmic space of *V. cholerae*. These data provide insight into the role of VCA0732 in both the general stress response and in responding to AMP damage. Ongoing investigation into the specific function of VCA0732 aims to better understand its role in the physiology of *V. cholerae*.

Grant Support: RO1

O5: Roles of BRD9 (bromodomain containing protein 9) in melanogenesis and melanoma proliferation

Tupa Basuroy and Dr. Ivana de la Serna

Department of Cancer Biology

Melanocytes are cells in the epidermis that produce the pigment melanin and protect skin against damage from ultraviolet radiation. Malignant melanoma, the most dangerous form of skin cancer, develops from the transformation of melanocytes. SWI/SNF chromatin remodeling complexes interact with master regulators of melanocyte differentiation and melanoma oncogenes to regulate the expression of genes important for melanogenesis and melanoma proliferation. Heterogeneous SWI/SNF complexes that contain either BRG1 or BRM as the catalytic subunit and an assortment of associated factors (BAFs) have been identified. BRG1 and BRM as well as some BAFs have bromodomains (BrDs) which bind to acetylated lysine residues in histone tails. Little is known about the role of bromodomain proteins in regulating SWI/SNF function. Small molecules that specifically inhibit the association of these BrD-containing proteins with chromatin can be used as tools to interrogate BrD function and may have therapeutic potential. I-BRD9 is a chemical inhibitor specific for BRD9, a newly identified BrD-containing component of SWI/SNF complexes that have BRG1 as the catalytic subunit. We found that BRD9 is highly expressed in melanocytes and melanoma cell lines. Co-immunoprecipitation studies indicated that BRD9 and BRG1 physically interact in melanoma cells. To test the hypothesis that BRD9 has a function in melanogenesis and melanoma proliferation, we treated melanocytes with chemical inhibitors of BRD9. Chemical inhibition of BRD9 resulted in decreased melanin synthesis and expression of genes that regulate melanocyte function. Decreased expression of genes that regulate melanin synthesis was associated with altered chromatin structure at regulatory sites. Depletion of BRD9 by siRNA had similar effects on gene expression as chemical inhibition. Furthermore, inhibition of BRD9 compromised proliferation and colony survival. In combination, our data indicate that Brd9 has an important role in regulating melanogenesis and melanoma proliferation and that chemical inhibition may be useful for treating melasma and melanoma.

Grant Support: University of Toledo Bridge Funding

O6: The CXCR4-LASP1-eIF4F Axis: A Novel Target in Triple Negative Breast Cancer

Cory Howard, Nicole Bearss, and Dayanidhi Raman

Department of Cancer Biology

Breast cancer is the most commonly diagnosed cancer among women and represents 15% of all new cancer cases in the United States. Despite the prevalence of the primary breast tumor, mortality mainly results from metastatic cancers. In addition, triple negative breast cancer is especially difficult to treat due to the limitation of current targeted therapies. Furthermore, CXCR4 has been identified to be one of the major chemokine receptors involved in breast cancer metastasis. Previously, our lab had identified LIM and SH3 Protein 1 (LASP1) to be a key mediator in CXCR4-driven invasion. Here, we report the novel interaction between LASP1 and the eukaryotic initiation 4F complex (eIF4F). Dysregulation in protein synthesis can be considered a hallmark of cancer. Moreover, the eIF4F complex is a critical component of translational initiation and consists of three core proteins: eIF4E, eIF4G, and eIF4A. Despite its ubiquitous nature, cancers more heavily rely on this complex to preferentially translate oncogenic mRNAs. In this study, we aimed to examine CXCR4's influence on the eIF4F complex. More specifically, we propose CXCR4 increases both the formation and activity of the eIF4F complex through: 1.) CXCR4-directed signaling and 2.) a direct interaction between LASP1 and eIF4A/B. Overall, our work identifies CXCR4 and LASP1 to be novel mediators in the protein translational machinery. We hypothesize the CXCR4-LASP1-eIF4F axis contributes to the preferential translation of oncogenic mRNAs to facilitate CXCR4-mediated breast cancer metastasis. Thus, the eIF4F complex represents an appreciable target for future cancer therapies.

