The Council of Biomedical Graduate Students

2017 Graduate Research Forum

COLLEGE OF MEDICINE AND LIFE SCIENCES

THE UNIVERSITY OF TOLEDO

BIOMEDICAL SCIENCES GRADUATE PROGRAM

THE UNIVERSITY OF TOLEDO

Sponsored by:

GUTSA

2017 Graduate Research Forum

March 22nd-23rd
Interprofessional Immersive Simulation Center (IISC)
Health Science Campus

Wednesday, March 22nd

Time	*Event*	*Location*
10:00am – 5:00pm	Poster Display	IISC Atrium
10:00am – 12:00pm	Preliminary Poster Session	IISC Atrium
12:00pm – 1:00pm	Lunch	IISC 1217
1:00pm – 4:15pm	Preliminary Oral Sessions	IISC 1200

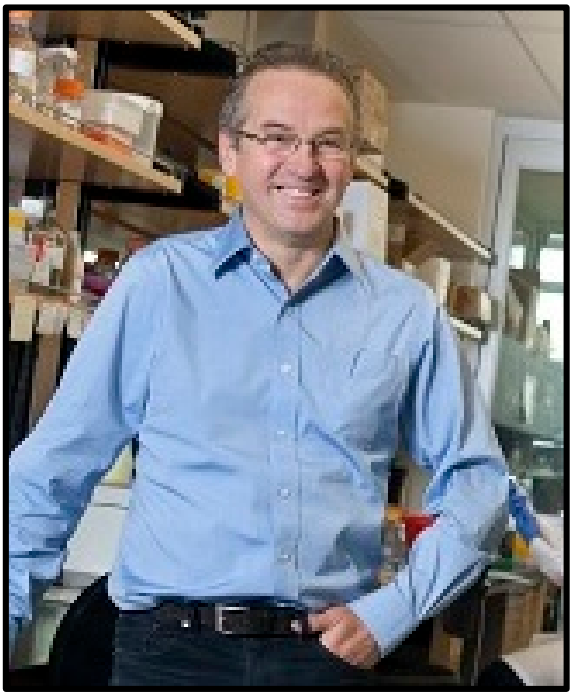
Thursday, March 23rd

Time	*Event*	*Location*
9:45am – 10:45am	Final Poster Session *(closed to public)*	IISC 1217
9:45am – 10:45pm	Final Oral Session	IISC 1200
12:00pm – 1:30pm	Lunch with Dr. Nagy *(RSVP'd participants only)*	CCE 0111
3:00pm – 3:30pm	Keynote Reception	HEB Lobby
3:30pm – 4:30pm	Keynote Lecture	HEB 100

Keynote Lecture

"Addressing Cell Therapy Safety with Genome Editing"

2017 Keynote Speaker



Dr. Andras Nagy, Ph.D.

Senior Investigator
Lunenfeld-Tanenbaum Research Institute
Mount Sinai Hospital
Joseph & Wolf Lebovic Health Complex

- Dr. Nagy primarily works on stem cells, tissue engineering and regenerative medicine.
- Established first human embryonic stem cells in Canada and was the only Canadian selected for the first annual *Scientific American* Top 10 Honor Roll in 2009
- Dr. Nagy's latest publication:

Complex Interdependence Regulates Heterotypic Transcription Factor Distribution and Coordinates Cardiogenesis; Cell, February 2016

The Council of Biomedical Graduate Students

The University of Toledo Council of Biomedical Graduate Students consists of officers and representatives from biomedically related graduate programs at the University of Toledo. This includes the Biomedical Science Graduate Program at the Health Science Campus and related graduate programs at the main campus, including Pharmacy, Medicinal & Biological Chemistry, Biology, Bioengineering, and so on.

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!

Poster Presentations

GROUP 1	Fawaz Alasmari	P1
	Oluwatomisin Atolagbe	P2
	Yetunde Badmus	P3
	Jin Chen	P4
GROUP 2	Shin Hae Cho	P5
	Amit Chougule	P6
	Daniel Craig	P7
	Rajib Dutta	P8
	Xiaoming Fan	P9
GROUP 3	Neeti Galwankar	P10
	Subhanwita Ghosh	P11
	Gardiyawasam Kalpana	P12
	Fatimah Khalaf	P13
	Daniel Koehler	P14
GROUP 4	Joseph Lee	P15
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	Kelsey Murphy	P18
GROUP 5	Kevin M Nash	P19
	Samyuktha Ravi	P20
	Shermel Sherman	P21
	Eric Starr	P22
	Augustus Tilley	P23
GROUP 6	Mengjie Wang	P24
	Jeff Xie	P25
	Shungang Zhang	P26
	Yuqi Zhang	P27
	Rose Zolondek	P28

Poster Contest Procedure

- See presentation guidelines on page 7.
- Preliminary Session- Each group will be judged by three faculty members and one finalist will be selected from each group.
- Final Session- Finalists will present to:
  - Dr. Andras Nagy, 2017 GRF Keynote Speaker
  - Dr. Kandace Williams, Associate Dean of the College of Medicine and Life Sciences
- The top three presenters will be awarded:
  - $300 for first place
  - $200 for second place
  - $100 for third place

Oral Presentations

GROUP 1	Amanda Blaker	O1
1:00-2:00pm	Alison Brandel	O2
	Saroj Chakraborty	O3
	Cara DeAngelis	O4
GROUP 2	Kaitlyn Dvorak	O5
2:00-3:00pm	Sarah Galla	O6
	Briana Zellner	O7
	Youjie Zhang	O8
GROUP 3	Angelique Nyinawabera	O9
3:00-4:15 pm	Gayatri Subramanian	O10
	Adaeze Izuogu	O11
	Claire Meikle	O12
	Jack Imbery	O13

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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

P.1: Effects of chronic inhalation of electronic cigarettes containing nicotine on glial glutamate transporters and α-7 nicotinic acetylcholine receptor in female CD-1 mice

Fawaz Alasmari, Laura E. Crotty Alexander, Jessica A. Nelson, Isaac T. Schiefer, Ellen Breen, Christopher A. Drummond, Youssef Sari,*

Department of Pharmacology and Experimental Therapeutics

Alteration in glutamate neurotransmission has been found to mediate the development of drug dependence, including nicotine. We and others have reported that exposure to drugs of abuse reduced the expression of glutamate transporter-1 (GLT-1) as well as cystine/glutamate antiporter (xCT), which consequently increased extracellular glutamate concentrations in the mesocorticolimbic area. However, previous studies did not reveal any changes in glutamate/aspartate transporter (GLAST). In the present study, for the first time, we investigated the effect of chronic exposure to electronic (e)-cigarette vapor containing nicotine, for one hour daily for six months, on GLT-1, xCT, and GLAST expression in frontal cortex (FC), striatum (STR), and hippocampus (HIP) in outbred female CD1 mice. In this study, we investigated the expression of alpha-7 nicotinic acetylcholine receptor (α-7 nAChR), a major pre-synaptic nicotinic receptor in the glutamatergic neurons, which regulates glutamate release. We found that inhalation of e-cigarette vapor for six months increased α-7 nAChR expression in both FC and STR, but not in the HIP. In addition, chronic e-cigarette exposure reduced GLT-1 expression only in STR. Moreover, e-cigarette vapor inhalation induced downregulation of xCT in both the STR and HIP. We did not find any significant changes in GLAST expression in any brain region. Finally, using LC-MS/MS techniques, we detected high concentrations of nicotine and cotinine, a major metabolite of nicotine, in the FC tissues of e-cigarette exposed mice. Our work may suggest that nicotine dependence via chronic inhalation of e-cigarette vapor may be mediated in part by alterations in the glutamatergic system.

Grant Support: National Institutes of Health (R01AA019458 to Y.S.), (1F32DK104615-01 to CAD), Veterans Affairs BLR&D Career Development Award (1IK2BX001313 to LCA), AHA Beginning Grant-in-aid (16BGIA27790079 to LCA), and O'Brien Center Daniel O'Connor Memorial Pilot Award to LCA.

P.2: Genome-wide rna-seq analysis of polarized macrophages derived from wild-type mice and from mice with macrophage-specific loss of trpc3 function

Oluwatomisin Atolagbe, Sivarajan Kumarasamy PhD, Bina Joe PhD, and Guillermo Vazquez PhD

Department of Physiology and Pharmacology and Bioinformatics Program

The Transient Receptor Potential Canonical 3 (TRPC3) channel is a member of the TRPC family of calcium-permeable non-selective cation channels important in a number of physiological processes. In the cardiovascular system, TRPC3 function has been associated with the pathogenesis of cardiovascular diseases such as hypertension and cardiac hypertrophy. Research in our lab has shown that TRPC3 modulates macrophage function in the setting of atherosclerosis. To better understand the underlying molecular mechanisms of macrophages that are affected by TRPC3, we conducted a comparative genome-wide RNA seq study of M1 (inflammatory) and M2 (anti-inflammatory) macrophages, which represent the polarized dominant types in atherosclerosis, derived from wild-type mice or from mice with macrophage-specific loss of TRPC3 function. A total of 2,127 transcripts were differentially expressed genes between the M1 and M2 wild-type macrophages, including 1,232 upregulated and 895 downregulated genes in M1 vs M2 cells. In comparing the transcriptome of wild-type vs. Trpc3-deficient M2 macrophages, 160 genes were found to be differentially expressed, of which 49 were upregulated and 111 downregulated. To validate the RNA-seq data, we conducted quantitative real-time PCR of 10 most significantly upregulated or downregulated genes from each group of macrophages. By conducting analysis of the biological processes enriched in M1 vs. M2 macrophages or in wild-type M2 vs. Trpc3 deficient M2 macrophages, we are currently gaining insight into molecular signatures and pathways that, directly or indirectly, are affected by TRPC3 function.

