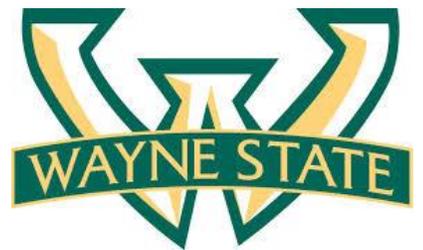


45th Annual Pharmacology Research Colloquium



Molecular Plant Sciences Building
Michigan State University
East Lansing, MI

Friday, June 1st, 2018

Table of Contents

Welcome – Page 3

Schedule – Page 4

Logistics – Page 5

Keynote Speaker Bio – Page 6

Chris Contag

Oral Session Abstracts – Pages 7-19

Poster Session Abstracts – Pages 20-108

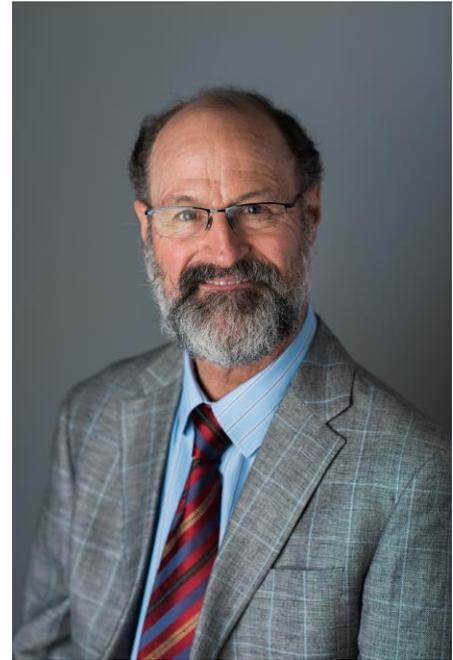
Welcome letter

June 1, 2018
East Lansing, MI

Dear Pharmacologists and Friends,

We are delighted to welcome you to East Lansing for the 45th Pharmacology Colloquium for Michigan State University, the University of Michigan, the University of Toledo, and Wayne State University.

It is such an exciting time in pharmacology and biomedical science. There have been tremendous advances in the understanding of disease mechanisms, development of precision medicine, and advances in treating disease through both small molecule drugs and other approaches such as gene therapy, cell-based therapies, etc. The power of computational approaches and the use of big data to identify new drugs is also advancing rapidly.



We welcome you to the newly expanding “South Campus” at MSU which is becoming a major hub for the biomedical sciences on our campus. Our Life Sciences Building has already been joined by the Institute for Quantitative Health Sciences and Engineering (IQ building) which houses Biomedical Engineering and many other scientists. Also, a new Integrative Sciences and Technology Building which will be completed next year which will include computational biologists, neuroscientists, and a new Precision Health Program. This expansion is driven in part by a major effort at MSU termed the Global Impact Initiative or GII – which aims to bring in 100 new faculty across MSU over 3-4 years.

I look forward to the student talks and posters by students and postdocs to learn more about the work going on at your three institutions. Also, I hope that we all get a chance to catch up with faculty and meet new members of the 4 departments to foster collaborations among our institutions.

Please enjoy this day of great science and connecting.

Rick Neubig
Chair of Pharmacology and Toxicology

Schedule

8:15 – 9:00	Registration/Breakfast
9:00 – 9:10	Welcoming Remarks (Dr. Rick Neubig)
9:10 – 10:50	Oral Session I Moderators: Kibrom Alula (MSU), Camille Akemann (WSU) 9:10 Di Zhang (MSU) 9:30 Ethan Brock (WSU) 9:50 Darren Mikael Gordon (U-T) 10:10 Kibrom Alula (MSU)
10:30 – 10:50	Coffee Break
10:50 – 11:50	Dr. Chris Contag, Keynote Address
11:50 – 12:45	Lunch
12:45 – 2:00	Poster Session
2:00 – 3:00	Oral Session II Moderators: Kaylin White (U-M), Amit Chougule (U-T) 2:00 Sudipta Baroi (U-T) 2:20 Vanessa Benham (MSU) 2:40 Alexandra Bouza (U-M) 3:00 Sarah Galla (U-T)
3:20 – 3:40	Coffee Break
3:40 - 5:00	Oral Session III Moderators: Danielle Meyer (WSU), Kelsey Fout (U-T) 3:40 Nicole Michmerhuizen (U-M) 4:00 Fausto Varela (WSU) 4:20 Usman Ashraf (U-T) 4:40 Kevin Baker (MSU)
5:00 – 5:30	Cocktails and Appetizers
5:30 – 7:00	Dinner and Awards

Logistics

Instructions for posters: Posters will be set up in rooms A149 and A155 of the Plant and Soil Sciences building, which is located down the hallway from Molecular Plant Sciences (MPS) 1200 (the Molecular Plant Sciences building and Plant and Soil Sciences building are connected). Please put up posters upon arrival or during the first break. Posters have been assigned numbers, which are indicated on the abstract in the program or can be found at the registration desk. These numbers correspond to the numbers on the poster boards—please set up posters according to the assigned numbers. Poster boards are 4' x 4'. Please use the Velcro provided. No push pins please!

Instructions for oral presentations: Oral presentations will be in MPS 1200. Please have your talk on an external USB drive in a form compatible with a PC computer. Talks should be loaded onto the computer in MPS 1200 by 8:30 AM (particularly for those in the first session).

Instructions for judges: Please check in during breakfast a registration for a packet containing scoring materials. Thanks for your help!

Instructions for moderators: Please check in during breakfast at registration for quick instructions. Your job will be to introduce each speaker. We have 2 moderators for each session, so you will need to divide the session between yourselves. Please be seated 5 minutes prior to the start of your assigned section. Thanks!

Registration will be outside of MPS 1200. Food and all breaks are in the Atrium of the MPS building.

Invited Speaker Biography

Dr. Chris Contag is the founding director of the Institute for Quantitative Health Science and Engineering (IQ) and chair of the new Department of Biomedical Engineering in the College of Engineering at Michigan State University.

Dr. Contag is also Professor emeritus in the Department of Pediatrics at Stanford University. Dr. Contag received his B.S. in Biology from the University of Minnesota, St. Paul in 1982. He received his Ph.D. in Microbiology from the University of Minnesota, Minneapolis in 1988. He did his postdoctoral training at Stanford University from 1990-1994, and then joined Stanford faculty in 1995 where he was professor in the Departments of Pediatrics, Radiology, Bioengineering and Microbiology & Immunology until 2016.



Current research in the Contag laboratory focuses on the development of macroscopic and microscopic optical imaging tools. Dr. Contag uses imaging to assess tissue responses to stress, reveal immune cell migration patterns, understand stem cell biology and advance biological therapies. The research mission of the lab is to develop and use noninvasive imaging tools that can simultaneously reveal the nuances of biological processes and provide an overall picture of disease states for the purpose of developing and redefining novel interventions.

Dr. Contag is a pioneer in the field of molecular imaging and is developing imaging approaches aimed at revealing molecular processes in living subjects, including humans, and advancing therapeutic strategies through imaging. He is a founding member and past president of the Society for Molecular Imaging (SMI), and recipient of the Achievement Award from the SMI and the Britton Chance Award from SPIE for his fundamental contributions to optics. Dr. Contag is a Fellow of the World Molecular Imaging Society (WMIS) and the recent past President of WMIS. Dr. Contag was a founder of Xenogen Corp. (now part of PerkinElmer) established to commercialize innovative imaging tools for biomedicine. He is also a founder of ConcentRx—a cancer therapy company, and PixelGear—a point-of-care pathology company.

Oral Session Abstracts:

Session 1:

Di Zhang (MSU): "Developing a nanoformulation of the PARP inhibitor Talazoparib as a novel delivery for treatment of BRCA-deficient breast cancer"

Ethan Brock (WSU) "Loss of Sprouty4 Promotes DCIS Progression through Increased ERK/MAPK Signaling"

Darren Mikael Gordon (U-T): "CRISPR Targeting of Biliverdin Reductase-A (BVRA) in Mouse Kidney Cells Advocates Lipotoxicity"

Kibrom Alula (MSU): "Transient increase in sympathetic constriction of mesenteric arteries from high fat diet-fed Dahl salt-sensitive female rats"

Session 2:

Sudipta Baroi (U-T): "PPAR γ : a sex-determined molecular brake for osteocyte energy metabolism and bone mass"

Vanessa Benham (MSU): "Mechanisms and Chemoprevention of Adiposity-Associated Breast Cancer"

Alexandra Bouza (U-M): "Voltage-gated sodium channel (VGSC) β 1 subunit is processed into a potential transcriptional regulator"

Sarah Galla (U-T): "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"

Session 3:

Nicole Michmerhuizen (U-M): "Small molecule profiling uncovers the landscape of combinatorial PI3K inhibitor responses in HNSCC"

Fausto Varela (WSU): "TMPRSS13 as a Modulator of Colorectal Cancer"

Usman Ashraf (U-T): "Novel look into COUP-TFII role in gluconeogenesis and β -oxidation in the Dahl salt sensitive (ss) rat"

Kevin Baker (MSU): "Direct activation of tissue factor procoagulant activity by bile acids drives intrahepatic coagulation in liver disease"

DEVELOPING A NANOFORMULATION OF THE PARP INHIBITOR TALAZOPARIB AS A NOVEL DELIVERY FOR TREATMENT OF BRCA-DEFICIENT BREAST CANCER

Di Zhang¹, Paige Baldwin², Srinivas Sridhar² and Karen T. Liby¹

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BRCA mutations are the leading cause of hereditary breast cancer. PARP inhibitors have shown promising activities in clinical trials for breast cancer by inducing synthetic lethality, particularly in patients with BRCA deficiency. Moreover, the utility of PARP inhibitors could potentially extend beyond BRCA mutations by targeting defects in homologous recombination, and thus impact up to 33% of breast cancer patients overall. However, conventional oral delivery of PARP inhibitors is hindered by limited bioavailability and significant off-target toxicities, thus compromising the therapeutic benefits and quality of life in patients. Therefore, we developed a new nanoparticle delivery system for PARP inhibitors and hypothesize that nanoformulated Talazoparib can enhance efficacy by increasing drug concentrations in the tumor and reduce off-target toxicities. The nanoparticle formulation includes polymer brushes to prolong the circulation time, enabling tumor accumulation through the enhanced permeability and retention effect. The therapeutic efficacy of Nano-Talazoparib (Nano-TLZ) was assessed after i.v. injection in Brca1Co/Co;MMTV-Cre;p53+/- mice with established tumors and compared to vehicle control (saline, i.v.), empty nanoparticles (i.v.), free Talazoparib (i.v.), and free Talazoparib (gavage). Treatment was started when the tumor was 4 mm in diameter and ended when the tumor size reached defined IACUC endpoints. Nano-TLZ significantly ($p < 0.05$) prolonged the life span of BRCA deficient mice compared to saline control, empty nanoparticle control, and gavage treatment group. Progression free survival is significantly improved in the Nano-TLZ treatment group ($p < 0.05$) compared to free TLZ groups (both i.v. and gavage). Moreover, Nano-TLZ is better tolerated than oral gavage and free Talazoparib, with no significant weight lost. In a biomarker study following 10 days of treatment, Nano-TLZ increased double strand DNA breaks (γ -H2AX) and decreased proliferation (PCNA) compared to controls. Interestingly, Nano-TLZ significantly ($p < 0.05$) decreased myeloid derived suppressor cells in both the tumor and spleen compared to the saline control. Nano-TLZ also significantly ($p < 0.05$) decreased the percentage of tumor-associated macrophages in the mammary gland compared to the saline control group. The changes in immune populations suggest potential immunomodulatory effects of Talazoparib. These results demonstrate that the delivery of Talazoparib as a nanoformulation induces superior treatment outcomes with reduced off-target toxicity in BRCA deficient mice, and provides a novel delivery strategy for PARP inhibitors in patients.