Grant Support: University of Toledo Start-up Funds, College of Graduate Studies, NIH R21, Ohio Cancer Research Grant

O7: Role of 14-3-3 zeta in IL-17 signalling

Cara Peter

Department of Surgery

Large Vessel Vasculitis (LVV) is a group of autoimmune diseases that are characterized by inflammation and tissue damage to large arteries including aorta. Etiology and molecular details of LVV pathogenesis are not known. Like other autoimmune diseases, increase in the inflammatory cytokines is common in LVV. IFN-gamma, TNF-alpha, IL-17 and IL-6 are some of the most commonly increased cytokines in the autoimmune diseases. Our group previously found that the majority of LVV patients carry increased level of antibodies against 14-3-3 proteins, particularly the ζ and η isoforms. There are seven isoforms of 14-3-3 in humans and they are well characterized for their adaptor functions. They are essential for cell survival and participate in several signaling mechanisms by interacting with phosphoproteins. We observed that inflamed human aorta show significant increase in 14-3-3 expression with specific localization to the inflamed regions. We questioned the role and significance of this increased protein expression. Using biochemical and genetic tools, we are currently investigating the role of 14-3-3 proteins in cytokine signaling. Our preliminary data suggest that, in addition to its antigenic role in LVV, 14-3-3 proteins play important role in promoting the inflammation. We believe this may be an important mechanism that connects both innate and humoral immune responses. On-going studies will shed light on the precise mechanisms by which 14-3-3 contributes to the sustained inflammation and molecular pathogenesis of LVV.

O8: BBB-Permeable Polysaccharide, Mini-GAGR, Activates Neuronal Nrf2 and Antioxidant Defense System

Kelsey Murphy, Joshua Park, Dong-Shik Kim and Kenneth Hensley

Department of Neurosciences

Oxidative stress triggers and exacerbates neurodegeneration in Alzheimer's disease (AD) brain. To reduce oxidative stress, various antioxidant agents have been used to treat AD while yielding little efficacy due to poor blood-brain-barrier (BBB)-permeability. Additionally, single-modal antioxidants are easily overwhelmed by global oxidative stress. It is believed that the best way to control global oxidative stress is activating nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2) and its downstream antioxidant system. Currently, few agents can penetrate the brain and activate Nrf2-dependent antioxidant system. We discovered a BBB-permeable Nrf2-activating polysaccharide in low acyl gellan gum. Mini-GAGR, a 0.8kD cleavage product of low acyl gellan gum showed a good BBB-permeability as it was accumulated $\sim 3 \mu\text{M}$ inside the brain 6 hours after intranasal administration. 14-day intranasal treatment of 3xTg-AD mice with 100 nmole mini-GAGR per day increased the protein levels of Nrf2-dependent antioxidant enzymes and the dissociation of Nrf2 from Kelch-like ECH-associated protein 1 (Keap1), the Nrf2 inhibitor. In cultured mouse cortical neurons, treatment with $1 \mu\text{M}$ mini-GAGR induced the nuclear translocation and Ser40 phosphorylation of Nrf2. The treatment also increased the protein expression and enzymatic activity of Nrf2-dependent enzymes. In accord with the increase, the intracellular levels of reactive oxygen species (ROS) were decreased and those of GSH increased. Moreover, mitotracker staining in mini-GAGR-treated neurons was not reduced by ROS exposure. Taken together, we discovered a BBB-permeable Nrf2 activating polysaccharide that is expected to be effective in reducing oxidative stress and neurodegeneration in AD brain.