Grant Support: NIH grants 1R56HL125619 to G.V., HL-076709, HL112641 and HL-020176 to B.J.

P.3: The Effects of Glutamate on Oxytocin Release

Yetunde Badmus, Dr. Richard Cantley, Dr. Jennifer Hill

Department of Physiology and Pharmacology

During pregnancy, weight gain creates a positive energy balance that serves the biological purpose of providing adequate energy stores for the upcoming extended period of lactation. However, excess adiposity is associated with a delay in the initiation of lactation and a reduced duration of breastfeeding. Glutamatergic neurons in the paraventricular nucleus (PVN) are implicated in the control of oxytocin release. Recent studies have also shown that glutamatergic target neurons in this nucleus mediate much of the ability of melanocortins to suppress feeding. Based on novel preliminary data, we hypothesize that glutamatergic neurons of the PVN influence prenatal and postnatal hyperphagia and modify the activity of oxytocin neurons involved in lactation. To test this hypothesis, we have created vglut2/sim1-cre female mouse, in which the gene for the vesicular glutamate transporter VGLUT2 has been deleted in the PVN. To examine dysfunctional lactation in this model we evaluated multiple parameters including neonatal survival and growth, and analysis of mammary tissue and ovary tissue. In addition, circulating oxytocin, prolactin, and other hormone levels were measured. The number of pups born to homozygous vglut2/sim1 dams that survive until weaning was dramatically down compared to controls; 0% survival was observed when fostering did not occur compared to approximately 100% survival when fostering did occur. Results to date suggest that homozygous vglut2/sim1 mice show a consistent and complete deficit in milk release and a buildup of milk protein and lipids within the mammary glands. In addition, we have observed a potential subfertility phenotype observed in the vglut2/sim1 females. These results highlight a potential pathway whereby obesity and lactational deficiency may develop concurrently. Additional studies will be needed to test whether the glutamatergic neuronal populations in the PVN controlling body weight and lactation overlap.

P.4: Complement is not involved in collagen- or vWF-mediated thrombi formation in human whole blood subjected to shear stress

Jin Chen and Viviana P. Ferreira

Department of Medical Microbiology and Immunology

Complement, platelet/leukocyte aggregates (PGA), and thrombi play key roles in cardiovascular disease pathogenesis. The contribution of complement, a central pro-inflammatory component of innate immunity, to thrombi formation remains unclear. We recently showed that properdin, the positive regulator of the alternative complement pathway (AP), enhances PGA formation, and that the effect of properdin is regulated by complement negative regulator Factor H (FH). Here we assess how complement influences thrombi formation under shear stress (a physiological factor in thrombi formation) by using the Fluxion Bioflux system that simulates vasculature shear rates under flow by perfusing blood through microfluidics chambers and allowing visualization of thrombi formation in real time. Calcein-labeled human whole blood was perfused through collagen- or von Willebrand factor (vWF)-coated microfluidic channels at varying shear stresses, in the presence or absence of the total complement inhibitor Compstatin, anti-properdin inhibitory antibodies, or complement promoters such as exogenous properdin or an inhibitor of FH. The data indicate that none of the reagents tested impede or exaggerate thrombi formation under low (20 dynes/cm2) and high (125-200 dynes/cm2) shear rates, suggesting that complement activation does not participate in collagen- or vWF-mediated thrombi formation. Current studies aim to understand the role of complement in thrombi formation under shear stress, including PGA in thrombi, on human aortic endothelial cells monolayers in the microfluidic channels, which more closely simulates human vasculature. These studies will advance the understanding of the functions of the AP in vascular disease pathogenesis by defining molecular mechanisms for platelet/leukocyte interactions in thrombi.

Grant Support: National Institutes of Health Grant R01HL112937

P.5: C5' oxidation and its adducts as potential biomarkers

Shin H. Cho, Amanda Bryant-Friedrich

Department of Medicinal and Biological Chemistry

The concept of endogenous exposome has enlightened the scientists to address the importance of understanding internal exposures as a result of biological processes throughout the lifetime. There have been various studies published in an effort to investigate adducts formed after the strand breaks in DNA from reactive oxygen species, a kind of endogenous exposome. Such work has been done by designing a synthesis of a radical pre-cursor or the subsequent intermediates from radicals generated at different carbon centers in deoxyribose moiety. Among the 5 carbons in the deoxyribose, the hydrogen at a 5' carbon is most susceptible to be influenced by the environment. Nonetheless, the actual mechanism to understand how the intermediates are degraded are yet to be investigated. Additionally, it is often degraded into furfural, which is carcinogenic. Developing a synthetic de-tour to tag the formation of furfural is crucial. Here we present the synthesis of the 5' aldehyde lesions in nucleosides as well as oligonucleosides. Then, the synthesized compounds are under subject of metabolism studies in a physiological condition with various mediums in hopes to develop a possible biomarker to measure the oxidative stress.

P.6: An important role of PPARα in regulation of bone mass via down-regulation of sclerostin protein

Amit Chougule, Lance Stechschulte, Peter Czernik, Beata Lecka-Czernik

Department of Physiology and Pharmacology

Bone remodeling is a vital process composed of bone resorption and formation. However, increased bone resorption and decreased bone formation lead to low bone mass and increase in fractures. Current available treatments for osteoporosis use mostly anti-resorptive approach, which prevents further loss but do not increase bone mass. Therefore, new osteoanabolic treatments are being developed including therapy with monoclonal antibodies against sclerostin protein. Sclerostin is expressed specifically in bone cells named osteocytes and by inhibiting canonical Wnt/β-catenin pathway it inhibits osteoblastogenesis and bone formation. We found that Peroxisome Proliferator-Activated Receptor alpha (PPARα), a known regulator of lipid metabolism, binds to the sclerostin promoter and downregulate sclerostin expression which results in augmented osteoblastic activity. PPARα KO animals have a larger bone cavity and thinner cortex as well as a decrease in bone formation rate compared to WT animals. Loss of PPARα also resulted in an increase in osteoclast number both in vivo and ex vivo, along with an increase in marrow fat. Finally, osteocytes from PPARα KO animals have increased expression of sclerostin and RANKL pro-osteoclastic cytokine, resulting in diminished Wnt signaling in osteoblast and decrease in bone formation, while simultaneous increase in osteoclast number and bone resorption. These findings suggest that PPARα regulates bone mass by suppressing osteoclast and adipocyte formation, and augmenting osteoblast formation and bone forming activity. Our approach is protein based and demonstrates for the first time that harnessing both osteoanabolic and antiresorptive PPARα activities may be a promising treatment for osteoporosis.

P.7: Using somatic mutation prevalence as a cancer risk biomarker

Daniel J. Craig

Department of Cancer Biology and Biochemistry

The human genome is exposed to a myriad of assaults capable of inducing DNA damage. Various DNA repair mechanisms are tasked with identifying these aberrant nucleotides and repairing them, maintaining the integrity of the DNA sequence. In spite of these protective mechanisms, damaged nucleotides are occasionally missed, resulting in somatic mutations during cell division. Stem cells may be particularly prone to accumulating mutations, as they undergo periods of quiescence, where they can accumulate DNA damaging events, followed by an induction to proliferate, which can turn damaged nucleotides into somatic mutations capable of being passed down to the next cell generation. As we age, the relative abundance of mutations increases, which may increase our risk for developing cancer. There is also inter-individual variation in somatic cell mutation prevalence. Therefore, somatic mutation prevalence may serve as a good biomarker for cancer risk, as it provides critical information regarding the state of the DNA repair mechanisms. However, quantifying mutation prevalence through Next Generation Sequencing (NGS) is limited by technical errors introduced during library preparation and sequencing. In our study, we utilize a novel technique termed Duplex Sequencing that enables distinction between true somatic cell mutation or technical error. Utilizing this method in normal human tissues, we will test the following hypotheses: (1) older individuals exhibit a higher mutation prevalence than younger individuals. (2) individuals with lung cancer exhibit a higher mutation prevalence than matched individuals without lung cancer. (3) mutation prevalence is directly correlated with specific cis-regulatory SNPs in DNA repair pathway genes.