LOSS OF SPROUTY4 PROMOTES DCIS PROGRESSION THROUGH INCREASED ERK/MAPK SIGNALING

Ethan J. Brock^{1,2}, Ryan Jackson², Julie L. Boerner¹, Quanwen Li², Bonnie F. Sloane^{1,2}, and Raymond R. Mattingly^{1,2}

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Breast ductal carcinoma *in situ* (DCIS) is a non-obligate precursor of invasive ductal carcinoma (IDC). Patients with DCIS often receive surgery and radiotherapy, but this early intervention has not produced substantial decreases in late-stage disease—highlighting overtreatment. This has resulted in a clinical need for reliable biomarkers or molecular determinants to assess disease progression. Sprouty proteins are considered important regulators of ERK/MAPK signaling, and have been studied in various cancers. We hypothesize that Sprouty4 is an endogenous inhibitor of ERK/MAPK signaling, and its loss or reduced expression is a mechanism by which DCIS lesions progress toward IDC, including triple-negative disease. Using immunohistochemistry we found that Sprouty4 expression was reduced in IDC patient samples compared to normal or DCIS tissues, and that ERK/MAPK activity had an inverse relationship to Sprouty4 expression. This corresponded with immunoblot data from our 3D culture model of progression where Sprouty4 expression was higher in DCIS compared to IDC cells. Over-expression of Sprouty4 reduced both ERK/MAPK activity and the aggressive phenotype of MCF10.CA1d IDC cells. Immunofluorescence experiments revealed data consistent with the relocation of E-cadherin back to the cell surface and the restoration of adherens junctions. To determine whether these effects were due to changes in ERK/MAPK signaling, IDC cells were treated with an allosteric MEK inhibitor. Nanomolar concentrations of drug restored an epithelial-like phenotype, similar to Sprouty4 over-expression. From these data we conclude that Sprouty4 may act to control ERK/MAPK signaling in DCIS, thus limiting the progression of these premalignant breast lesions.

CRISPR TARGETING OF BILIVERDIN REDUCTASE-A (BVRA) IN MOUSE KIDNEY CELLS ADVOCATES LIPOTOXICITY.

Darren Gordon¹, Samuel Adeosun², Mary Weeks², Kyle Moore², John Hall², Terry Hinds¹, David Stec²

(1) Department of Physiology and Pharmacology, University of Toledo (2) Department of Physiology & Biophysics, University of Mississippi Medical Center.
University of Toledo

Obesity and increased lipid availability have been implicated in the development and progression of chronic kidney disease. One of the major sites of renal lipid accumulation is in the proximal tubule cells of the kidney, suggesting that these cells may be susceptible to lipotoxicity. We previously demonstrated that loss of hepatic BVRA causes fat accumulation in livers of mice on a high-fat diet. To determine the role of BVRA in mouse proximal tubule cells, we created a CRISPR targeting BVRA for a knock-out in mouse MCT proximal tubule cells (BVRA KO). The BVRA-KO cells had significantly less metabolic potential and mitochondrial respiration, which was exacerbated by treatment with saturated fatty acid, palmitic acid. The BVRA KO cells also showed increased intracellular triglycerides which were associated with increased fatty acid uptake gene cluster of differentiation 36 (CD36) as well as increased de novo lipogenesis as measured by higher neutral lipids. Additionally, apoptotic and cellular function assays demonstrated that BVRA KO cells are more sensitive to palmitic acid-induced lipotoxicity than wild-type cells. Phosphorylation of BAD, which plays a role in cell survival pathways, were significantly higher in non-treated BVRA KO cells, but was reduced with palmitic acid treatment. These data demonstrate the protective role of BVRA in proximal tubule cells against saturated fatty acid-induced lipotoxicity and suggest that activating BVRA could provide a therapeutic in protecting from obesity-induced kidney injury.

TRANSIENT INCREASE IN SYMPATHETIC CONSTRICTION OF MESENTERIC ARTERIES FROM HIGH FAT DIET-FED DAHL SALT-SENSITIVE FEMALE RATS

Kibrom Alula¹, Hui Xu¹, Stephanie Watts¹, Gregory Fink¹, James Galligan^{1,2}

¹Department of Pharmacology & Toxicology and ²the Neuroscience Program, Michigan State University, E. Lansing MI 48824

Hypertension (HTN) is a risk factor for cardiovascular diseases, stroke, type 2 diabetes and kidney failure. Obesity (BMI ≥ 30) is associated with increased sympathetic nerve activity (SNA) that leads to HTN. SNA increases blood pressure in part by causing norepinephrine (NE) and adenosine-5'-triphosphate (ATP) release and increased constriction of resistance mesenteric arteries (MAs). HTN is less common in premenopausal women than in age-matched men. The mechanisms by which blood pressure may be differentially regulated in men versus women are not well understood. We hypothesized that sympathetic neurotransmission in adiposity-related HTN is greater in MAs of male versus female rats. We used male and female Dahl salt-sensitive rats (Dahl SS) that were fed either control (CD) or high fat (HFD) diet beginning at 3 weeks of age. Body weight was measured when rats were euthanized at 10, 17 and 24 weeks on diet. Males gained more weight than females irrespective of their diet. Mean arterial pressure (MAP) was measured weekly by tail-cuff plethysmography. MAP was higher in HFD than CD rats at all time points. Plasma estradiol (E2) level was measured by ELISA. E2 level was greater, similar and lesser in HFD males than HFD females at 10, 17 and 24 weeks on diet, respectively. MAs (Inner diameter; male=275-350 μm , female=245-315 μm) were harvested and mounted in a pressure (60 mmHg) myograph and inner diameter was measured continuously by video microscopy. MA contractile responses to electrical field stimulation was measured at 0.2-30Hz. Neurogenic constriction of MA from HFD females transiently shifted leftward at 17 weeks on diet. Constrictions caused by exogenous NE and ATP were similar in MA from HFD male and female rats. Prejunctional $\alpha 2$ -adrenergic receptor ($\alpha 2$ -AR) function was evaluated using the $\alpha 2$ -agonist UK 14,304 in the presence of 20 Hz stimulation. Macrophage infiltration was also assessed by confocal immunohistochemistry to further evaluate $\alpha 2$ -AR function. No indication was found to implicate $\alpha 2$ -AR impairment. NE content from MA tissue, plasma, and mesenteric perivascular adipocyte tissue (MPVAT) were measured by HPLC. NE contents in MAs were similar between males and females at 17 weeks on diet, but females had greater MPVAT and plasma levels than males at all time points. Taken together, the transient increase in neurogenic response in 17 weeks on diet females is not due to $\alpha 2$ -AR dysfunction, changes in vascular reactivity, and difference in NE content in MAs. Further inquiry is warranted in the role of E2 and MPVAT in relation to this transient shift in females.

PPAR γ : A SEX-DETERMINED MOLECULAR BRAKE FOR OSTEOCYTE ENERGY METABOLISM AND BONE MASS

Sudipta Baroi, Lance A. Stechschulte, Amit Chougule, Beata Lecka-Czernik
University of Toledo

Diabetes impairs systemic energy metabolism and affects bone which is reflected in low material quality and susceptibility to fractures. The etiology of diabetic bone disease is unclear, however it includes attenuation of bone remodeling – a process which is responsible for bone homeostasis. Plethora of evidence indicate that bone and energy metabolism share the same regulatory mechanisms including an activity of peroxisome proliferator-activated receptor gamma (PPAR γ), which simultaneously regulates cellular and systemic energy metabolism and is a key regulator of bone remodeling process. We hypothesized that, PPAR γ plays an important role in regulation of energy metabolism in osteocytes (OT), which comprise 95% of bone cells and play an essential role in regulation of bone remodeling. To test this hypothesis, we developed osteocyte specific PPAR γ knock out animals (γ OTfl/fl mice) using Cre-LoxP technology. We monitored these mice from 2 to 6 months of age for bone mineral density (BMD) using DEXA, body composition using NMR, and energy expenditure using indirect calorimetry measured in CLAMS metabolic cages. In addition, the PPAR γ transcriptome in OT was analyzed using next generation sequencing (NGS). In a separate set of experiments, PPAR γ effect on cellular energy metabolism was measured using Seahorse technology in OT-like MLO-A5 cells with PPAR γ knocked down with shRNA. As compared to WT, male γ OTfl/fl mice had higher oxygen consumption, carbon dioxide production, heat generation and increased locomotion at all analyzed ages. A similar phenotype was observed in female γ OTfl/fl mice until age of 3 months which subsequently diminished at later time points. Increased energy metabolism in γ OTfl/fl males (but not in females) correlated with higher lean mass and higher BMD, as compared to age-matched WT control. This indicates sexual divergence in OT-specific PPAR γ activity. The NGS analysis of OT transcriptome upon PPAR γ activation showed upregulation of key genes associated with lipid and carbohydrate metabolism. In contrast, an analysis of cellular respiration and ATP production in MLO-A5 cells with PPAR γ KD showed higher basal oxidative phosphorylation. We conclude that PPAR γ in OT acts as a sex-determined molecular brake that regulates the extent of energy production and utilization, and OT function. When this brake is no longer active, as in γ OTfl/fl mice, there is an increase in energy demand by OT which is reflected in elevated systemic energy metabolism. In support to this conclusion, another group has recently reported increases glucose uptake in bone in mice with PPAR γ deficiency in OT. It is unclear at this point whether increased BMD is a result of increased energy metabolism or it results from different, but PPAR γ -specific, mechanism in OT. The phenotypic differences observed between male and female mice are indicative of a possible cross-talk between PPAR γ and sex hormones in OT. These specific questions and relationships are currently under study.

MECHANISMS AND CHEMOPREVENTION OF ADIPOSITY-ASSOCIATED BREAST CANCER

V Benham, D Chakraborty, B Bullard, TS Dexheimer, JJ Bernard
Michigan State University

The extrinsic factors that contribute to breast cancer, especially pre-menopausal breast cancer, are poorly understood. A higher body mass index (BMI) increases the risk of post-menopausal breast cancer, but is negatively associated with pre-menopausal breast cancer. Epidemiological studies have demonstrated inconsistent associations between pre-menopausal breast cancer risk and body fat distribution. However, when separated by ER status, abdominal adiposity (defined by waist circumference and the waist to hip ratio) significantly increases the estrogen receptor negative (ER-), pre-menopausal breast cancer. This implicates hormone-independent mechanisms in adiposity-associated pre-menopausal breast cancer. The objective of this study was to determine how abdominal obesity increases breast cancer risk and discover a path to prevention. Our results demonstrate a hormone-independent mechanism of mammary epithelial cell transformation. We found that fibroblast growth factor-2 (FGF2) released from fat cells (adipocytes) in abdominal adipose tissue induces malignant transformation of human mammary epithelial cells (MCF-10A) by activating the proto-oncogene c-Myc. We also demonstrated that picropodophyllin (PPP) dose-dependently inhibited stimulation of MCF-10A cell transformation by FGF2 at low micromolar concentrations. PPP is a cyclolignan plant alkaloid with anti-neoplastic activity. Collectively, these data suggest a mechanism linking excess abdominal adipose tissue with malignant transformation and a way to prevent or treat adiposity-associated breast cancer.