Grant Support: MOHW | Korea National Institute of Health (KNIH);, R21 AG053590-02; University of Toledo Foundation (UT Foundation);, deArce-Koch Memorial Endowment Fund; University of Toledo Foundation (UT Foundation);, University of Toledo Medical Research Society

O9: Bilirubin is a PPAR α Modulator to Regulate Fat Deposition in Adipocytes

Darren M Gordon, Kezia John, Christopher J. Trabbic, Amarjit Luniwal, Michael W. Hankins, Justin Baum, and David E. Stec, Terry D. Hinds, Jr.

Department of Molecular Medicine

Fat deposition in adipocytes has become key to understanding how obesity and insulin resistance impacts morbidity and mortality. Several studies have shown a decrease in the amount of body fat in patients with increased plasma bilirubin, a molecule derived from the breakdown of red blood cells previously implicated only as a potent antioxidant. Increasing plasma bilirubin levels have been shown to correlate with weight loss and protection against diabetes as well as cardiovascular events. Our current studies revealed this phenomenon is via bilirubin's binding to the nuclear receptor peroxisome proliferative-activated receptor alpha (PPAR α) to increase transcriptional activity. We have previously shown that PPAR α knockout (KO) mice when fed a high-fat diet and treated with PPAR α ligand fenofibrate or bilirubin, caused significantly less glucose compared to wild-type mice. Also, the impact of fenofibrate and bilirubin on weight loss was reduced in the PPAR α KO mice. To determine the mechanistic role of the bilirubin-PPAR α axis, we treated adipocytes with biliverdin, a precursor of bilirubin, and known PPAR α agonist WY 14,643, and identified by chromatin immunoprecipitation (ChIP) and Seahorse mitochondrial energy analysis. Interestingly, bilirubin activates PPAR α to bind to the promoter of genes involved in the mitochondrial activity. Altogether, these data point to the importance and newfound role that bilirubin in the burning of fat in adipose via binding to PPAR α .

Grant Support: National Institutes of Health (K01HL-125445, T.D.H.) and (PO1HL-051971 and HL088421, D.E.S.)

O10: Antibodies against properdin prevent complement-mediated lysis of red blood cells from paroxysmal nocturnal hemoglobinuria patients

Jin Y. Chen, Neeti S. Galwankar, Samuel A. Merrill, Robert A. Brodsky, and Viviana P. Ferreira

Department of Medical Microbiology and Immunology

Paroxysmal nocturnal hemoglobinuria (PNH), an acquired clonal disorder caused by a somatic mutation in a hematopoietic stem cell clone, leads to lack of GPI-anchored membrane proteins, including cell-surface complement regulators CD55 and CD59, on red blood cells (RBCs), leukocytes, and platelets derived from the deficient stem cell. PNH RBCs succumb to complement-mediated lysis and chronic hemolysis is the primary disease manifestation. Eculizumab, a humanized monoclonal antibody (MAb) that inhibits complement terminal pathway C5, is the only FDA-approved drug for PNH treatment, is the most expensive orphan drug, and is not completely effective. Properdin, the only complement positive regulator, is an attractive target for complement alternative pathway (AP) inhibition. We developed murine MAbs, 6E11A4 and 3A3E1, that inhibit properdin function and here we evaluated their ability to protect PNH RBCs. Using an in vitro PNH-like hemolysis assay, where complement regulation is blocked on normal RBCs, the data indicate 6E11A4 and 3A3E1 are more efficient at inhibiting PNH-like RBC lysis versus other complement modulators, including Cp20, anti-factor B, C5 inhibitor OmCI, and even eculizumab. Moreover, the anti-properdin MAbs also completely protect PNH patient-derived RBCs from hemolysis, while eculizumab protection is incomplete. The data also confirms that total percent lysis of patient PNH RBCs in the hemolysis assay correlates with the percent of defective PNH cells (determined by FACs), as previously reported by us, suggesting our assay may serve as an alternative diagnostic tool. Finally, further studies aimed at inhibiting properdin as a therapeutic target for PNH and other AP-mediated diseases are warranted.