P.8: Intricacies in arrangement of SNP haplotypes suggest "Great Admixture" that created modern humans

Rajib Dutta, Joseph Mainsah, Yuriy Yatskiv, Sharmistha Chakrabortty, Patrick Brennan, Basil Khuder, Shuhao Qiu, Larisa Fedorova, Alexei Fedorov

Department of Physiology and Pharmacology

Inferring history from genomic sequences is challenging and problematic because chromosomes are mosaics of thousands of small Identical-by-descent (IBD) fragments, each of them having their own unique story. However, the main events in recent evolution might be deciphered from comparative analysis of numerous loci. A paradox of why humans, whose effective population size is only 104, have nearly three million frequent SNPs is formulated and examined. We studied 5398 loci evenly covering all human autosomes. Common haplotypes built from frequent SNPs that are present in people from various populations have been examined. We demonstrated highly non-random arrangement of alleles in common haplotypes. Abundance of mutually exclusive pairs of common haplotypes that have different alleles at every polymorphic position (so-called Yin/Yang haplotypes) was found in 56% of loci. A novel widely spread category of common haplotypes named Mosaic has been described. Mosaic consists of numerous pieces of Yin/Yang haplotypes and represents an ancestral stage of one of them. Scenarios of possible appearance of large number of frequent human SNPs and their habitual arrangement in Yin/Yang common haplotypes have been evaluated with an advanced genomic simulation algorithm. Computer modeling demonstrated that the observed arrangement of 2.9 million frequent SNPs could not originate from a sole stand-alone population. A "Great Admixture" event has been proposed that can explain peculiarities with frequent SNP distributions. This Great Admixture presumably occurred 100-300 thousand years ago between two ancestral populations that had been separated from each other about a million years ago. Our programs and algorithms can be applied to other species to perform evolutionary and comparative genomics.

P.9: Partial Nephrectomy Induces Cardiac Fibrosis through a Na/K-ATPase-Src-NF-kappa B-microRNA-29b-3p Mediated Pathway in Mice

Xiaoming Fan, Christopher A. Drummond, Jeffrey X. Xie, Huilin Shi, Steven T. Haller, David Kennedy, Jiang Liu, Zijian Xie, Joseph I. Shapiro, Christopher J. Cooper, and Jiang Tian

Department of Medicine

Cardiac fibrosis is a common pathological process in different cardio-renal diseases. Activation of Na/K-ATPase signaling by its ligands, cardiotonic steroids (CTS), contributes to cardiac fibrosis in animal models with chronic kidney disease (CKD). Our recent in vitro studies revealed that Na/K-ATPase-mediated Src signaling regulates collagen synthesis in cardiac fibroblasts through microRNA-29b-3p (miR-29b-3p). In this study, we performed 5/6th partial nephrectomy (PNx) surgery on wild type (WT) and Na/K-ATPase α1 reduced heterozygous (α1+/-) mice. Twelve weeks after surgery, half of the mice were treated with pNaKtide or saline. The results showed that in WT mice, PNx significantly decreased miR-29b-3p expression by about 2 fold, resulting in significantly increased cardiac fibrosis (5.3±1.5% vs 0.6±0.2% in sham-operated group, $p<0.01$). PNx also significantly increased total expression as well as nuclear fraction of NF-kappa B p65 (NF-κB), a miR-29b-3p suppressive regulator. pNaKtide treatment significantly reduced cardiac fibrosis after PNx surgery. It also restored miR-29b-3p expression to the level comparable to sham-operated mice. However, in α1+/- mice, the basal level of Src and NF-κB were higher and miR-29b-3p was lower compared to that in WT. PNx surgery did not affect the NF-κB expression or activation in α1+/- mice. In cardiac fibroblasts isolated from WT mice, Na/K-ATPase ligand, ouabain, decreased miR-29b-3p, and NF-κB inhibition blocked such decrement. Whereas in the cells isolated from α1+/- mice, ouabain or NF-κB inhibition had no effect on miR-29b-3p expression. These results suggest that Na/K-ATPase, Src, and NF-κB signaling contribute to PNx-induced cardiac fibrosis via regulation of miR-29b-3p expression.

P.10: Anti-properdin monoclonal antibodies: Relevance in detecting functional differences between properdin polymers and in inhibiting properdin function

Neeti Galwankar and Viviana P. Ferreira

Department of Medical Microbiology and Immunology

The alternative pathway (AP) of the complement system can be activated excessively in several inflammatory diseases, particularly when there is a defect in regulatory components of the complement system. For instance, defects of complement regulatory protein factor H (FH) are associated with atypical hemolytic uremic syndrome (aHUS), which leads to severe prothrombotic pathologies. Understanding molecular mechanisms involved in complement activity are essential for developing new treatments. Properdin, the positive regulator of complement, is essential for complement amplification by stabilizing enzymatic convertases. It exists as head-to-tail associations of dimers, trimers and tetramers (P2:P3:P4) in 1:2:1 ratio and the convertase-stabilizing function of P4 is greater than P3 and P3 greater than P2. Although properdin is produced by different leukocytes in response to local stimuli it remains unknown whether leukocytes secrete properdin forms into the microenvironment in the same physiological ratio found in plasma. Here we have developed and characterized monoclonal antibodies against properdin and standardized a functional ELISA that will allow us to determine whether the distribution of properdin polymeric forms in the inflammatory microenvironment has been altered. Our results so far have indicated that there is a difference in how quickly the properdin forms activate the AP pathway. In addition, when tested in an in-vitro model of aHUS the inhibitory monoclonal antibodies had lower IC50 values than all other complement inhibitors, including Soliris, which is currently used to treat patients with this disease. Further studies aimed at determining the therapeutic value of inhibiting properdin in human inflammatory diseases are warranted.

P.11: Regulatory Mechanisms of Cardiotonic Steroids in Chronic Kidney Disease

Subhanwita Ghosh MS, Bruce S. Levison PhD, Sadik Khuder PhD, Fatimah K. Khalaf MBBS, Andrew L. Kleinhenz BS, Erin L. Crawford MS, Deepak Malhotra MD PhD, David Kennedy PhD

Department of Medicine and Bioinformatics, proteomics and genomics

Cardiotonic steroids (CTS) are steroid hormones which are elevated in chronic kidney disease (CKD). The 2-pyrone ring structure of CTS is critical for its binding to the Na+/K+-ATPase and subsequent initiation of profibrotic signaling which promotes tissue fibrosis and organ dysfunction underlying cardiac and renal disease. We have recently discovered a family of hydrolytic enzymes, Paraoxonases (PONs), which are capable of regulating 2-pyrone structures like those found in CTS and which demonstrate decreased activity in CKD. We hypothesized that dysregulated expression in the PON gene family is associated with renal disease and that PON's, via their hydrolytic activity, participate in the metabolism and regulation of CTS. We analyzed peripheral blood whole genome microarray data from a case-control study of 63 stage 5 CKD patients and 20 age and sex matched normal controls. We found that both PON-1 ($p = 7.49E-5$) and PON-3 ($p = 3.01E-5$) expression was increased in CKD vs. controls, while PON2 expression ($p = 7.55E-15$) was significantly decreased in CKD vs. controls. Next, we developed an LC-MS based assay to measure and quantify CTS as well as to monitor the potential metabolism of CTS by PON's. Incubation of the CTS Telocinobufagin (TCB) with PON-1 over expressing HEPG2 cells lead to a 2-fold decrease in the intact 2-pyrone form of TCB vs control ($p<0.01$). PON expression is significantly dysregulated in patients with chronic kidney disease. Furthermore, CTS appear to be novel physiologic substrates for PON hydrolytic activity and this may represent a novel regulatory mechanism for CTS.

Grant Support: CSCTR

P.12: Role of RKIP– RhoA Axis in Regulating Breast Cancer Metastasis

Gardiyawasam Kalpana, Kam C. Yeung

Department of Cancer Biology and Biochemistry

Tumor metastasis is a multistep process that includes local invasion, intravasation, survival in the circulation, extravasation and colonization in distant sites. Tumor metastatic suppressors are proteins that have the ability to inhibit metastasis by inhibiting any of these steps. Raf-1 kinase inhibitory protein (RKIP) is a one such protein that inhibits metastasis in breast, prostate and several other types of cancers. The molecular mechanisms through which RKIP executes its anti-metastasis effects is not yet completely defined, although it appears to follow more than one approach. Rho family of proteins is small GTPases that belong to the Ras superfamily, and consist of RhoA, RhoB, and RhoC. They have important physiological functions in regulating actin cytoskeleton, cell polarity, cell movements, and microtubule dynamics. Regulating by activator guanine nucleotide exchange factor (GEF) proteins and inhibitory GTPase activating proteins (GAP), these GTPases cycle between GTP-bound active form and GDP-bound inactive form. Overexpressed or aberrantly activated GEFs are often reported to drive tumorigenesis and consider as oncogenic, while activated GAPs are assumed to inhibit the cancer formation. Hence, Rho GTPases are widely accepted as oncogenes. Despite this notion, several studies in recent years suggested a possible tumor suppressive function for RhoA in colorectal, diffuse-type gastric and basal-like breast carcinomas and T-cell lymphomas. In this study, we discovered that RKIP specifically enhances RhoA activity in breast cancer cells thereby affecting the cell motility phenotype. The functional importance of RKIP-RhoA axis in breast cancer metastasis is currently under investigation.