VOLTAGE-GATED SODIUM CHANNEL (VGSC) β 1 SUBUNIT IS PROCESSED INTO A POTENTIAL TRANSCRIPTIONAL REGULATOR

Alexandra A. Bouza*, James Offord, Alexa M. Pinsky, and Lori L. Isom
University of Michigan

Variants in *SCN1B*, encoding the non-pore forming VGSC β 1 subunit, are linked to Dravet Syndrome (DS), a pediatric epileptic encephalopathy. *Scn1b* null mice model DS. β 1 is a multifunctional protein that modulates VGSC gating and kinetics and participates in cell adhesion, cell signaling, and neurite outgrowth. Previous work has shown that β 1-mediated neurite outgrowth is regulated by phosphorylation of β 1 by fyn kinase at residue Y181. In addition, β 1 is post-translationally modified by sequential cleavage by BACE1 and γ -secretase. The mechanisms regulating β 1 sequential cleavage and subsequent downstream signaling pathways are not understood. We developed an *in vitro* cell culture model to study the biochemical mechanisms regulating β 1 cleavage and subsequent downstream signaling. We examined the role of β 1Y181 phosphorylation in sequential cleavage using phosphorylation-null (β 1Y181A and β 1Y181F) and phosphomimetic (β 1Y181E) mutants. Cell surface biotinylation showed that, similar to WT- β 1, each of these mutants is trafficked to the cell surface. In addition, each of the mutants was sequentially cleaved *in vitro*, indicating that β 1Y181 phosphorylation does not regulate cleavage. Thus, the mechanism of β 1-mediated neurite outgrowth may include multiple signaling pathways. Sequential cleavage of other substrates by BACE1 and γ -secretase generates intracellular domains (ICDs) that can be translocated to the nucleus to regulate transcription. Our preliminary results suggest similar nuclear translocation of the β 1-ICD. We hypothesize that this β 1-mediated signaling mechanism may underlie differential gene expression of VGSCs in brain and contribute to neuronal development. The absence of this signaling pathway in *Scn1b* null mice may contribute to DS.

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
University of Toledo

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.

SMALL MOLECULE PROFILING UNCOVERS THE LANDSCAPE OF COMBINATORIAL PI3K INHIBITOR RESPONSES IN HNSCC

Nicole L. Michmerhuizen, Elizabeth Leonard, Chloe Matovina, Micah Harris, Gabrielle Herbst, Susan K. Foltin, Aditi Kulkarni, Thomas E. Carey, Ph.D., Carol R. Bradford, M.D., Hui Jiang, Ph.D., J. Chad Brenner, Ph.D.
University of Michigan

Recent sequencing studies of head and neck squamous cell carcinomas (HNSCCs) have identified the phosphatidylinositol 3-kinase (PI3K) pathway as the most frequently mutated, oncogenic pathway in this cancer type. Despite frequent genetic aberrations in the PI3K pathway, targeted PI3K inhibitors have shown limited clinical efficacy as monotherapies. To identify factors that might predict response to PI3K inhibitors, we tested a panel of more than 20 patient-derived HNSCC cell lines and observed varying inhibitor sensitivity. While all cell lines were much more sensitive than fibroblasts to these drugs and responses varied widely across the panel, PI3K inhibitor responses were generally characterized by resistance with micromolar IC₅₀ values. In order to characterize potential mechanisms of PI3K inhibitor resistance in HNSCC, we have developed and optimized an unbiased, small molecule profiling approach. We have used this assay to test a library of ~1400 inhibitors as monotherapies and in combination with PI3K inhibitors HS-173 and BKM120 in ten HNSCC models. Our initial screening data suggested that the combination of PI3K inhibitor and irreversible EGFR/HER2 inhibitor afatinib is synergistic in a subset of cell lines. After testing this combination in additional models, we identified several cell lines that were more responsive to this combination than to PI3K inhibitor and reversible EGFR and HER2 inhibitors. Future studies will also examine additional factors that may predict PI3K inhibitor responses through validation of other effective combinations identified in small molecule profiling studies. In all, our efforts seek to better understand promising combination treatments and advance them for effective use in HNSCC patients.

TMPRSS13 AS A MODULATOR OF COLORECTAL CANCER

Fausto A Varela¹, Thomas Hyland¹, Victoria Foust¹, & Karin List^{1,2}

¹Department of Pharmacology; ²Department of Oncology, Wayne State University

Colorectal cancer (CRC) is one of the most common forms of cancer in both men and women, with inflammatory bowel disease (IBD) patients at increased risk for developing tumors. Many cancer types present with proteolytic dysregulation, which can have pro-oncogenic consequences. TMPRSS13 is a largely uncharacterized type II transmembrane serine protease (TTSP), that is hitherto unstudied in cancer. Through *in silico* analysis and immunohistochemistry, we have observed TMPRSS13 to be upregulated in human CRC. To answer the question of whether TMPRSS13 plays a pro-oncogenic role in CRC, we utilized human CRC cell lines to identify pro-oncogenic processes affected by TMPRSS13 expression. We also incorporate a TMPRSS13-deficient mouse with the azoxymethane/dextran sulfate sodium (AOM/DSS) model of CRC to probe the role of TMPRSS13 *in vivo*. We have found that loss of TMPRSS13 expression via siRNA-mediated knockdown promotes apoptosis, decreases cellular invasion, sensitizes cells to chemotherapeutic drug treatment, and decreases protein levels of claudin-2, a tight-junction protein also upregulated in CRC. Additionally, with our mouse model we have observed increased weight loss following DSS treatment in TMPRSS13-deficient mice when compared to controls. Together, these findings suggest that TMPRSS13 promotes or modulates CRC, potentially through interaction with claudin-2, and may contribute to for proper maintenance of barrier function in the colon.

NOVEL LOOK INTO COUP-TFII ROLE IN GLUCONEOGENESIS AND B-OXIDATION IN THE DAHL SALT SENSITIVE (SS) RAT

Usman Mohammad Ashraf and Sivarajan Kumarasamy
University of Toledo

The Interaction between genetic and environmental factors plays an important role in disease progression. One of the major environmental factor includes diets, which governs significant attention based on the broad spectrum of effect in cardiovascular and associated diseases. Consumptions of diets that are rich in calories and salts can significantly increases the risk for developing life threatening cardiovascular events including hypertension and metabolic syndrome. Through GWAS studies, a locus with significant association for hypertension was found in human chromosome 15 containing the gene Chicken Ovalbumin Upstream Promotor Transcription Factor II (*COUP-TFII*). To validate this gene, a mutant rat was developed with a 15-base pair deletion in Dahl Salt Sensitive (SS) rat background. Under high salt diet regimen, the *Coup-TfII*^{mutant} rat had an improved cardiac and renal function when compared to SS rats. Being a known transcriptional regulator, *Coup-TfII* also plays a role in other physiological events including glucose homeostasis and energy metabolism. In H4IIE rat hepatoma cells, *Coup-TfII* expression modulated in response to glucose levels; under high glucose condition *Coup-TfII* expression was significantly down-regulated ($p \leq 0.05$); whereas in low glucose condition *Coup-TfII* expression was significantly up-regulated ($p \leq 0.05$). In addition, *Coup-TfII* protein expression is found to negatively correlate with insulin regardless on the amount of glucose present in H4IIE cells ($p \leq 0.05$). Similar to our in-vitro findings, *Coup-TfII* gene expression was significantly up-regulated in SS rat liver during fasting as compared to re-fed condition ($p \leq 0.05$). *Coup-TfII*^{mutant} rat have also showed a significant decrease in fasting blood glucose levels ($p \leq 0.05$) compared to SS rat along with a significant decrease in their body weight ($p \leq 0.05$). Furthermore, the *Coup-TfII*^{mutant} rat demonstrated a superior glucose response and insulin response as measured by glucose tolerance test (GTT) and Insulin tolerance test (ITT) ($p \leq 0.05$). Further, under fasting conditions the *mRNA* expression analysis in livers of SS and *CoupTfII*^{mutant} rats revealed that the key enzymes involved in both beta oxidation and gluconeogenesis was significantly down regulated in *Coup-TfII*^{mutant} rats compared to SS rats ($p \leq 0.05$). Overall, our preliminary findings suggest that *Coup-TfII* is important for glucose homeostasis in SS rats. Further understanding of *Coup-TfII* function will help to dissect the relationship between metabolic syndrome and blood pressure regulation in SS rats.

DIRECT ACTIVATION OF TISSUE FACTOR PROCOAGULANT ACTIVITY BY BILE ACIDS DRIVES INTRAHEPATIC COAGULATION IN LIVER DISEASE

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The procoagulant activity of tissue factor (TF) is controlled by several mechanisms, including post-translational regulation (i.e., encryption). This regulatory mechanism is especially critical in the liver, where plasma is in direct contact with TF-expressing hepatocytes. Previous studies have shown that hepatocyte TF drives coagulation during liver disease. Here, we sought to identify mechanisms regulating hepatocyte TF procoagulant activity using an experimental setting of cholestatic liver disease and samples from patients with cholestasis. In mice, common bile duct ligation triggered hepatocyte TF-dependent intrahepatic coagulation within 30 minutes, in the absence of liver damage. Similarly, increased coagulation was evident in cholestatic patients, even in the absence of elevated serum liver enzymes. Exposure of primary mouse hepatocytes to pathological concentrations of the bile acid glycochenodeoxycholic acid (GCDCA) increased TF-dependent procoagulant activity within 15 minutes. Bile acid-stimulated increase in TF procoagulant activity was independent of necrosis, apoptosis, and transcription. GCDCA significantly increased the procoagulant activity of TF embedded in unilamellar vesicles made using phosphatidylcholine. Moreover, GCDCA, in a concentration-dependent manner, substituted for procoagulant phospholipids (i.e., phosphatidylserine) to increase the procoagulant activity of soluble, recombinant full-length TF. The results indicate that GCDCA directly activates hepatocyte TF activity. This is the first study to demonstrate decryption of TF by a physiological mediator elevated in a disease state, and suggests a novel mechanism whereby TF-driven coagulation could be triggered by bile acids, which are elevated in patients with liver disease.