Grant Support: National Institutes of Health Grant R01HL112937 (VF) and American Heart Association Student Scholarship in Cardiovascular Disease (NG)

O11: ROLE OF CXCR4-DEPENDENT LASP1-AGO2 AXIS IN BREAST CANCER

Augustus M.C. Tilley and Dayanidhi Raman

Department of Cancer Biology

The goal of our research is to investigate the role of CXCR4-LASP1 axis in modulating the activity of Argonaute2 (Ago2) in breast cancer. CXCR4 is primarily involved in metastasis and elevated levels of CXCR4 predicted poorer survival in patients. Expression of a constitutively active mutant of CXCR4 in MCF7 cells resulted in enhanced motility and lung metastasis in mice. Ago2 is a key component of the RNA-induced silencing complex shaping the transcriptome and proteome. We previously reported that CXCR4-mediated migration/invasion could be ablated by stable knockdown of LIM and SH3 protein 1 (LASP1). We investigated the mechanisms by which the CXCR4-LASP1 axis could mediate this phenotype. We hypothesized that altering the proteome through changes in mRNA level via repression of translation could lead to defective cell migration and invasion. In this study, we demonstrate for the first time that Ago2 directly binds to LASP1 through its LIM domain. The interaction of LASP1 with Ago2 was further validated by co-immunoprecipitation, GST-pull down and proximity ligation assays. These results show that endogenous LASP1 is capable of interacting with Ago2 both basally and upon stimulation with CXCL12. Interestingly, Ago2 prefers the non-phosphorylated form of LASP1. To discern the functional significance behind this interaction, Ago2 was stably knocked down and evaluated. We observed a mesenchymal to epithelial conversion, reduced cell proliferation, and surface adhesion. Further analysis will be performed in knock down cells with dominant negative Ago2 that does not interact with LASP1. Translationally, the CXCR4-LASP1-Ago2 axis could be a novel target for chemotherapy.

Grant Support: Ohio Cancer Research Associates Grant

O12: BORRELIA BURGDORFERI CheY1, CheY2, AND CheY3 POSSESS DISTINCT CHEMOTAXIS AND/OR VIRULENCE FUNCTIONS DURING THE NATURAL ENZOOTIC CYCLE IN TICK AND MOUSE RESERVOIRS

Muhammed Saad A. Moledina, Padmapriya Sekar, Syed Z. Sultan, Elizabeth A. Novak, Aaron Yerke, Md A. Motaleb, R. Mark Wooten

Department of Medical Microbiology and Immunology

Borrelia burgdorferi (Bb) is a vector-borne spirochetal bacterium that must complete a complex enzootic cycle between tick- and vertebrate-hosts. Bb possess 7-11 endoflagella that provide them a powerful corkscrew-like motility, which is essential for them to disseminate and persist within these hosts. Our current studies are directed towards understanding the chemotaxis pathways that allow them to complete each phase of their enzootic cycle. This study focuses on the role of 3 different putative response-regulator proteins (CheY), which are usually a single gene and allow direction reversal in most bacteria.

We hypothesize that each CheY protein is essential for at least one of the transmission events within or between vertebrate and tick hosts. In this study, we will use Δ cheY1, Δ cheY2, and Δ cheY3 mutants to study in vitro chemotaxis/motility, as well as their abilities to complete the natural infection cycle.

Δ cheY3 lacked the ability to reverse direction or complete chemotaxis both in vitro and in vivo, and specifically was deficient in their ability to persist in fed ticks, or be transmitted to or persist within mice. Δ cheY2 displayed no defects in vitro, and persisted within tick hosts similar to WT. However, they were not transmitted from ticks to mice and could not persist/disseminate in vivo. Interestingly, Δ cheY1 demonstrated no motility or chemotaxis defects in vitro or within mice, but were defective in their ability of tick-acquisition after feeding on infected mice.

These findings indicate that the three CheY proteins have distinct, non-overlapping functions in the Bb enzootic cycle. CheY3 protein acts like a classic response regulator, whereas the functions of CheY1/CheY2 are still under study.