P.13: Cardtiotonic steroids mediate inflammation in renal epithelium via the Na/K-ATPase alpha-1-Src kinase signaling pathway

Fatimah K. Khalaf , Andrew L. Kleinhenz , Erin L. Crawford , Jiang Tian , Deepak Malhotra, Zijian Xie, and David J. Kennedy

Department of Medicine

Cardiotonic steroids (CTS) are Na/K-ATPase alpha-1 isoform (NKA α-1) ligands that are increased in renal disease. Binding of CTS to NKA α-1 activates Src kinase and directs a number of important cellular functions including cell growth and ROS production. While initiation and resolution of inflammation is an important component of cellular injury and repair in renal disease, it is unknown whether CTS activation of the NKA α-1-Src signaling axis regulates the inflammatory response in these settings.
We tested the hypothesis that CTS can promote pro-inflammatory effects in renal epithelial cells and this process is mediated by the NKA α-1-Src signaling mechanism.
We found that while the CTS Telocinobufagin (TCB) activated Src at 15 minutes in a renal epithelial cell line (P11 control cells), the effect was abolished in renal epithelial cells with 90% NKA α-1 knock-down (PY17 α-1 knock-down) or by pretreatment of P11 cells with a specific peptide inhibitor of the NKA α-1-Src kinase pathway, pNaKtide (1 uM, 30 minute pretreatment). TCB also enhanced collagen expression in P11 control cells and this was attenuated by pretreatment with the Src inhibitor PP2. Furthermore, TCB activated multiple pro-inflammatory cyto/chemokines in P11 control cells and these effects were attenuated in the PY17 α-1 knock-down line. Finally, TCB activation of pro-inflammatory cyto/chemokines was significantly attenuated by pNaKtide in human HK2 renal epithelial cells.
These findings suggest that CTS can stimulate inflammatory cytokine/chemokine production in renal epithelium and that this is mediated via the NKA α-1 Src kinase signaling pathway.

P.14: Development of a Novel Pharmacological Model of Okadaic Acid-induced Alzheimer's Disease in Zebrafish

Daniel Koehler

Department of Pharmacology and Experimental Therapeutics

The present study aimed at establishing a new pharmacologically-induced Alzheimer's (AD) model in zebrafish by administering okadaic acid (OKA). This model involves most of the pathophysiological conditions predominant in AD while also cutting down time constraints and cost which hinder previously established AD models. Zebrafish were divided into 5 groups containing 5-6 fish per group. Each group was exposed to a respective concentration of OKA of 10nM, 100nM, 500nM, and 1μM along with a control group. Each exposure lasted for 9 days with a learning and memory test performed at the end. Fish were tested individually in a spatial alternation task in order to assess learning and memory capabilities. Fish were placed in a 10 L aquarium that was divided into two equal sections by a white divider that allowed for adequate room for the fish to swim from one side of the aquarium to the other. Each trial was initiated with a light tap (discriminative stimulus) at the center of each aquarium. After the light tap, there is a 5 second delay followed by food presentation on one side of the tank. In 20 minutes intervals, food presentation continued on alternating sides for a total of 28 trials (14 on each side). A response was considered correct if the fish was present on the side of food presentation during or shortly after food presentation. Each trial ended 5 seconds after the food was presented. Zebrafish are deemed to have learned the task when 75% or more of the responses are correct.
Behavioral analysis was done using a non-linear regression equation of:
P = .5 + b(t/c)**d / [1 + (t/c)**d]
After completing the learning and memory tests, fish were euthanized and their brains were further analyzed by conducting immunohistochemistry analysis for phospho-glycogen synthase-3 α/β (p-GSK-3 α/β), GSK-3 α/β, Aβ, p-tau, and tau. All parameters of expression of p-GSK-3α/β, GSK-3α/β, Aβ, p-tau, and tau were analyzed using a student's t-test. A value of $p<0.05$ was considered to be significant.
It was observed that brain hemorrhages and higher mortality rates were caused in fish with increasing concentration of OKA. The 500nM and 1μM exposure groups observed the highest mortality rates of 60%-70%. The control group demonstrated rapid learning with maximal learning shown to be between 75%-80%. The exposed groups never demonstrated learning with their probabilities of correctness continuously staying around 50%. OKA exposure resulted in the increased phosphorylation of GSK-3 α/β, increased phosphorylation of tau, Aβ deposition, and the formation of Aβ plaques.
These experiments were able to establish an AD model that incorporates most of the hallmarks of AD pathophysiology. This model can now be used to study drug discovery for AD and the molecular mechanisms involved in AD without the time and cost constraints that other AD models entail.

P.15: Role of GABA Receptors on Parotid Secretory Activity

Joseph Lee and David Giovannucci

Department of Neurosciences

The parotid gland secretes salivary components critical for oral health. Secretory output is controlled by autonomic neural activity on parotid acini, the basic units of saliva production. This process can be disrupted by benzodiazepines, leading to the severe oral condition of xerostomia, or dry mouth. Neural inputs to the acini are primarily cholinergic and noradrenergic, but the discovery of gamma-aminobutyric acid (GABA) and its receptors in the salivary glands hints at an unexplored mechanism for benzodiazepine-induced xerostomia. The purpose of this study was to describe the GABAergic system in the mouse parotid gland and define its effect on acinar regulation. RT-PCR detection of mRNA transcripts for multiple GABA receptor subunits and positive immunofluorescence strongly indicate the expression of GABA receptors in mouse parotid tissue. However, both live cell calcium imaging and whole-cell recordings of dispersed acinar cells revealed no acute impact on cellular physiology. In contrast, immunohistochemical analysis of the autonomic innervations to the parotid gland aligned with the identification and characterization of GABA-evoked chloride currents in a subset of neurons recorded from primary ganglionic cultures. Our data suggest GABA has no direct effect on the parotid acini but may downregulate acinar function by inhibiting neurotransmitter release from autonomic neural inputs.

P.16: Insulin action in non-neuronal cells (Glial) and the regulation of puberty and reproduction

Iyad Manaserh, Jennifer W. Hill*

Department of Physiology and Pharmacology

Infertility is a major problem in the United States and occurs in approxiately 10% of reproductive aged women. Hyperinsulinemia and obesity are associated with infertility and reduced GnRH release. Bruning et al., 2000 demonstrated that male and female mice lacking insulin signaling in the brain (both neuronal and non-neuronal cells) during their development exhibit subfertility. However, the specific cell types mediating insulin effects on fertility remain unidentified. To determine whether hypothalamic insulin sensing plays an important role in the regulation of energy hemostasis and fertility, we generated mice lacking insulin receptors in astrocytes (GFAP-cre, IR flox-flox). Male and female GFAP-IR mice showed a delay in balanopreputial separation and vaginal opening and first estrous, respectively. Female GFAP-IR mice also exhibited longer estrus cycle lengths and irregular estrus cyclicity. Adult body weight, body growth and fat composition were comparable between the two groups. These data suggest that impaired insulin sensing in astrocytes delays the initiation of puberty and affects adult reproductive function. Additional investigation is needed into the mechanisms underlying these effects.

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

P.17: *Borrelia burgdorferi* chey1, chey2, and chey3 possess distinct chemotaxis and/or virulence functions during the natural enzootic cycle in tick and mouse reservoirs

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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 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 ΔcheY1, ΔcheY2, and ΔcheY3 mutants to study in vitro chemotaxis/motility, as well as their abilities to complete the natural infection cycle.
ΔcheY3 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. ΔcheY2 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, ΔcheY1 demonstrated no motility or chemotaxis defects in vitro or in vivo.
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.

P.18: Identification of Neurotrophic and Antioxidant Mechanisms of BBB-Permeable Mini-GAGR

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Though much effort has been placed on finding effective therapeutic agents for neurodegenerative diseases such as Alzheimer's disease (AD), many current therapies exhibit limited improvement and fail to stop disease. The shortcomings of current AD therapeutics necessitate an alternative means of slowing or stopping AD. Our novel polysaccharide, Midi-GAGR, showed promising results as a potential AD therapeutic. Midi-GAGR is a cleavage product of low acyl gellan gum, an FDA-approved human food additive. Midi-GAGR possesses neurotrophic and neuroprotective effects and also has a sustainable half-life and good blood brain barrier permeability. All of these properties point to a great potential of Midi-GAGR for AD treatment. Previously, Midi-GAGR enhanced neuritogenesis and activated nuclear cAMP-responsive element binding protein, suggesting that Midi-GAGR is neurotrophic. Midi-GAGR also protected cortical neurons against oxidative stress, suggesting that Midi-GAGR exerts neuroprotection through an antioxidant system. To identify this system, we performed treatment with Mini-GAGR, the basic unit of low acyl gellan gum. Mini-GAGR not only increased the expression of antioxidant enzymes, heme oxygenase-1 and superoxide dismutase but also increased nuclear translocation of nuclear factor erythroid-2-related factor 2 (Nrf2), a major inducer of antioxidant enzymes. Thus, Mini-GAGR uses the antioxidant Nrf2-antioxidant response element (ARE) pathway. We found that Mini-GAGR upregulated pAkt, suggesting Akt comes upstream of the Nrf2-ARE pathway. We also found that Mini-GAGR bound to neurotrophic TrkB receptor. All of the results elucidate that TrkB-Akt-Nrf2-ARE pathway underlies the antioxidant and neurotrophic effects of Mini-GAGR. These results indicate that Mini-GAGR has a great therapeutic potential for AD treatment.