Poster Session Abstracts

Behavior Pharmacology:

- P-1 Madison O'Donnell (U-M): "The role of ankyrin G in diseases of the epithelial origin"
- P-2 Rachel Altshuler (U-M): "Protein kinase c β inhibitors attenuate amphetamine-stimulated behaviors through a direct and indirect mechanism in different brain regions"
- P-3 Ram Kandasamy (U-M): "Positive allosteric modulators of the μ -opioid receptor produce antinociception in assays of pain-evoked and pain-depressed behaviors"
- P-4 Sherrica Tai (U-M): "Effects of the CB1/2 agonist CP-55, 940 in mice that express G α O proteins that are insensitive to RGS GAP activity"
- P-5 Stephen Robertson (U-M): "The effects of Remifentanyl dose on the acquisition and persistence of responding for drug-paired cues"
- P-6 Anna Moszczynska (WSU): "The role of *PARK2* gene in vulnerability to abuse methamphetamine"

Cardiovascular:

- P-7 Craig Nash (U-M): "An internal pool of b-adrenergic receptors activates PLC-mediated PI4P hydrolysis in cardiac myocytes"
- P-8 David Ferland (MSU): "Liver acquitted, fat indicted: hepatic chemerin knockdown does not reduce blood pressure while whole-body knockdown does"
- P-9 Dawn Henderlong (MSU): "Determining the efficacy and safety of dt-678, a novel conjugate of clopidogrel, in rabbits"
- P-10 Fatimah Khalaf (U-T): "Evidence of hepatotoxicity following chronic low dose exposure to Microcystin-LR in a murine model of non-alcoholic fatty liver disease"
- P-11 Hoa Phan (MSU): "Loss-of-function mutations in human rgs2 differentially regulate vascular reactivity of resistant vasculature"
- P-12 Janice Diaz-Otero (MSU): "Mineralocorticoid receptor signaling regulates parenchymal arteriole vasodilation and cognitive function"

- P-13 Loren Brown (U-M): "Phospholipase c epsilon's role in GQ-coupled GPCR mediated cardiac hypertrophy"
- P-14 Nnamdi Edokobi (U-M): "Characterizing the role of voltage-gated sodium channel $\beta 1/ \beta 1b$ subunits in neuro-cardiac mechanisms of SUDEP"
- P-15 Ramya Kalyana Kumar (MSU): "Is PVAT-dependent fenfluramine-induced contraction 5-HT dependent?"
- P-16 Saroj Chakraborty (U-T): "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"
- P-17 Shuang Zhang (U-M): "Characterizing the effect of chronic stress on the secretory response of the adrenal medulla"
- P-18 Teri Lansdell (MSU): "Association of hdac9 expression with the estrogen receptor and the risk of intracranial aneurysm"
- P-19 Yajing Ji (MSU): "Identifying the Rho/MRTF-A/SRF signaling axis as a new therapeutic target for pulmonary arterial hypertension"

Cellular and Molecular:

- P-20 Adrian Campbell (U-M): "Biasing μ opioid receptors with g protein inhibitors to improve opioid analgesics"
- P-21 Carly Martin (WSU): "Targeting the transmembrane protease tmprss13 in breast cancer"
- P-22 Jessica Blount (WSU): "The various fates of unanchored poly-UB"
- P-23 Jiuling Yang (U-M): "Targeted mdm2 degradation as a novel and efficacious cancer therapy"
- P-24 Joel Maust (U-M): "Co-targeting MEK and CDK4/6 to treat pancreatic cancer"
- P-25 Markisha Banies (WSU): "Evaluating the invasiveness of plexiform neurofibroma cell lines using a 3D reconstituted membrane invasion assay"
- P-26 Mounir Bendahmane (U-M): "Synaptotagmin 7 is necessary for a normal secretory response to basal cholinergic stimulation in the adrenomedullary chromaffin cell"

- P-27 Oluwademilade Nuga (WSU): "Identification and characterization of novel MET fusion gene amplifications in glioblastoma"
- P-28 Sarah Dubaisi (WSU): "Expression of multiple sulfotransferase (SULT) 1C4 transcript variants in developing human liver"
- P29 Sean Misek (MSU): "Rho-mediated MRTF and YAP activation contributes to BRAF1 resistance in melanoma"
- P-30 Naincy Chandan (U-M): "Inhibiting the interaction of phospholipase-C-e with muscle specific-A kinase anchoring protein-b as a potential cardiac hypertrophy treatment"
- P-31 Julie Philippe (U-M): "The role of Ankyrin-G s-Palmitoylation in neuronal cell function"
- P-32 Alina Chapman-Morales (U-M): "A potential role for synaptotagmin-7 in plasticity of the sympatho-adrenal synapse"
- P-33 Jaquelyn Sanchez (U-M): "Novel HSP90 inhibitors to overcome drug resistant melanomas"
- P-34 Vincent Shaw (MSU): "Role of protein dynamics in selectivity of thiadiazolidinone inhibition of RGS proteins"

Endocrine and Diabetes:

- P-35 Ahmed Al-Khudhair (U-T): "Promoter region polymorphisms in PDL-1 gene associated with risk of type 1 diabetes"
- P-36 Amit Sopan Chougule (U-T): "Sclerostin, a novel target to treat low bone mass diseases is under negative control of PPAR α "
- P-37 Irina Zhang (U-M): "The effects of ER stress on beta cell function in pancreatic islets"
- P-38 Jonnelle Edwards (U-T): "Biliverdin reductase in the liver to gut axis"
- P-39 Ruta Jog (WSU): "Potential role of altered glycine metabolism in type II diabetes"
- P-40 Noah Schenk (U-M): "The cholesterol-regulatory protein, ABCG1, controls sorting of synaptotagmins to dense core granules in pancreatic beta cells"

GI, Hepatic, Pulmonary and Renal:

- P-41 Abdul-rizaq Hamoud (U-T): "Biliverdin reductase a (BVRA) attenuates the progression of alcoholic liver disease via the liver to gut axis"
- P-42 Anna Kopec (MSU): "Treatment with a direct thrombin inhibitor interrupts the progression of obesity-associated pathologies in mice fed a high fat diet"
- P-43 Asmita Pant (MSU): "Interleukin-10 inhibits liver repair in experimental acetaminophen-induced liver injury"
- P-44 Chrysan J Mohammed (U-T): "Paraoxonase regulation of cardioprotective steroids in chronic kidney disease"
- P-45 Jenna Strickland (MSU): "Upregulation of stromal-derived factor-1 by hypoxia requires hypoxia-inducible factor-1alpha and transforming growth factor-1"
- P-46 Katherine Roth (MSU): "Critical role of plasmin in macrophage activation during liver injury"
- P-47 Lauren Poole (MSU): "Thrombin-mediated fibrin polymer formation does not exacerbate experimental hepatic fibrosis"
- P-48 Shungang Zhang (U-T): "A novel CD40-target peptide inhibits pro-inflammatory signaling in renal proximal tubule epithelial cells"
- P-49 Dafna Groeneveld (MSU): "Intrahepatic fibrin(ogen) deposition drives liver regeneration after partial hepatectomy in mice and humans"

Inflammation, Immune and Asthma:

- P-50 Alexandra Turley (MSU): "Determination of the role of NRF2 and the NRF2 activator TBHQ in primary human CD4 T cell activation"
- P-51 Lyndsey Reich (MSU): "Immune-modulatory activity of a mixed lineage kinase inhibitor in a preclinical model of pancreatic cancer"
- P-52 Maja K Blake (MSU): "ERAP-1 reduction in natural killer (NK) cells induces anti-tumor activity"
- P-53 Robert Freeborn (MSU): "The NRF2-activating food additive, TBHQ, impairs both the primary and secondary immune response to influenza virus infection"

- P-54 Yining Jin (MSU): "The effect of the common food additive TBHQ in ova-elicited food allergy"
- P-55 Nupur Raychaudhuri (MSU): "The RXR agonist LG100268 alters polarization of bone marrow-derived macrophages"
- P-56 Allison Boss (MSU): "The food additive tBHQ inhibits natural killer cell activation *in vitro*"
- P-57 Jaijun Zhou (MSU): "Lymphocyte-specific protein tyrosine kinase (LCK) is involved in the aryl hydrocarbon receptor (AHR)-mediated impairment of immunoglobulin secretion in human primary B cells"
- P-58 Kyungmin Ji (WSU): "Paracrine cytokine pathways mediate metastasis of breast cancer to lymphatics"
- P-59 Joseph Heriquez (MSU): "Suppression of plasmacytoid dendritic cell activation by cannabinoid receptor 2-selective agonists"

Neuropharmacology:

- P-60 Alex Stanczyk (U-M): "The delta opioid receptor positive allosteric modulator BMS 986187 is a G protein biased allosteric agonist"
- P-61 Andrew Nelson (U-M): "A loss-of-function variant in ANK3 from a family with bipolar disorder causes altered forebrain circuitry"
- P-62 Angela Carter (U-M): "Evaluating the effects of ANK3 variants identified in an epileptic encephalopathy patient cohort"
- P-63 Casandra Larrivee (MSU): "The use of a mouse model in understanding GNAO1 associated mutations"
- P-64 Ekta J Shah (WSU): "Determining the effect of tau in response to traumatic brain injury"
- P-65 Elizabeth Williams (MSU): "Sex differences in hippocampal physiology: circuit-specific mechanisms underlying stress susceptibility"
- P-66 Huijie Feng (MSU): "A mouse model of GNAO1 associated movement disorder"
- P-67 Kaylin Marie White (U-M): "Development of a cell adhesion assay for SCN1B-

linked DRAVET syndrome variants"

- P-68 Nicholas Griggs (U-M): "Opioid analgesics with reduced side effects: merging delta-opioid receptor inhibition with mu-opioid receptor activation"
- P-69 Sean Johnson (WSU): "Importance of protein context in spinocerebellar ataxia 3"
- P-70 Heidi Matos (U-M): "Sex differences in intrinsic properties of developmentally defined medial amygdala neuronal sub-populations"
- P-71 Keith Olson (U-M): "The non-selective opioid diprenorphine produces delta-opioid receptor-mediated rapid antidepressant-like effects in mice"

Toxicology:

- P-72 Apurva Lad (U-T): " Evidence of hepatotoxicity following chronic low dose exposure to Microcystin-LR in a murine model of non-alcoholic fatty liver disease"
- P-73 Camille Akemann (WSU): "Lead-induced transgenerational neurobehavioral toxicity and transcriptomic changes in zebrafish brains"
- P-74 Danielle Meyer (WSU): " Sublethal TCDD exposure during zebrafish development produces multigenerational testicular abnormalities in histology and gene expression"
- P-75 Debrup Chakraborty (MSU): "Visceral adiposity-stimulated genotoxicity and malignant transformation of epithelial cells"
- P-76 Duanghathai Wiwatratana (MSU): "Assessment of the antioxidant compounds in protection of spinal cord astrocytes from methylmercury toxicity"
- P-77 Monica Rios-Cabanillas (MSU): "Effects of acute methylmercury-induced calcium changes in the ventral lumbar spinal cord region of the c57BL6J mouse"
- P-78 Rebekah Kennedy (MSU): "Transcriptional regulation of GATA3 is dependent on NRF2"
- P-79 Robin Su (U-T): "Assessment of Microcystin induced hepatotoxicity in the setting of preexisting liver disease"

THE ROLE OF ANKYRIN G IN DISEASES OF THE EPITHELIAL ORIGIN

Madison P. O'Donnell, Kathleen M. Ignatoski, Emily M. Jutkiewicz, Paul M. Jenkins University of Michigan

Depression is a disorder characterized by a state of low mood and a person's aversion to activities. Depression can affect a person's thoughts, feelings, and overall sense of well-being. It is observed that "leaky gut" is comorbid with depression. Ankyrin G is a cytoplasmic protein that is necessary to maintain the integrity of the lateral membrane in epithelial cells. Preliminary data from the Jenkins lab has showed that an insult to the epithelial cell layer results in de-localization of Ankyrin G and a decrease in resistance of particle flow through. Our focus is to determine if Ankyrin G de-localization is concomitant with leaky gut and depression. Naive rats were fed feces of depressed rats or non-depressed rats for five days, euthanized, and their intestines were resected. Staining for Ankyrin G indicated that Ankyrin G is de-localized in depressed rats when compared to the naive rats. Our results suggest a correlation between Ankyrin localization and leaky gut in depression. Future studies will look at the mechanism of Ankyrin localization and possible drug targets.