P.19: A Novel ROS-Sensitive NOS Inhibitor for the Treatment of Ischemic Stroke

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Ischemic stroke is one of the leading causes of death in the United States, and is characterized by hypoxia leading to inflammatory and free-radical mediated cell death. Reactive oxygen species (ROS) are formed in excess under hypoxic conditions which cause protein, DNA and lipid oxidation. Nitric oxide (NO) formed by NO synthase (NOS) is necessary to restore blood flow, and has been shown to be protective in ischemic stroke, however NO can react with O2.- to form the highly oxidizing peroxynitrite (ONOO-). Additionally, NOS has been shown to 'uncouple' under oxidative conditions to instead produce O2.-, further exacerbating the initial insult. Nitrones are antioxidant molecules that are shown to spin trap' free radicals to then decompose into a benzaldehyde species and release NO. In this study, the nitrone KN-1-35 was designed such that its decomposition product is a NOS inhibitor, effectively leading to NOS inhibition specifically at the site of ROS production while simultaneously neutralizing ROS and releasing NO. The ability of KN-1-35 to spin-trap radicals and decompose into the putative NOS inhibitor was observed using electron paramagnetic resonance (EPR) and LC-MS/MS. The pro-drug concept was tested in vitro by measuring cell viability and inhibitor formation in SH-SY5Y cells subjected to oxygen glucose deprivation (OGD). KN-1-35 was found to be more efficacious, and more potent than the conventional nitrone, PBN, in models of ischemia-reperfusion and inflammation. Lastly, the efficacy of the proposed nitrone in reducing ischemic damage will be tested in an in vivo mouse model of permanent ischemia.

Grant Support: American Foundation for Pharmaceutical Education (AFPE) Pre-Doctoral Fellowship

P.20: Sexual Behavior in POMC Neuronal Insulin and Leptin Resistant Female Mice

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Pro-opiomelanocortin (POMC) is a common precursor protein expressed in the anterior and intermediate lobes of the pituitary gland. It is cleaved to form alpha, beta, and gamma melanocyte stimulating hormone (MSH) peptides, adrenocorticotropic hormone (ACTH), as well as other important peptides such as beta-endorphin. The alpha-MSH produced by neurons in the arcuate nucleus has important roles in the regulation of appetite and sexual behavior. Insulin and leptin are hormones that act on the arcuate nucleus to stimulate the POMC neurons, thereby stimulating alpha-MSH production. Insulin and leptin resistance, often seen in obesity and Type 2 Diabetes, results in a decrease in alpha-MSH production. Based on this understanding, we hypothesize that melanocortin deficiency as a result of leptin and insulin resistance leads to reduced sexual behavior. We used POMC LepR/IR DKO female mice and control females negative for the cre. Vaginal wet smears are utilized to assess the reproductive status of the mouse two weeks after priming with vasectomized males. On the day the mouse indicates proestrus stage, the mice are paired and the copulatory behavior is filmed. The filmed behaviors are then analyzed for specific hallmarks of female sexual behavior. The results provide further insight into the relationship between insulin/leptin resistance and sexual behavior in female mice.

P.21: Prenatal Androgen Exposure of Single-Injection of Testosterone Induces Cardiovascular and Metabolic Dysfunction in Adult Female Wistar Rats.

Shermel Sherman, Nadeen Sarsour, and Jennifer Hill, Ph.D.

Department of Physiology and Pharmacology

Cardiovascular disease is the leading cause of death in men and women within the United States. Individuals at risk for developing cardiovascular disease possess morbidity characteristic of metabolic dysfunction including diabetes, hypertension, and obesity. Previous studies have found that the origins of cardiovascular disease may arise from the prenatal environment and exposure to excess sex steroids such as testosterone produce adverse consequences that places male and female adult offspring at risk for the development of insulin resistance, infertility, obesity, and heart disease. However, the effects of excess testosterone on female cardiovascular function remains to be understood. In this study, we hypothesized that a single-injection of testosterone in the late gestation of Wistar rats would establish a metabolic phenotype characteristic of hypertension in adult female rats. Sixty-six female Wistar rats were separated into two groups, vehicle and prenatal androgen (PNA) after 21 days of age. Our lab investigated the effects of prenatal exposure to excess testosterone on cardiovascular function with telemetry, echocardiogram, and immunohistochemistry. We also assessed insulin and glucose tolerance and utilized quantitative gene expression analysis to determine differential gene and protein expression. Our data suggests that the prenatal exposure to excess androgen alters systemic expression of genetic markers associated with diabetes and hypertension and negatively impacts cardiovascular function by increasing systolic and diastolic blood pressure and decreasing heart rate. Therefore, suggesting that prenatal androgen exposure during fetal programming may negatively impact cardiovascular health of females in adulthood.

P.22: PACAP induces persistent transcription-dependent, activity-independent synaptic plasticity.

Eric R. Starr and Joseph F. Margiotta

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a secretin family neuropeptide, found in presynaptic terminals throughout the nervous system. In autonomic ciliary ganglion (CG) neurons, PACAP exposure engages a PACAP type 1 receptor signaling cascade that enhances the function of nicotinic acetylcholine receptor- (nAChR-) mediated synapses on the neurons within minutes. Consistent with our previous findings that PACAP alters synapse-specific gene expression, striking changes persisted at synapses for 48 hours after the initial PACAP exposure (15 min) and washout that were dependent on gene transcription. Here, we report that the physiological correlates underlying this persistent PACAP-induced plasticity reflect an activity-independent increase in both post- and presynaptic strength. First, reversible inhibition of synaptic activity, achieved by blocking nAChR-mediated synaptic transmission with curare or action potentials with tetrodotoxin prior to and following PACAP treatment, failed to inhibit subsequent plasticity reflected in elevated spontaneous excitatory postsynaptic current (sEPSC) frequency and amplitude. Second, in conjunction with increased sEPSC frequency and amplitude the sustained PACAP-induced plasticity was accompanied by significant increases in both miniature EPSC amplitude (postsynaptic quantal size) and ACh release (presynaptic quantal content). No detectable differences were observed in excitability suggesting that the physiological hallmarks underlying the sustained PACAP-induced plasticity are confined to presynaptic terminals and postsynaptic densities. Third, analysis of confocal images revealed that PACAP significantly enhanced the size of individual pre- and postsynaptic clusters labeled with SV2 and nAChR specific antibodies, respectively, as well as the size and number of colocalized pre- and postsynaptic puncta. These results indicate that PACAP induces persistent synaptic plasticity by inducing transcription-dependent, activity-independent alterations in synaptic function and in the structural arrangement of associated pre- and postsynaptic components.

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P.23: Role of CXCR4-LASP1 Axis in Translational Repression in Breast Cancer

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The C-X-C chemokine receptor family members CXCR1, CXCR2, CXCR3 and CXCR4 are involved in primary breast cancer progression, maintenance of cancer stem/initiating cell niche, chemoresistance, and metastasis. CXCR4 is primarily involved in directional migration, local invasion, and metastasis of triple negative breast cancer (TNBC) cells and is associated with a poor patient outcome. Previously our lab demonstrated that LIM and SH3 protein1 (LASP-1) directly bound to the carboxy-terminal domain of CXCR1-4, and more importantly that LASP-1 binding to CXCR4 influenced cell migration and Matrigel invasion. By employing proteomic and subsequent validation approaches, LASP-1 was found to associate with several components of the RNA-induced silencing complex (RISC) including XRN1/2 (ribonucleases), TNRC6A (a deadenylating enzyme), and DCP1/2 (decapping activators). Additionally, recombinant LASP-1 bound directly to the silencing domain of TNRC6A, potentially modulating its deadenylating capability. Further exploration revealed that LASP-1 interacted with argonaute2 (Ago2), the central RISC component, in an RNA-independent manner. Ago2 mediates small RNA-directed silencing of RNAs, potentially down regulating RNAs involved in cell-cell adhesion (e.g., E-cadherin). Next we examined the domains of LASP-1 to identify which mediates the interaction with Ago2 through a GST-pull down assay. Our results demonstrated that LASP-1 associates with Ago2 primarily through its src homology 3 (SH3) domain. This suggests that LASP-1 may mediate invasion and migration through altering the RNA profile of cells via interacting with Ago2. Future experiments include co-immunoprecipitation and proximity ligation assay in addition to RNA-seq or Nanostring to further characterize this interaction and its effect on TNBC metastasis.

P.24: Roles of Insulin Receptors and IGF-1 Receptors in Leptin-Responsive Neurons in Regulation of Body Weight, Growth, Pubertal Development and Fertility

Mengjie Wang and Jennifer W. Hill

Department of Physiology and Pharmacology

Growth and reproduction are tightly linked. Growth hormone deficiency results in a profound suppression of postnatal growth accompanied by delayed puberty (of a week or more in mice) and delayed reproductive senescence, while GH excess is correlated with the reverse. The specific mechanism underlying this delay is undefined. IGF-1 administration advances pubertal timing, however deletion of IGF-1R from GnRH neurons only delays puberty by 3-4 days. Thus, upstream, metabolically active neurons may play a role in the effects of IGF-1 on pubertal timing. Although neurons in the hypothalamus that express leptin receptors (LepRb) are known to modulate the timing of puberty, whether IGF-1 receptor (IGF-1R) signaling in these neurons controls pubertal development is unknown. To test whether IGF-1 action specifically in LepRb expressing cells affects pubertal development and fertility, we used Cre-loxp technology to generate female transgenic mice lacking IGF-1R exclusively in LepRb expressing cells (termed IGF-1RLepRb). Because IGF-1R and insulin receptor (IR) signaling overlap, we also generated double knockout female mice (termed IGF-1R/IRLepRb). IGF-1RLepRb mice experienced delayed pubertal development and impaired fertility. Surprisingly, the vaginal opening age, first estrus age, and fertility were comparable between the IGF-1RLepRb and IGF-1R/IRLepRb female mice. IGF-1RLepRb mice also exhibited metabolic abnormalities including reduced body weight and body growth. However, the body weight and body length in IGF-1R/IRLepRb female mice were significantly lower than IGF-1RLepRb mice. These findings identify divergent roles of IR and IGF-1R signaling in LepRb neurons. IGF-1R signaling in leptin responsive neurons plays a dominant role in the control of body weight, body length, and pubertal development and fertility, while IR signaling contributes to the control of body weight and body length.

P.25: Na/K-ATPase Signaling Stimulates CD40 Activation in Renal Proximal Tubular Epithelial Cells

Jeffrey X. Xie, , Shungang Zhang, Andrew Kleinhenz, Erin Crawford, Deepak Malhotra, Jiang Tian, Steven T. Haller

Department of Medicine

Background: Studies from our group and others have demonstrated that in addition to its canonical role as an ion-transporter, the Na/K-ATPase can form a functional signaling complex with Src, a non-receptor tyrosine kinase. We have established that infusion of cardiotonic steroids (CTS), ligands of the Na/K-ATPase, cause renal fibrosis both in vitro and in vivo. In addition, we have demonstrated that in vivo infusion of CTS causes an increase in renal CD40 expression. Recent studies have also established an important role for CD40 in renal fibrosis as well. We sought to test the hypothesis that CTS, via signaling through the Na/K-ATPase, can activate Src and regulate CD40 expression.

Methods: We treated LLC-PK1 cells, a pig proximal tubule cell line, with nanomolar concentrations of the cardiotonic steroid telocinobufagin (TCB) for 24 hrs and assayed for changes in CD40 expression by Western blot and Real-time PCR (qPCR). We also utilized a novel Na/K-ATPase knockdown cell-line and Src inhibitor.

Results: Twenty-four hour treatment with 10 nM TCB caused an increase in CD40 expression at the protein level ($p<0.05$). Pre-treatment with our novel Na/K-ATPase antagonist, pNaKtide, attenuated TCB mediated increases in CD40 expression ($p<0.05$), implicating Src as a key signaling molecule in this process. Finally, the effects of TCB on CD40 expression were abolished in the Na/K-ATPase knockdown cell line, which suggests that the effects of TCB are mediated specifically through the Na/K-ATPase.

Conclusions: Our results suggest that CTS signaling through the Na/K-ATPase-Src signaling axis may be a novel regulatory mechanism of CD40.

P.26: Targeted Disruption of Cd40 Significantly Reduces Renal Fibrosis Following Renal Transplantation in Experimental Renal Ischemia

Shungang Zhang, Stanislaw M. Stepkowski, Jeffrey X. Xie, Andrew Kleinhenz, Erin Crawford, Jiang Tian,Deepak Malhotra,Steven T. Haller,.

Introduction: In our novel Cd40 mutant created on the background of the Dahl S rat (S rat), we have previously demonstrated a significant reduction in renal fibrosis in the ischemic kidneys compared to S rats following induction of renal ischemia by the two-kidney, one clip (2K1C) method. We used reciprocal kidney transplants performed between Cd40 mutants and S rats followed by 2K1C to test the hypothesis that renal expression of Cd40 significantly contributes to the development of ischemic renal fibrosis in a process mediated by activation of renal fibroblasts.

Methods: Male Cd40 mutants and S rats were subjected to reciprocal kidney transplants followed by 2K1C ischemia. Western blot analysis and trichrome staining were performed on kidney tissue four weeks following 2K1C surgery. Primary rat renal fibroblasts were treated with soluble CD40 ligand (sCD40L) to stimulate CD40 signaling. Western blot and real-time PCR assays were performed for markers of inflammation and fibrosis 24hrs after treatment.

Results: Following reciprocal renal transplants, kidneys from Cd40 mutants transplanted into S rats and subjected to 2K1C ischemia demonstrated a significant decrease in renal fibrosis (as assed by trichrome staining) compared to ischemic kidneys from S rats transplanted into Cd40 mutants ($p<0.01$). In addition, renal fibroblasts stimulated with sCD40L demonstrated a significant increase in Cd40, monocyte chemotactic protein-1 (MCP-1), and procollagen-1 expression ($p<0.01$).

Conclusions: Reciprocal renal transplantation confirms that Cd40 expressed within the kidney contributes to renal fibrosis in ischemic renal disease, and this process is potentially mediated by activation of Cd40 in renal fibroblasts.

P.27: Role of mDia2 at Adherens Junctions in Epithelial Ovarian Cancer Cells

Yuqi Zhang, Kathryn Eisenmann.

Department of Cancer Biology and Biochemistry

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 mass involves the disruption of cell-cell adhesions in a process commonly known as epithelial-mesenchymal transition (EMT). 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 αE-catenin. However the specific players involved in the interaction between the junctional E-cadherin complex and the underlying junctional actin is complex and 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 αE-catenin to linear F-actin inhibits association of the branched-actin nucleator Arp2/3, while favoring linear F-actin bundling. Our previous work specifically implicated mDia2 and not mDia1 as a downstream effector of RhoA in maintaining EOC spheroids. Loss of mDia2 was associated with spheroid invasive egress. Our current work indicates that mDia2 and RhoA enhances junctional localization of β-catenin and actin in EOC monolayers, supporting the AJ-stabilizing effect of RhoA-activated mDia2. Immunoprecipitation studies show mDia2 interacts specifically with αE-catenin, yet not with E-cadherin or b-catenin. This suggests that αE-catenin recruits mDia2 to stabilize AJs by increased formation of linear actin bundles in the perijunctional region. Functional inhibition of mDia2 with small molecule inhibitors of formin homology 2 domains (SMIFH2s) does not alter associations either between β-catenin and αE-catenin or β-catenin and E-cadherin though cell-cell junctional disruption is observed, suggesting that mDia2's role in junctional stabilization may be restricted to interaction with peri-junctional αE-catenin, which does not associate with b-catenin or E-cadherin. Immunofluorescence studies will be performed to confirm spatial and temporal co-localization of αE-catenin and mDia2 in EOC cells. Specific domains through which mDia2 interacts with αE-catenin will be determined.

P.28: Identification of Lung Cancer Associated cis-Regulatory SNPs

Rose Zolondek

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Lung cancer kills about 160,000 people/year. High risk subjects tend to be ages 55-80, have smoked thirty or more pack years, and quit smoking less than fifteen years previous. The United States Preventative Safety Task Force recommends annual low dose CT scans for early lung cancer diagnosis in people meeting these high risk epidemiologic criteria. However, these criteria are non-specific and include people who will never die of lung cancer. Therefore, there is a need to identify biomarkers that define lung cancer risk better than epidemiologic criteria alone. There is increasing evidence that many common, low effect single nucleotide polymorphisms (SNPs) are associated with COPD and/or lung cancer. For many risk SNPs, the effect is so small that 10-100,000 subjects or more would be required in classic GWAS to reach power. The purpose of this study is to test strategies that promise to have higher power to identify common low effect lung cancer risk SNPs and thereby better define hereditary risk. Based on preliminary data, <200 subjects are necessary to assess a) function of putative cis-rSNPs in NBEC or b) altered NBEC regulation associated with risk. In other studies, we identified SNPs that replicate at $p<0.05$ threshold in COPD GWAS, and are doing same for lung cancer GWAS. We have assessed 600 lung cancer risk associated putative cis-rSNP for functionality of these replicated SNPs in existing GTEx database, and, in future studies, experimentally in cultured primary normal bronchial epithelial cells (NBEC) using the Massively Parallel Report Assay (MPRA).