PROTEIN KINASE C β INHIBITORS ATTENUATE AMPHETAMINE-STIMULATED BEHAVIORS THROUGH A DIRECT AND INDIRECT MECHANISM IN DIFFERENT BRAIN REGIONS

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Amphetamine (AMPH) are a class of stimulants that elicit reinforcing behaviors through an increase in extracellular dopamine levels. Protein kinase C β (PKC β) is necessary for the increase in extracellular dopamine levels and inhibition of PKC β results in a decrease in AMPH-stimulated dopamine release. When PKC β inhibitors are administered directly into the nucleus accumbens (NAc) of rats, the drugs act immediately to reduce AMPH's behavioral effects. When PKC β inhibitors are administered into the ventricles of rats, the drug must be administered 18 hrs prior to AMPH to alter AMPH's behavioral effects. In this study, we sought to assess the actions of the PKC β inhibitors in distinct brain regions to better understand the mechanism of action of the inhibitors. Male Sprague-Dawley rats were administered 10 pmol of ruboxistaurin, a PKC β -selective inhibitor, directly into the NAc or the ventral tegmental area (VTA). Following a 30-min or 18-hr pretreatment time, rats were injected with 1 mg/kg AMPH (s.c.) and measured their locomotor activity. In a separate group of rats, ventral striatal and VTA tissue were collected following an 18-hr pretreatment of ruboxistaurin in the VTA or a 30-min pretreatment in the NAc. Levels of PKC β were evaluated by immunoblotting. An injection of ruboxistaurin into the NAc attenuated AMPH-stimulated locomotor activity following a 30-min pretreatment, but not an 18-hr pretreatment. Conversely, an 18-hr pretreatment of ruboxistaurin, but not a 30-min pretreatment, in the VTA attenuated AMPH-stimulated locomotor activity. PKC β levels were decreased in the VTA following an 18-hr pretreatment. This suggests that an 18-hr pretreatment of ruboxistaurin results in a downregulation of PKC β levels, which could contribute to the decrease in AMPH-stimulated locomotor activity at this timepoint. These studies demonstrate that PKC β inhibitors act through at least two mechanisms; a direct and immediate mechanism at the NAc and an indirect mechanism at the VTA through regulation of PKC β in the cell bodies. Future work will examine PKC β levels and activity in the NAc.

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POSITIVE ALLOSTERIC MODULATORS OF THE μ -OPIOID RECEPTOR PRODUCE ANTINOCICEPTION IN ASSAYS OF PAIN-EVOKED AND PAIN-DEPRESSED BEHAVIORS

Ram Kandasamy and John R. Traynor
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Traditional opioids (e.g., morphine) produce analgesia by acting at the orthosteric site on the μ -opioid receptor (MOR); however, G protein-coupled receptor function can also be controlled by compounds acting at a separate (allosteric) site on the receptor. We have identified two such positive allosteric modulators, BMS-986122 and BMS-986187, that bind to the allosteric site on the MOR and enhance the affinity and potency of MOR agonists *in vitro*. Thus, we hypothesized that BMS-986122 and BMS-986187 will produce antinociception by enhancing endogenous and exogenous opioid activity *in vivo*. Male and female mice were administered BMS-986122, BMS-986187, or vehicle via intraperitoneal injections (0-10 mg/kg). Antinociception against acute, inflammatory, and visceral pain was assessed using tests of pain-evoked and pain-depressed behaviors. Thirty min pretreatment of either BMS-986122 or BMS-986187 potentiated methadone (10 mg/kg) antinociception on the hot plate test. Systemic administration of low doses of either BMS-986122 or BMS-986187 (1 and 3.2 mg/kg, i.p.) produced antinociception on the tail withdrawal test which lasted 15 min. In contrast, the high dose of both BMS compounds (10 mg/kg) produced sustained antinociception lasting 120 min. Similarly, systemic administration of the high dose of either BMS compound reversed mechanical hypersensitivity produced by intraplantar injection of 2.5% carrageenan, as measured by the von Frey test. Interestingly, 20 min pretreatment of the orthosteric antagonist naloxone (10 mg/kg) blocked antinociception produced by the high dose of BMS-986122 and BMS-986187 on both the tail withdrawal and von Frey tests. Lastly, 30 min pretreatment of either BMS compound (10 mg/kg) prevented visceral pain-induced depression of nesting behavior, a translational measure of pain-induced functional impairment. Collectively, these data suggest that positive allosteric modulators of MOR produce antinociception against various types of acute and persistent pain. Further, given that the orthosteric antagonist naloxone blocks BMS-produced antinociception, BMS compounds likely produce antinociception by enhancing the activity of endogenous opioids. Thus, positive allosteric modulators of the μ -opioid receptor may provide safe and effective analgesia simply by enhancing the body's endogenous pain-relieving system.

EFFECTS OF THE CB1/2 AGONIST CP-55,940 IN MICE THAT EXPRESS G α O PROTEINS THAT ARE INSENSITIVE TO RGS GAP ACTIVITY

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The cannabinoid CB1 receptor, is a 7-transmembrane domain receptor and couples to members of the Gai/o family of heterotrimeric G proteins. As such, signaling downstream of the receptor is negatively regulated by RGS proteins, or Regulator of G protein Signaling proteins. RGS proteins act as GAPs or GTPase Accelerating Proteins and bind to activated G α -GTP subunits to enhance the hydrolysis of bound G α -GTP to G α -GDP, leading to inactivation of G α subunits and the reformation of inactive G protein heterotrimer. Therefore, we hypothesize that inhibition of RGS proteins will prolong cannabinoid CB1 receptor signaling and potency of ligands that bind to the CB1 receptor. However, there are over 20 members of the RGS protein family and there can be redundancy among members of the family. Consequently, we employed a mouse line that expresses G α o protein that is insensitive to the action of all RGS GAP activity (RGSi). Here, we examine the effects of the CB1 agonist CP-55,940 in mice that express G α o proteins that are insensitive to RGS GAP activity. This project is supported by NIH R01 DA035316.

THE EFFECTS OF REMIFENTANIL DOSE ON THE ACQUISITION AND PERSISTENCE OF RESPONDING FOR DRUGPAIRED CUES

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Drug-paired cues take on reinforcing properties that can promote drug seeking and taking. Previous research has shown that, following response-independent infusions of remifentanil paired with a cue, rats learned to make a novel response for presentations of a drug-paired cue to a greater extent than control rats. In the current experiment, we first established a cue as a conditioned reinforcer by pairing i.v. infusions of remifentanil (1.0, 3.2, or 10.0 ug/kg/infusion) with the cue. Twenty infusions of remifentanil were paired with 20 cue presentations that were delivered according to a variable time 3-min schedule each day for 5 days. The control group received the same number of drug infusions and cue presentations, but they were not explicitly paired. For the next 28 sessions, rats were allowed to freely respond on a nose poke manipulandum for presentations of the drug-paired cue, which were delivered according to a random ratio 2 schedule. We found that responding to produce drug-paired cues was elevated in the experimental group relative to the control group. The number of responses emitted was dose-dependent such that following 3.2 and 10 ug/kg/infusion, but not 1 ug/kg/infusion, animals in the experimental group responded more than controls. For animals conditioned with 3.2 ug/kg/infusion, responding persisted for 24 days; whereas responding following conditioning with 10 ug/kg/infusion persisted for 7 days. These findings demonstrate that drug-paired cues sustain drug seeking behavior, suggesting that Pavlovian conditioning processes contribute to prolonged drug use.

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THE ROLE OF *PARK2* GENE IN VULNERABILITY TO ABUSE METHAMPHETAMINE

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Risk for abuse of psychostimulant methamphetamine (METH) has a genetic component that is not fully understood. Discovery of new genes predisposing individuals to METH abuse will aid development of new pharmacotherapies against addiction to this highly reinforcing drug. Currently, there are no effective FDA-approved pharmacotherapies for METH addiction. This study investigated the consequences of *Park2* gene knockout (*Park2*^{-/-}) on rewarding and reinforcing properties of METH in rats, employing conditioned place preference (CPP) and long-access self-administration (LA SA) paradigm. *Park2* encodes parkin, a ubiquitin-protein ligase with neuroprotective properties, which plays a role in modulating dopaminergic (DAergic) neurotransmission in the nucleus accumbens (NAc) and dorsolateral striatum (DLS), key brain areas mediating the rewarding and reinforcing effects of METH, respectively. We hypothesized that *Park2* knockout would increase rewarding and reinforcing properties of METH. We further hypothesized that overexpression of parkin in the NAc or DLS would attenuate these properties.

We found a significant increase in METH intake in *Park2*^{-/-} rats as compared to WT rats during the second half of the LA METH SA ($p < 0.05$, RM ANOVA). In contrast, rats overexpressing parkin in the NAc or DLS did not differ from the WT rats in METH SA. Surprisingly, neither *Park2*^{-/-} rats nor parkin overexpressing rats developed significant preference for METH-associated context during the CPP as did the WT rats. The results suggest that the observed augmentation of LA METH SA in *Park2*^{-/-} rats as well as paradoxical decrease in METH CPP in these rats as compared to WT controls are a consequence of adaptive changes induced by brain-wide *Park2* knockout. Importantly, our results also suggest that parkin is a potential drug target in METH abuse as it can decrease rewarding properties of METH.

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**AN INTERNAL POOL OF B-ADRENERGIC RECEPTORS ACTIVATES
PLC-MEDIATED PI4P HYDROLYSIS
IN CARDIAC MYOCYTES**

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Cardiac hypertrophy and subsequent heart failure is a leading cause of morbidity and mortality in the USA. Previously, our laboratory identified a new prohypertrophic pathway where PLC ϵ , scaffolded to mAKAP β at the nuclear envelope, hydrolyzes phosphatidylinositol 4-phosphate (PI4P) at the Golgi, locally generating DAG and drive hypertrophic gene expression. This pathway is activated by multiple upstream signals including the Epac-selective cAMP analogue, cpTOME. However, stimulation of β -adrenergic receptors with Isoproterenol does not activate this pathway despite strongly raising cAMP. We were able to uncover Iso stimulated PI4P hydrolysis only in the presence of a cAMP phosphodiesterase 3 inhibitor. Irannejad et al 2 demonstrated that β 1-adrenergic receptors are present in the Golgi membrane of Hela cells and can generate cAMP there. Also, Boivin et al 3 demonstrated that β 1-adrenergic receptors are present in the perinuclear region of adult cardiomyocytes. To determine if internal β -adrenergic receptors can stimulate PI4P hydrolysis, we treated NRVMs with the membrane permeable β -adrenergic agonist, dobutamine. In contrast to the membrane impermeable Iso, dobutamine induced rapid and sustained PI4P hydrolysis that was at least partially blocked by the cell permeable β -adrenergic antagonist, metoprolol (10 μ M). The cell impermeable β AR antagonist, sotalol (5mM) could not block dobutamine effects, suggesting that a pool of internal β -adrenergic receptors can induce PI4P hydrolysis. Over expression of the RA1 domain of PLC ϵ , which competes PLC ϵ away from the mAKAP β scaffold, also inhibited dobutamine stimulation of PI4P hydrolysis. These data suggest that β -adrenergic stimulation in internal membranes, potentially at the Golgi apparatus or nuclear envelope, can stimulate PI4P hydrolysis. These observations demonstrate a novel mechanism for PLC activation through an internal GPCR which may be responsible for cardiac hypertrophy and heart failure. Targeting internal β -adrenergic receptors may allow for the development of selective therapies which can treat heart failure with little to no effect on other cardiac functions.