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Oral Presentation Abstracts

O.1: Alcohol interactions with Methamphetamine through inflammation

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Alcohol and methamphetamine (Meth) are often co-abused. Individually, both cause neurotoxicity evidenced by long-term depletions of dopamine and serotonin but the consequences of their co-abuse are unknown. We hypothesized that serial exposure to alcohol and Meth enhances the neurotoxicity produced by either drug alone. Male Sprague Dawley rats voluntarily drank 10% ethanol (EtOH) for 24-hr periods, every other day, for 4 weeks and then were exposed Meth. The results show that EtOH intake and preference increased over 4 weeks. EtOH drinking increased inflammation as depicted by increases in serum and brain LPS, and brain COX2. Meth alone but not EtOH alone depleted dopamine and serotonin in the striatum. In contrast, serial exposure to EtOH followed by Meth depleted dopamine and serotonin in a manner that was greater than that produced by Meth itself suggesting a synergistic relationship between EtOH and Meth. Moreover, the ability of EtOH to enhance Meth-induced neurotransmitter depletions in the striatum was dependent on the amount of EtOH that was consumed. Administration of the COX inhibitor, ketoprofen during EtOH drinking did not alter EtOH intake but prevented the increases in LPS and COX2 and the subsequent enhancement of dopamine and serotonin depletions in the striatum produced by Meth. The behavioral consequences of the enhanced striatal dopamine depletions were revealed by motor deficits measured by performance in the roto-rod test, the deficits also being prevented by ketoprofen administration during EtOH drinking. Overall, these results provide a role for inflammation in mediating the synergistic effects of EtOH and Meth.
Grant Support: NIH DA07606

O.2: Isolation and Characterization of Lake Erie Bacteria that Degrade the Microcystin Toxin MC-LR

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Department of Medical Microbiology and Immunology

Microcystin-LR (MC-LR) is a hepatotoxin produced by lake-dwelling cyanobacteria, including Microcystis sp. and Planktothrix sp. Harmful algal blooms (HABs) in Lake Erie have become a major human health concern in recent years, highlighted by the August 2014 “do not drink” order issued by the City of Toledo to roughly half a million residents over the course of three days. Given that MC-LR is a molecule containing 7 amino acids, we hypothesized that naturally-occurring Lake Erie bacteria could use MC-LR as a ‘free’ energy source. Further, isolation of such MC-LR-degrading bacteria could lead to the development of safe and cost-effective methods (i.e. biofilters) to remove MC-LR from drinking water. To test this hypothesis, we collected water samples from various locations in Lake Erie during the summers of 2014, 2015, and 2016, added MC-LR to each water sample 2-3 times weekly, for 4-6 weeks, to enrich for MC-LR-degrading bacteria, and assessed MC-LR degradation over time. Our data demonstrated that MC-LR-degrading bacteria are present in Lake Erie and that these bacteria rapidly degrade MC-LR. In addition, the vast majority of these MC-LR degrading bacterial clones were found to produce robust biofilms, indicating that biofilter development may be feasible. Finally, DNA sequencing analysis has revealed that a diverse array of bacteria degrade MC-LR, none of which appear to be human pathogens. Current studies are testing the ability of groups of clones, or individual clones, to degrade MC-LR and to test the ability of laboratory-scale biofilters to remove MC-LR from water.

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O.3: A Novel CRISPR/Cas9 Knock-in Rat Model Defines A Polymorphic Variant of Secreted Phosphoprotein 2 as A Quantitative Trait Nucleotide Linked to The Heritability of Blood Pressure

Saroj Chakraborty, Ying Nie, Xi Cheng, Blair Mell, Sarah Galla, Bina Joe

Department of Physiology and Pharmacology

Hypertension is a complex polygenic disease caused by a combination of inherited (genetic) and environmental factors. In rat model, one such candidate gene prioritized through systematic linkage and substitution mapping is Spp2 or Secreted Phosphoprotein 2. 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) 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 the resultant Spp2 knock-in rescue model. Following transgenesis, pups born were genotyped and grouped into founders and non-founders. The founder and non-founder rats were isogenic except for the Spp2 locus, wherein the founder S rats contained the 'G' allele and the non-founder S rats contained the 'T' allele. Both founder and non-founder rats (n=12 male rats/group) were fed a 2% NaCl containing diet and their BP was monitored by radiotelemetry. Both systolic and diastolic BP of the Spp2 knock-in rescue model were significantly lower compared to that of the non-founder S 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.

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

O.4: Characterization of the phage shock protein response in Vibrio cholerae

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Cholera is a severe intestinal infection characterized by voluminous, watery diarrhea that can be fatal within hours. Ingestion of water or food contaminated by the marine bacterium Vibrio cholerae leads to human disease. While rare in the United States and other industrialized nations, cholera is endemic in more than 50 countries. In both the aquatic and intestinal life cycles, V. cholerae will encounter various stressful conditions, such as fluctuating pH, bacteriophage predation, and exposure to antimicrobial peptides, which may negatively affect the integrity of the inner membrane. The phage shock protein (Psp) system is a stress response mechanism that senses and responds to such insults. The Psp system is conserved in a number of clinically relevant Gram-negative bacteria, including V. cholerae, and has been implicated in a variety of virulence-related processes. However, most of the current knowledge about the Psp system stems from in vitro studies from Escherichia coli and Yersinia enterocolitica. In fact, the Psp response in V. cholerae has remained completely uncharacterized. Therefore, we are using several methods, including chromosomal transcriptional reporters, various growth conditions, and specific protein inducers to begin characterizing the Psp system in V. cholerae.

O.5: The tumor microenvironment drives tumor progression through an mdia2-mediated mechanism

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The tumor microenvironment (TME) is a heterogeneous region containing tumor cells, stromal cells, and secreted factors that make the local tissue favorable for cancer formation and progression. An important shift in a pro-cancerous microenvironment is the transformation of normal fibroblasts to carcinoma-associated fibroblasts (CAFs). CAFs can directly enhance adjacent tumor-cell motility via their innate ability to secrete factors into the TME. We sought to understand how CAF-derived chemokines impact breast tumor-cell motility through F-actin cytoskeletal regulation. To do this, we collected cultured media (CM) from WS19T fibroblasts, a transformed patient-derived breast CAF cell line, and studied its impact upon tumor-cell motility. We previously linked chemokine signaling with a critical F-actin cytoskeleton regulator, mammalian Diaphanous-related formin (mDia2). Here we assess changes in formin expression in response to chemokine-enriched CAF-CM. CAF-CM dramatically enhances tumor-cell wound-closure in breast adenocarcinoma MDA-MB-231 cells, relative to normal fibroblast-CM. Western blotting CAF-CM-treated MDA-MB-231 cells reveals a significant loss of mDia2 protein. We verify mDia2 loss is at the protein level via a proteasome-dependent mechanism. Using a membrane-based cytokine array to assess CAF-CM cytokine content, we show significant increases in the chemokine CXCL12 within CM. Exogenous CXCL12 treatment results in increased wound closure and loss of mDia2 protein. Blocking CXCL12 signaling augments motility and rescues mDia2 protein expression in the presence of CM. These findings indicate a novel mechanism for driving tumor-cell invasive capacity and highlight a crucial gap in the therapeutic approach of treating primary lesions with high invasive potential.

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O.6: Quantitative proteomic analysis of endothelial cells in G-protein coupled estrogen receptor (Gper1) knockout rats

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G-protein coupled estrogen receptor (Gper1), a previously identified orphan receptor, now known to be a receptor for estrogen and aldosterone, has been localized to blood vessels, heart, and the central nervous system. Gper1 knockout models developed in mice and rats have shown increased blood pressure (BP) as compared to controls. In addition, a Gper1 agonist, G1, caused decreased blood pressure when injected into mice. Women with a GPERP16L amino acid substitution in their GPER1 protein have increased BP. In order to study the effects of this receptor further, we created a novel CRISPR/Cas9 gene deletion model of the Gper1 gene on a Dahl Salt-Sensitive (S) rat background. Hemodynamic and vascular phenotypic studies demonstrated that Gper1 knockout rats had lower BP and improved vascular function compared to the S rat, in an endothelial dependent manner. Therefore, we hypothesize that deletion of Gper1 in endothelial cells causes quantitative alterations in proteins that are directly impacted by the function of Gper1. To test this hypothesis, a quantitative proteomic analysis was conducted on endothelial cells isolated and cultured from the thoracic aorta of Gper1 knockout rats and wildtype S rats. Endothelial cell cultures were digested and quantitatively analyzed by Mass Spectrometry. A total of 101 statistically significant differentially expressed proteins were identified, of which 97 were with higher expression in the Gper1 knockout rats. Data prioritizing three of the most significantly upregulated proteins, Fibrillin-1, Ephrin B1, and Cd99, as potential mechanistic links downstream of the functionality of Gper1 in endothelial cells will be presented.

O.7: γ-Glutamyl Cyclotransferase Is Required for Francisella tularensis Virulence

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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 ability to cause rapid, fatal disease in many species, including humans. In previous studies, we demonstrated that Ft encodes a disulfide bond formation protein ortholog, DsbA, that is required for infection of macrophages and mice. Using a molecular trapping approach, we identified over 50 Ft DsbA substrates, including outer membrane proteins, known virulence factors, and many hypothetical proteins. Here, we performed a detailed analysis of one DsbA substrate, FTL1548 in Ft Type B strain LVS and FTT0509c in Ft Type A strain SchuS4, demonstrating that this previously-unstudied hypothetical protein contains γ-glutamyl cyclotransferase (GGCT) domains. Although little has been reported about bacterial GGCT proteins, human GGCT plays important roles in glutathione homeostasis and amino acid recycling. Isogenic mutants of both FTL1548 and FTT0509c were found to be attenuated in macrophages and mice. Additionally, site-directed mutagenesis of the conserved glutamic acid catalytic residue also resulted in Ft attenuation. In vitro assays with recombinant FTT0509c demonstrated GGCT enzymatic activity. Finally, immunofluorescence studies in macrophages are defining the host cell mechanisms resulting in GGCT mutant clearance. Taken together, these studies have defined the molecular function of a previously uncharacterized protein and have identified a new Ft virulence factor.