**LIVER ACQUITTED, FAT INDICTED: HEPATIC CHEMERIN
KNOCKDOWN DOES NOT REDUCE BLOOD PRESSURE WHILE
WHOLE-BODY KNOCKDOWN DOES.**

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Chemerin is an inflammatory adipokine positively associated with hypertension and obesity with the majority of chemerin thought to derive from the liver and adipose tissue. The contributions of liver-derived chemerin to plasma chemerin levels and blood pressure regulation are unknown. We compared whole-body vs liver chemerin inhibition using antisense oligonucleotides (ASO) with liver-restricted activity (GalNAc) or liver and fat activity (Gen 2.5). We hypothesized that in normotensive male SD rats, circulating chemerin is predominately liver-derived and regulates blood pressure. A scrambled ASO control and phosphate-buffered saline (PBS) were used as controls and radiotelemetry was used to monitor blood pressure. Baseline mean arterial blood pressure (MAP) was recorded for one week, beginning 5 days after surgery to establish a baseline. ASOs were given weekly by subcutaneous injections for four weeks. Two days after the final injection, animals were sacrificed for tissue RT-PCR and plasma chemerin measurements using Western analysis. Gen 2.5 chemerin ASO treatments (compared to PBS control) reduced chemerin mRNA in liver, retroperitoneal fat, and mesenteric perivascular adipose tissue (mPVAT) by $99.5\% \pm 0.1\%$, $95.2\% \pm 0.3\%$, and $69\% \pm 2\%$, respectively, and plasma chemerin was reduced to undetectable levels. GalNAc chemerin ASO treatments (compared to PBS control) reduced chemerin mRNA in liver by 97.9% but had no effect on chemerin expression in retroperitoneal fat and mPVAT; plasma chemerin was reduced by $90\% \pm 5\%$. Gen 2.5 chemerin ASO treatment reduced MAP, which reached a nadir of 7 ± 2.1 mmHg 48 – 72 hours after each dose compared to the scrambled ASO controls. By contrast, MAP was unchanged in animals receiving the GalNAc chemerin ASO. Thus, although most circulating chemerin is liver-derived, plasma chemerin does not play a role in blood pressure regulation. This study suggests that while chemerin is still generally associated with increased blood pressure, circulating chemerin levels cannot directly predict this effect. In addition, local effects of chemerin from fat may explain this discrepancy and support chemerin's association with both hypertension and obesity.

DETERMINING THE EFFICACY AND SAFETY OF DT-678, A NOVEL CONJUGATE OF CLOPIDOGREL, IN RABBITS

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Clopidogrel (Plavix®) is often prescribed in combination with aspirin as dual antiplatelet therapy to prevent stroke, heart attack and unstable angina. It is a prodrug that requires cytochrome P450s (CYPs) for oxidative bioactivation to form its active metabolite. The active metabolite irreversibly blocks the P2Y₁₂ receptor on platelets thereby reducing platelet aggregation in response to ADP. In many individuals, insufficient bioactivation of clopidogrel due to polymorphisms in the CYPs leads to 1/3 of patients not responding effectively. Furthermore, approximately 85% of clopidogrel is converted into inactive carboxylic acid derivatives by esterases. DT-678 is a novel conjugate of the clopidogrel active metabolite that does not require bioactivation. Instead, DT-678 releases the active metabolite via a thiol exchange reaction with glutathione. Preliminary studies have shown that the active metabolite is released ~6-fold quicker and its maximum plasma concentration is ~30-fold higher with DT-678 than with clopidogrel. It has also been shown that DT-678 is effective in preventing arterial thrombosis at doses as low as 0.1mg/kg in mice while clopidogrel is effective only at doses greater than 10mg/kg.

The purpose of the present study was to further define the efficacy and safety of DT-678 in comparison to other clinically available antiplatelet drugs, clopidogrel and ticagrelor, in New Zealand white rabbits. Ticagrelor, a reversible P2Y₁₂ inhibitor, is the newest drug in this class and is widely prescribed due to its improved efficacy and rapid onset of action in comparison to clopidogrel. However, serious bleeding effects are observed with ticagrelor. The drugs were administered intravenously to the rabbits through the jugular vein. Citrated whole blood was drawn at various time points to assess light transmission platelet aggregation and the expression of surface markers of platelet activation by flow cytometry. Tongue bleeding times were measured using a Surgicutt® device which creates a uniform 5mm long and 1mm deep incision on the top surface of the tongue. Bleeding times were assessed before and after drug treatment.

DT-678 reduced platelet aggregation in a dose dependent manner similar to clopidogrel and ticagrelor, however, clopidogrel required a higher dose. At the effective doses required for inhibition of platelet aggregation, DT-678 did not cause a significant increase in bleeding time while clopidogrel and ticagrelor induced a 1 to 2-fold increase in bleeding time. These results suggest that DT-678 may be equally effective without significant bleeding risks. Furthermore, DT-678 does not require metabolism by CYPs to produce the active metabolite and its activation would be unaffected by loss of function polymorphisms. In the future we hope to determine the mechanistic differences that might explain the observed reduction in bleeding tendency with DT-678.

CARDIOTONIC STEROID SIGNALING THROUGH NA/K-ATPASE-A-1 AND SRC KINASE ENHANCE FUNCTIONAL INTERACTIONS BETWEEN IMMUNE CELLS AND ENDO/EPITHELIAL CELLS

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Introduction: Cardiotoxic steroids (CTS) are Na/K-ATPase alpha-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 responses in both renal proximal tubular cells and macrophages upon binding and signaling through the NKA α -1. Inflammation and oxidative stress play a central role in the onset and progression of renal injury associated with CKD. 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 cell recruitment and adhesion to endo/epithelium that ultimately advance inflammation.

Methods/Results: First, we examined the effect of CTS on the expression of the biological markers that are associated with adhesion in both immune and endo/epithelial cells. 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 (10 nM, 24 hours) induced the expression of intercellular adhesion molecules I-CAM and V-CAM (both $p < 0.05$) in a human endothelial cell line. Next, we used a functional monocyte adhesion assay to investigate the effect of CTS on immune cell adhesion to endothelial and epithelial cells under physiologically relevant conditions. We found that TCB (10 nM, 24 hrs) 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 levels of NKA α -1 (wild type) or 90% NKA α -1 knock-down. In these experiments TCB induced macrophage adhesion was diminished $> 80\%$ in NKA α -1 knock-down cells ($p < 0.01$). Further, pretreatment of wild type cells with a specific peptide inhibitor of the NKA α -1-Src kinase pathway (pNaKtide, 1 μ M) yielded a 75% reduction in macrophage adhesion ($p < 0.01$). Finally, we used a series of in vivo models to study the effect of CTS on inflammatory cells. Here we found that rats injected with TCB (100 μ g/Kg/day i.p. for 4 weeks) 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 (100 μ g/Kg/day i.p.) or vehicle into mice expressing wild type NKA- α -1 (WT), as well as mice with either knock-down of NKA- α -1 (NKA-Het) or those expressing a transgenic human NKA- α -1 (NKA-Tg), which renders them more sensitive to CTS. Peritoneal macrophages collected from TCB infused mice expressed increased adhesion markers such as CD11b/CD18 compared to the control group

($p < 0.05$). We also found that these adhesion markers induced by TCB were decreased in NKA-Het mice while they were increased in NKA-Tg vs WT ($p < 0.05$).

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

LOSS-OF-FUNCTION MUTATIONS IN HUMAN RGS2 DIFFERENTIALLY REGULATE VASCULAR REACTIVITY OF RESISTANT VASCULATURE

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Background: Regulator of G protein signaling 2 (RGS2) plays a significant role in alleviating vascular contraction and promoting vascular relaxation due to its GTPase accelerating protein activity toward Gαq. Through a Ca²⁺ mobilization assay, we identified 4 loss-of-function (LOF) mutations (Q2L, D40Y, R44H and R188H) out of 16 rare, missense mutations in RGS2 selected from various human exome sequencing projects. This study is to investigate whether these LOF RGS2 mutations disrupt the protein function in regulating vascular reactivity.

Methods and results: Pulse blood pressure is elevated in RGS2^{-/-} mice by ~5mmHg (p < 0.05). Isolated mesenteric arteries from RGS2^{-/-} mice showed elevated contractile response to 5 nM angiotensin II (AngII) stimulation in pressure myograph while isolated aortic rings from those animals generated more tension upon phenylephrine (PE) stimulation compared to aortic rings from RGS2^{+/+} mice. Reintroduction of wild-type RGS2-GFP plasmids into RGS2^{-/-} mesenteric arteries by reversible permeabilization suppressed the vasoconstrictor response to 50 nM AngII (RGS2 WT plasmid -4% vs pcDNA control -35% of basal diameter, p = 0.01). Transfection with RGS2 LOF mutation plasmids failed to suppress Ang II constriction compared to those expressing WT-RGS2 (Q2L -20%, D40Y, R44H and R188H -30% of basal diameter, p < 0.05). RGS2 WT expression restored relaxation to maximal level. Q2L and R188H expressing arteries showed less relaxation compared to RGS2WT (75% and 64%, p < 0.05 and p < 0.001, respectively) expressing arteries. D40Y and R44H expressing arteries, however, showed equivalent relaxation response to RGS2WT. Phosphorylation of RGS2 at Ser46 and Ser64 rescued localization and function of D40Y and R44H mutants, which may explain the fully restored relaxation response of D40Y and R44H mutants in mesenteric arteries.

Conclusions: The results demonstrate that RGS2 attenuates vasoconstriction in the mesenteric arteries and that RGS2 mutations disrupt this effect. Among these LOF mutations, the RGS2 Q2L and R188H supported less relaxation to acetylcholine while relaxation responses of the D40Y and R44H mutant proteins were equal to WT protein. Although these mutations are rare, knowledge about their function may provide insights into pathophysiology and treatment.