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O.8: Gut microbiotal dysbiosis and increased risk for complex polygenic diseases emerge with genomic selection for low aerobic exercise capacity

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Bi-directional host-microbiotal interactions are increasingly being recognized as important factors contributing to the pathophysiology of complex polygenic diseases. While host genomes are inherited, the microbiome is acquired as a result of the composition of the microbiota that choose to reside within the host. The identities of the factors that determine which microbes choose to reside in a given host are unknown. Given the evidence for host-microbiotal interactions in health and disease, we hypothesized that host genomes exert a selection pressure to influence the types of microbiota that reside in the host and that the resultant microbiotal composition is a pivotal determinant of the divide between health and disease of the host. To test this hypothesis, we developed models of health and disease by inbreeding rats divergent in aerobic exercise capacity. These novel inbred strains were developed by >20 generations of brother-sister mating of the selectively-bred low aerobic capacity rats and high aerobic capacity rats and named as LCR/BJ and HCR/BJ, respectively. Inbred HCR/BJ rats had significantly higher aerobic exercise capacity than inbred LCR/BJ rats as recorded by their total running distance to exhaustion of 1151 m vs. 130 m, respectively, $p < 0.001$. Next-generation sequencing of the whole genomes of inbred HCR/BJ and LCR/BJ rats revealed considerable differences in their genomic sequences. To test whether this selection for differential genomic sequences resulted in any alterations in microbiota, fecal microbial communities of inbred LCR/BJ and HCR/BJ rats were profiled using 16S rRNA sequences. Interestingly, principal coordinates analysis displayed distinct clustering of the two cohorts (ANOSIM analysis, weighted UniFrac, $P = 0.002$), indicating significant phylogenetic differences of the microbial community structures between inbred LCR/BJ and HCR/BJ rats. Firmicutes/Bacteriodetes ratio was higher in the LCR/BJ rats compared with HCR/BJ rats suggesting the possibility of the increased risk for diseases in the LCR/BJ rats. Compared with inbred HCR/BJ rats, inbred LCR/BJ rats exhibited elevated blood pressure, increased body weight, lower circulating mean corpuscular hemoglobin, and higher neutrophil-to-lymphocyte ratio. Metabolic and behavioral phenotyping is currently underway. Overall, these studies provide clear evidence for the ability of the host genome to shape the microbiome and identify an inverse co-evolutionary relationship between host genomic factors conferring enhanced aerobic exercise capacity and microbiotal dysbiosis driving disease risks.

O.9: Targeting dysregulated mitochondrial fission pathways in triple negative breast cancer therapy

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Triple Negative Breast Cancer (TNBC) is the most aggressive, fatal and highly metastatic, accounting for about 15 percent of all forms of breast cancers. TNBC tumors lack expression of three cell-surface therapeutic targets (estrogen receptor-α, progesterone receptor, and HER2/ERBB3) and have no known therapeutic target. We found mitochondrial fission protein GTPase dynamin-related protein-1 (Drp1), to be highly dysregulated in metastatic TNBC for which one Drp1 inhibitor (Mdivi-1) is under active investigation. Mdivi-1, however, has shown inconsistent antineoplastic activity, poor potency and selectivity. Consequently, using the Specs compound library of 200,000 commercial molecules, in conjunction with Drp1-Mdivi-1 complexes, we computationally identified 10 potent and selective Drp1 inhibitors. In our preliminary work we found one lead compound ANT-4 with IC50 value of 0.38μM that has up to three-fold selectivity in different TNBC cells (MDA-MB 231, MDA-MB468, BT-20) compared to normal breast cells (HMEC) and up to 20-fold compared to other normal cells (HEK-293, CRL-1459). ANT-4 was also found to be ten-fold selective to TNBC cells compared to other cancer cell lines i.e. colon cancer cell line (HCT-116, HCT-15), ovarian cancer cell line (OV2008, A2780). ANT-4 prevented migration and inhibited the invasive characteristics of TNBC cell lines confirming its anti-metastatic property. The ANT-4 significantly downregulated the major proteins involved in mitochondrial fission pathway such as Drp1, its phosphorylated form, pDrp1, cyclin B1 and mitochondrial fission factor (MFF). Further studies are underway to understand how mitochondrial dysfunction results in selective TNBC targeting.

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O.10: AMPK, a novel checkpoint to control Herpesvirus replication

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Herpes Simplex Virus-1 (HSV-1), a member of the herpesvirus family, is a human pathogen, which spreads by human contact and replicates in mucosal surface. HSV-1 infection is a major concern due to its ability to remain latent and cause chronic infections. Like other viruses, HSV- 1 takes advantage of specific host factors to promote its replication. Our results suggest, for the first time, that HSV-1 replication is dependent on adenosine monophosphate kinase (AMPK). AMPK is a sensor of cellular energy and plays a role in homeostasis and regulates several cellular processes. HSV- 1 replicates poorly in AMPK knockout mouse embryonic fibroblasts (MEFs) and AMPK knockdown human epithelial cells (HEK293). Moreover, increasing levels of cellular AMPK expression leads to enhanced viral replication. To investigate whether the kinase activity of AMPK is required for HSV-1 replication, we used a pharmacological inhibitor of AMPK, known as Compound C. The inhibition of HSV-1 replication with the use of Compound C, in both human and mouse cells suggests that AMPK activity is essential for HSV-1 replication and not its physical presence. Since, AMPK is known to promote autophagy, we used chemical inhibitors and activators of autophagy and found that HSV replication is independent of the AMPK-mediated autophagy pathway. Therefore, our results have clearly identified a novel mechanism of HSV-1 replication. Future studies will reveal whether AMPK inhibition is a potential therapeutic strategy to control HSV-1 infection in vitro and in vivo.

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O.11: Investigating the antiviral response to tick-borne flaviviruses in the white-footed mouse

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Tick-borne flaviviruses (TBFVs), including Powassan virus and tick-borne encephalitis virus cause encephalitis or hemorrhagic fevers in humans with case-fatality rates ranging from 1-30%. Despite severe disease in humans, TBFV infection of natural rodent hosts has little noticeable effect. Currently, the basis for this resistance to disease is not known. We hypothesize that the coevolution of flaviviruses with their respective hosts has shaped the evolution of potent antiviral factors that suppress virus replication and protect the host from lethal infection. In the current study, we compared virus infection between reservoir host cells and related susceptible species. Infection of primary fibroblasts from the white-footed mouse (*Peromyscus leucopus*, a representative host) with multiple TBFVs showed up to a 10,000-fold reduction in virus titer compared to control *Mus musculus* cells. Stepwise comparison of the virus infection cycle revealed a significant block to viral RNA replication, but not virus entry, in *P. leucopus* cells. To understand the role of the type I interferon (IFN) responses in virus restriction, we depleted signal transducer and activator of transcription 1 (STAT1) or the type I IFN receptor (IFNAR1) by RNA interference. Loss of IFNAR1 or STAT1 significantly relieved the block to virus replication in *P. leucopus* cells. Collectively, this work demonstrates that the IFN response of *P. leucopus* imparts a strong and virus-specific barrier to flavivirus replication. Future identification of the IFN-stimulated genes responsible for virus restriction in *P. leucopus* will yield mechanistic insight into efficient control of virus replication and may inform the development of antiviral therapeutics.

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O.12: Platelet hypersensitivity as a potential mechanism of thrombosis in lung cancer

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Cancer patients are known to be at high risk for thrombosis. 20% of all patients experiencing venous thromboembolism (VTE) have cancer, and the death rate due to venous thromboembolism for cancer patients actively undergoing chemotherapy is 47 times greater than the general population. Moreover, cancer-associated thrombosis is associated with poor prognosis and is a leading cause of death among cancer patients. Cancer may influence platelet function, which may predispose cancer patients to thrombotic events. We tested the hypothesis that platelets from lung cancer patients are hypersensitive to thrombotic stimulation which may lead to thrombosis. To test this, whole blood samples are collected from patients undergoing a chest PET scan at the University of Toledo Medical Center Dana Cancer Center, including individuals diagnosed with lung cancer (non-small cell) and those without cancer. Platelets from healthy volunteers are evaluated as an additional control population. Purified platelets from each subject were exposed to several concentrations of thrombin, a potent platelet agonist, and analyzed for expression of activation markers by flow cytometry. We observed no difference in baseline platelet activation among any groups. Interestingly, lung cancer patients not taking antiplatelet drugs demonstrate significantly higher sensitivity to thrombin (EC50) when compared to healthy controls and patients taking antiplatelet drugs. We seek to determine mechanisms underlying platelet hypersensitivity in lung cancer patients, which we hope may lead to a biomarker of thrombosis risk.

O.13: cAMP dependent recruitment of acidic organelles for calcium signalling in the salivary gland

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Autonomic neural activation of intracellular Ca2+ release in parotid acinar cells induces the secretion of the fluid and protein components of primary saliva critical for maintaining overall oral homeostasis. In the current study, we profiled the role of acidic organelles in shaping the Ca2+ signals of parotid acini using a variety of imaging and pharmacological approaches. Results demonstrate that zymogen granules predominate as an apically polarized population of acidic organelles that contributes to the initial Ca2+ release. Moreover we provide evidence that indicates a role for the intracellular messenger NAADP in the release of Ca2+ from acidic organelles following elevation of cAMP. Our data are consistent with the “trigger” hypothesis where localized release of Ca2+ sensitizes canonical intracellular Ca2+ channels to enhance Ca2+ signals from the ER. Release from acidic stores may be important for initiating saliva secretion at lower levels of stimulation and a potential therapeutic target to augment secretory activity in hypofunctioning salivary glands.