MINERALOCORTICOID RECEPTOR SIGNALING REGULATES PARENCHYMAL ARTERIOLE VASODILATION AND COGNITIVE FUNCTION

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Hypertension-associated parenchymal arteriole (PA) dysfunction reduces blood flow and impairs cognitive function. We have shown that: 1.) Transient Receptor Potential Vanilloid 4 (TRPV4) channels are important regulators of PA endothelium-dependent dilation and 2.) mineralocorticoid receptor (MR) signaling contributes to the impaired PA endothelium-dependent dilation in hypertension. However, if MR signaling regulates the TRPV4-mediated dilation of PAs is less clear. We tested the hypothesis that MR activation impairs PA TRPV4-mediated dilation in stroke-prone spontaneously hypertensive rats (SHRSP). Pressure myography was used to assess PA endothelium-dependent dilation from 20-22-week-old male normotensive Sprague Dawley (SD) rats and SHRSP \pm the MR, antagonist, eplerenone (EPL; 100mg/kg/day for 4 weeks). Data are presented as mean \pm SEM; SD vs SHRSP vs SHRSP+EPL unless otherwise stated; n=4-8/group. EPL prevented the increased myogenic tone (30.1 \pm 3.5 vs 47.4 \pm 5.1 vs 37.0 \pm 3.4% tone; p=0.02) and impaired carbachol (CCh)-induced dilation in SHRSP (39.1 \pm 4.4 vs 18.8 \pm 5.1 vs 35.2 \pm 5.8% dilation; p<0.0001). The TRPV4 inhibitor, GSK2193874 (10⁻⁷M) was used to confirm the importance of TRPV4 activation in CCh-mediated dilation. TRPV4 inhibition blunted the CCh-mediated dilation in all groups (SD: 39.0 \pm 4.4 vs 5.9 \pm 4.2, SHRSP: 18.7 \pm 7 vs -12.0 \pm 18.7, SHRSP+EPL: 35.2 \pm 5.8 vs 5.9 \pm 3.6% dilation; CCh vs CCh+GSK2193874, p<0.05). Interestingly, GSK2193874 caused a loss of myogenic tone in hypertensive rats (50.8 \pm 8.3 vs 22.5 \pm 5.7% tone; Baseline tone vs +GSK2193874, p=0.0005), this effect was also observed in the EPL treated rats (36.1 \pm 4.1 vs 19.0 \pm 2.1% tone; Baseline tone vs +GSK219387, p=0.004). Removing the endothelium prevented the GSK2193874 induced loss of tone suggesting endothelial TRPV4 is important for PA myogenic tone (41.2 \pm 18.1 vs 38.2 \pm 19.1% tone; Baseline tone vs +GSK219387, p>0.05). Hypertension also reduced the TRPV4 mRNA expression and this was not prevented by EPL treatment (1.0 \pm 0 vs 0.2 \pm 0 vs 0.3 \pm 0.1; p<0.0001). We confirmed these findings in a mouse model of Angiotensin II-dependent hypertension, where we also found that EPL treatment attenuates hypertension associated cognitive dysfunction. TRPV4 $-/-$ rats were used to assess if TRPV4 channels are important regulators of cognitive function; Wistar rats were used as control. Cognitive function was assessed using the novel object recognition test. Although there was no difference in the novel exploration coefficient between groups (0.4 \pm 0.1 vs 0.3 \pm 0.2; p=0.9), the TRPV4 $-/-$ rats moved significantly less (1062 \pm 43 vs 526 \pm 18cm; p=0.004) and were slower (3.6 \pm 0.1 vs 1.8 \pm 0.3cm/s; p=0.004) than control rats (Wistar vs TRPV4 $-/-$). Preliminary data suggests TRPV4 deletion does not change systolic blood pressure (147 \pm 0 vs 146 \pm 13mmHg). PAs from TRPV4 $-/-$ had blunted CCh-mediated dilation (3.9% dilation, compared to approximately 40% in

a control rat) and did not respond to the TRPV4 agonist, GSK1016790A. Our data suggest that MR activation regulates TRPV4 signaling in PAs.

**PHOSPHOLIPASE C EPSILON'S ROLE IN GQ-COUPLED GPCR
MEDIATED CARDIAC HYPERTROPHY**

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Pathological cardiac hypertrophy is the abnormal enlargement of the heart due to an increase in the size of cardiac myocytes often resulting from heart diseases such as hypertension. Cardiac hypertrophy can lead to heart failure which is a leading cause of mortality in the USA. Activation of Gq-coupled G-protein coupled receptors (GPCRs) (i. e. endothelin receptor (ETR), angiotensin I receptor (ATR), and α -adrenergic receptors (α -AR)) results in cardiac hypertrophy *in vitro*. Previous research from our lab has indicated that phospholipase C epsilon (PLC ϵ) is an important pro-hypertrophic signaling molecule downstream of ETR in the cardiac myocyte. Activation of PLC ϵ by ET leads to PI4P hydrolysis resulting in nuclear PKD activation which leads to cardiac hypertrophy. Whether activation of other Gq-coupled receptors require PLC ϵ to drive hypertrophy is not known. Neonatal rat ventricular myocytes (NRVM) were isolated and treated with either ET, angiotensin II (AT), or phenylephrine (PE) to activate the ETR, ATR, and α -AR, respectively, to induce hypertrophy. Cell size and expression of a cardiac hypertrophy marker, atrial natriuretic factor (ANF), were assessed to determine hypertrophy. ET, AT, and PE all induced hypertrophy and this was prevented using shRNA targeting PLC ϵ . To further understand how knock-down of PLC ϵ prevents hypertrophy a nuclear-localized PKD FRET sensor (DKAR-NLS) was used to assess PKD activation in the nucleus. Knockdown of PLC ϵ decreased PKD activation in the nucleus induced by ET, AT, and PE. Furthermore, NRVM treated with the PKD inhibitor, kb nb142-70, were resistant to hypertrophy induced by ET, AT, and PE. This indicates that Gq-coupled GPCRs induce cardiac hypertrophy in part through a PLC ϵ /PKD dependent manner. Better understanding of the signaling pathways involved in cardiac hypertrophy may lead to new therapeutic approaches.

CHARACTERIZING THE ROLE OF VOLTAGE-GATED SODIUM CHANNEL $\beta 1/ \beta 1B$ SUBUNITS IN NEURO-CARDIAC MECHANISMS OF SUDEP

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Severe epilepsy syndromes are associated with mutations in Voltage Gated Sodium Channels (VGSC). Dravet Syndrome (DS), a pediatric epilepsy, is linked to mutations in *SCN1B*, the gene encoding VGSC $\beta 1/ \beta 1B$ subunits. Children with this disorder have frequent and prolonged febrile seizures, multiple comorbidities, and a high rate of mortality due to sudden unexpected death in epilepsy (SUDEP). Clinically, no diagnostic test exists for SUDEP, and autopsy have shown no pathognomonic for the condition. Interestingly, *SCN1B* mutations have also been linked to inherited cardiac arrhythmia syndromes, in which patients are also at risk for sudden death. Although the underlying mechanism(s) of SUDEP remains unknown, evidence suggests that, in addition to seizures, cardiac arrhythmias and autonomic imbalances are involved. Classically, the beta subunits were characterized as modulators of the VGSC ion conducting pore. However, the β subunits are now known to have an important role in human physiology and pathophysiology. Therefore, understanding the cellular mechanisms resulting from DS linked *SCN1B* mutations in human cardiomyocytes will provide the framework to identify novel biomarkers predictive of SUDEP risk in DS patients. I plan to investigate the role of $\beta 1/ \beta 1B$ subunits in sodium channel modulation using induced pluripotent stem cell cardiomyocytes (iPSC-CMs) derived from *SCN1B*-linked DS patients. Upon completion, these results will have a broader impact by establishing a mechanism of arrhythmias resulting from mutations in the $\beta 1/ \beta 1B$ subunits and propose novel therapeutics targets.

IS PVAT-DEPENDENT FENFLURAMINE-INDUCED CONTRACTION 5-HT DEPENDENT ?

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One of the long-standing goals of our lab is to study the vasoactive potential of 5-hydroxy tryptamine (5-HT). We discovered that the perivascular adipose tissue (PVAT) surrounding the thoracic aorta (RA) and the superior mesenteric artery (SMA) contain significant amounts of 5-HT: 177 ± 31 and 95 ± 16 ng/g tissue, respectively. Also, the RA-PVAT (30 times) and SMA-PVAT (5 times) contain a higher concentration of 5-HT than their respective vessels. We hypothesized that the 5-HT contained within the PVAT was functional and vasoactive. Isolated tissue baths were used for isometric contractility studies and high performance liquid chromatography was used to quantitatively measure amines in the PVAT and release studies. Fenfluramine was used as it is well known to induce 5-HT release. Fenfluramine (10 nM-100 μ M) was tested for its ability to contract arteries with and without PVAT. The RA with PVAT ($98\pm 10\%$) contracted to a greater magnitude than RA without PVAT ($24\pm 4\%$) while no prominent differences in contraction of SMA with ($78\pm 2\%$) and without ($75\pm 6\%$) PVAT were observed. This suggests that there is a PVAT-dependent fenfluramine-induced contraction mechanism in the rat aorta, which was next examined. This maximum contraction of RA with PVAT was diminished with alpha-1 adrenoreceptor antagonist prazosin (100 nM; by $30\pm 4\%$), the norepinephrine (NE) transporter inhibitor nisoxetine (1 μ M; by $40\pm 2\%$) but not with 5HT₂ receptor antagonist ketanserin (10 nM). To understand if 5-HT was releasable, the RA-PVAT was incubated with vehicle or fenfluramine (10 μ M-10 mM), and amines released into the incubating buffer were quantified. Semicarbazide hydrochloride (1 mM) and pargyline (10 μ M) were used to minimize amine metabolism. Surprisingly, a pronounced concentration dependent NE-release was recorded. Together, this suggests a novel role of fenfluramine to trigger NE release (and not 5-HT) and in turn reiterates PVAT as being an important reservoir of amines.

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**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**

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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.

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CHARACTERIZING THE EFFECT OF CHRONIC STRESS ON THE SECRETORY RESPONSE OF THE ADRENAL MEDULLA

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Physiological homeostasis is constantly challenged by external and internal stressors in all living organisms. One of the principle peripheral effectors to stress is catecholamines released from the adrenal medulla in the sympathoadrenal system. These secreted hormones are initially beneficial and essential in response to acute stress, whereas they may alter the normal physiological functions when stress is persistent and/or repetitive. This maladaptation of the sympathoadrenal system leads to the organisms being susceptible to a number of disorders, such as cardiovascular, metabolic, and immune disorders. To characterize the alteration in the adrenal medulla induced by chronic stress, we assessed gene expression, morphology, and stimulus-secretion coupling function changes in adrenal medullas from the chronic stressed mouse models. A variety of molecular cell biology, biochemistry, and live cell imaging approaches were employed including immunostaining, quantitative PCR, and TIRF imaging, mass spectrometry, and behavioral tests. Force swim and elevated plus maze tests were used to evaluate stress model. Live cell imaging results indicate that the adrenomedullary chromaffin cells from stressed mice are more sensitive to stress-associated presynaptic signal pituitary adenylate cyclase-activating polypeptide (PACAP) on top of physiological stimulus acetylcholine (ACh), but not in the presence of ACh alone. Consistently, the expression level of PACAP receptor (PAC1) located on chromaffin cells is found to be increased in chronic stress models. In addition, mRNA levels of calcium sensors, synaptotagmin subunits, in chromaffin cells mediating exocytosis and cargo release are increased as well. Taken together, our data indicate that gene expression of the critical components of stimulus-secretion coupling and dynamics of exocytosis were altered in the adrenal medulla to cope with long-term chronic stress.

ASSOCIATION OF HDAC9 EXPRESSION WITH THE ESTROGEN RECEPTOR AND THE RISK OF INTRACRANIAL ANEURYSM

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Introduction: An intracranial aneurysm (IA) is an outpouching of a cerebral artery. Continuing wall shear stress can cause IAs to rupture resulting in subarachnoid hemorrhage; a condition with high mortality and morbidity. Understanding risk factors is important in understanding the likelihood that an IA will rupture. An important risk factor for IA is sex. There is a disproportionate number of women with IA compared to men. Genetic factors may also present a risk for the development of IA. A risk allele (rs10230207) located nearby the HDAC9 gene has been associated with IA. HDAC9 is an epigenetic transcriptional regulator through the deacetylation of histones which prevents the transcription of genes including the estrogen receptor. We tested the hypothesis that the published GWAS association of IA with rs10230207 in the Finnish and Dutch population could be replicated in the United States population. Furthermore, we tested the hypotheses that patients harboring this risk allele will have increased HDAC9 and decreased estrogen receptor β (ER β) expression compared to those that do not.

Methods: Epstein-Barr Virus immortalized B-lymphocytes from either patients with unruptured IA (50) or population controls (50) were obtained from the NINDS repository and cultured to passage 3 in RPMI-1640 medium supplemented with 10% fetal calf serum. Subject genomic DNA was isolated and genotyped for the presence of the risk allele using Taqman SNP genotyping assays directed toward either the lead SNP rs10230207, or rs11767221, which is in high linkage disequilibrium with the lead SNP. HDAC9 and ER β protein were analyzed by Western blot. HDAC9 and ER β mRNA expression were analyzed using Taqman expression assays.

Results: While HDAC9 was increased in the IA patient population compared to population controls, HDAC9 expression was not associated with the presence of the rs10230207 risk allele. However, decreased ER β was associated with the presence of the rs10230207 risk allele (OR = 3.0; 95%CI 1.32-6.68).

Conclusions: Changes in HDAC9 expression were not associated with the presence of the rs10230207 risk allele while ER β expression was. It is possible that the presence of more than one risk allele is necessary to change HDAC9 expression. Future studies will be directed toward identifying multiple risk alleles which in combination may be affecting HDAC9 expression. We will also test to see if inhibiting HDAC9 activity rescues ER β expression. These studies may have important implications for detection and treatment of unruptured IA in patients.

IDENTIFYING THE RHO/MRTF-A/SRF SIGNALING AXIS AS A NEW THERAPEUTIC TARGET FOR PULMONARY ARTERIAL HYPERTENSION

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Background: Pulmonary arterial hypertension (PAH) is a progressive disease that can lead to right heart failure and ultimately death. PAH is one of the most common complications of scleroderma and is associated with pathological vascular remodeling. Current therapies for PAH fail to alter the progression of PAH. It is critical to further clarify the molecular mechanisms underlying the pathological process of PAH in order to develop new therapeutic strategies. TGF β 1 is one of the main drivers of PAH pathogenesis. TGF β 1 stimulates the differentiation of smooth muscle cells and several contractile genes, including α SMA, SM22 and CNN1 are upregulated by TGF β 1 in smooth muscle cells. This upregulation is dependent on both the canonical SMAD pathway and on non-canonical Rho kinase (ROCK) signaling. Increased expression of contractile genes increases smooth muscle contractility and vascular resistance. In our studies, we aim to further define mechanisms of contractile activity regulation by TGF β 1 to identify new therapeutic targets for PAH.

Methods and Results: We confirmed that TGF β 1 upregulates mRNA for genes (α SMA, SM22, CNN1) in human pulmonary arterial smooth muscle cells (HPASMC) at 6 hour. Inhibition of either SMAD3 phosphorylation or ROCK activity was sufficient to block TGF β 1-induced upregulation. Myocardin-related transcription factors (MRTF-A/B) are transcriptional coactivators for serum response factor (SRF). MRTF/SRF activity is regulated by Rho GTPases and ROCK. A role for this mechanism is evidenced by effects of the MRTF/SRF pathway inhibitor CCG-222740 to reduce expression of contractile genes activated by TGF β 1. Interestingly, siRNA studies revealed that MRTF-A, but not MRTF-B, was necessary for these genes expression induced by TGF β -1. We then wanted to identify how the canonical SMAD pathway interacted with the noncanonical Rho/MRTF-A/SRF mechanism. TGF β 1 increased the level of sphingosine kinase (SphK1) mRNA. SphK1 is the enzyme that produces sphingosine-1-phosphate, S1P, a known mediator of PASMCM contractility. TGF β 1 also increased levels of mRNA for CTGF and ET-1, which also activate the Rho/MRTF-A/SRF signaling pathway. Increased expression of ET-1 mRNA depends on phosphorylated Smad3. These data are consistent with an autocrine/paracrine mechanism whereby TGF- β 1 increases the expression of Rho pathway activators to stimulate contractile gene expression in PASMCM.

Conclusions: MRTF-A/SRF is a critical downstream signaling node for multiple activators (TGF β 1, S1P, ET-1, etc.) that regulate contractile genes in HPASMC. This mechanism may be a promising therapeutic target for PAH. The beneficial effects of MRTF/SRF inhibitors similar to CCG-222740 have already been demonstrated in skin and lung fibrosis models providing multiple benefits in scleroderma-related PAH.

BIASING μ OPIOID RECEPTORS WITH G PROTEIN INHIBITORS TO IMPROVE OPIOID ANALGESICS

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The development of “safer” opioids is difficult owing to the fact that the undesirable effects of opioid analgesics are all on-target effects at the μ opioid receptor (MOR). A new exciting approach to avoiding side effects has been development of “biased” MOR agonists that alter receptor conformation to preferentially activate analgesic pathways downstream, and away from pathways that promote undesirable side effects.¹ One such drug, Oliceridine, developed by Trevena pharmaceuticals has shown analgesic efficacy in acute pain with limited side effects in Phase IIb clinical trials. Our laboratory has pioneered an alternate approach to biasing GPCR signalling with small molecules that bind to the G $\beta\gamma$ subunits downstream of receptor activation to selectively modify signals downstream of GPCRs. Two prototype molecules, gallein and M119 potentiate the analgesic potency of morphine, without potentiating side effects typically associated with opioid use including acute tolerance, respiratory depression, constipation, hyperlocomotion, reward preference or physical dependence.²⁻⁴ To identify novel small molecule inhibitors of G $\beta\gamma$ with more drug-like potential we have screened small molecule libraries of over 140,000 compounds. We have identified 2 compounds that show promise as inhibitors of G $\beta\gamma$. The first, compound 67, shows efficacy in a competition binding Alphascreen assay and in vitro PLC activity assay, as well as direct binding to G $\beta\gamma$ by biolayer interferometry (BLI). This compound has also shown potentiation of morphine analgesia in a tail withdrawal assay using a 55°C waterbath. Another, compound 54, shows efficacy in the competition Alphascreen assay, the PLC activity assay and also shows direct binding via BLI. We have embarked on a medicinal chemistry campaign to explore structure-activity relationships of these compounds to improve the pharmacokinetics and physicochemical properties.

TARGETING THE TRANSMEMBRANE PROTEASE TMPRSS13 IN BREAST CANCER

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Despite advances in screening, diagnosis, and treatment, breast cancer remains the second leading cause of cancer death in U.S. women, with nearly 41 thousand deaths estimated in 2018 alone. The five-year survival rate of women diagnosed with breast cancer at the localized disease stage is over 90% but decreases to around 30% for women diagnosed at the metastatic stage. For this reason, it is important to understand the factors that drive breast cancer progression into invasive disease and to potentially target those factors to increase survival. TMPRSS13 is a member of the type II transmembrane serine protease (TTSP) family that has been shown to be significantly increased in patient samples of invasive ductal carcinoma (IDC). We therefore hypothesized a role for TMPRSS13 in breast cancer cell proliferation, survival and invasiveness. *In vivo* experiments show reduced mammary tumor size and lung metastases in TMPRSS13 deficient mice, indicating a direct correlation between TMPRSS13 expression and disease progression. Silencing TMPRSS13 by RNAi *in vitro* using the human breast cancer cell lines MCF7, BT20, and HCC1937, increases apoptosis and is associated with expression changes of epithelial-to-mesenchymal transition (EMT) proteins including the zinc-finger transcription factor Slug. EMT is a hallmark of breast cancer metastasis, as the cells lose their adhesive properties and gain a more invasive phenotype. Therefore, TMPRSS13 may be used as a prognostic marker of more aggressive breast cancer and is a candidate target for therapeutics.

THE VARIOUS FATES OF UNANCHORED POLY-UB

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The modifier protein, ubiquitin (Ub) regulates various cellular pathways by controlling the fate of substrates to which it is conjugated. Ub moieties are also conjugated to each other, forming chains of various topologies. In cells, poly-Ub is attached to proteins and also exists in unanchored form. Accumulation of unanchored poly-Ub is thought to be harmful and quickly disassembled by deubiquitinases (DUBs). We wondered whether disassembly by DUBs is necessary to control unanchored Ub chains *in vivo*. We generated *Drosophila melanogaster* lines that express chains non-cleavable into mono-Ub by DUBs. These chains are rapidly modified with different linkages and represent various types of unanchored species. We found that unanchored poly-Ub is not devastating in *Drosophila*, under normal conditions or during stress. The DUB-resistant, free Ub chains are degraded by the proteasome, at least in part through the assistance of VCP and its cofactor, p47. Also, unanchored poly-Ub that cannot be cleaved by DUBs can be conjugated *en bloc, in vivo*. Our results indicate that unanchored poly-Ub species need not be intrinsically toxic; they can be controlled independently of DUB-based disassembly by being degraded, or through conjugation onto other proteins.

TARGETED MDM2 DEGRADATION AS A NOVEL AND EFFICACIOUS CANCER THERAPY

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The E3 ubiquitin ligase MDM2 (Murine Double Minute 2) is the most important negative regulator of p53 tumor suppressor primarily by targeting it for ubiquitination and proteasomal degradation. Amplification or overexpression of the *MDM2* gene occurs in many human cancers contributing to tumor development, progression and metastasis. Targeting MDM2 to restore p53 function has become an attractive therapeutic strategy for cancers harboring wild-type *TP53*. Currently, numerous drugs inhibiting the MDM2-p53 interaction, such as RG7112 (Hoffmann-La Roche), MI-773 (Sanofi) and DS-3032b (Daiichi Sankyo), have entered different stages of clinical trials. However, even in p53 wild-type tumors, MDM2 inhibitors can exert limited efficacy as monotherapy in some models, probably due to inadequate p53 induction and side effects. Therefore novel and improved strategies to effectively target the MDM2-p53 pathway are needed. Recently, we have designed a series of MDM2 degraders by conjugating a small molecule MDM2 inhibitor to phthalimide. The phthalimide moiety interacts with its target protein Cereblon (CRBN) and recruits the CUL4-DDB1-CRBN (also known as CRL4CRBN) E3 ubiquitin ligase complex to promote ubiquitination and proteasome degradation of MDM2. Our initial evaluation focused on acute leukemias that are mostly p53 wild-type and express high levels of MDM2. The MDM2 degraders exert significantly improved growth inhibitory activity compared to the inhibitor in human acute leukemia cell lines. We performed Western Blots for MDM2 and p53 protein levels and qRT-PCR analysis for mRNA levels of p53 target genes to demonstrate MDM2 protein degradation and activation of p53 downstream pathways. Flow cytometry analysis confirmed the induction of apoptosis. In agreement with our design, the action of our MDM2 degraders depends on binding to Cereblon, as demonstrated by competition with free phthalimide, and on proteasome function, as demonstrated by proteasome inhibition. Our studies demonstrate that the MDM2 degraders robustly activate wild-type p53 by inducing rapid degradation of MDM2 leading to strong apoptosis in leukemia cell lines in a CRBN-binding and proteasome dependent manner. More importantly, the MDM2 degraders showed great efficacy in inducing complete tumor regression or strong tumor growth inhibition in human leukemia xenograft models and significantly improved survival in disseminated human leukemia models in mice at well-tolerated dose-schedules. Our data provide strong preclinical rationale to further develop MDM2 degraders as a new class of therapy for the treatment of human acute leukemia and potentially other types of human cancer.

CO-TARGETING MEK AND CDK4/6 TO TREAT PANCREATIC CANCER

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Hyperactivation of KRAS and inactivation of *CDKN2A* [p16] play a prominent role in tumor initiation and progression in a broad spectrum of human cancers. Based on the high incidence of these genomic events in pancreatic adenocarcinoma, the present study addresses the hypothesis that dual targeting of MEK and CDK4/6 represents a viable therapeutic strategy for the treatment of pancreatic adenocarcinoma. A panel of primary and high passage xenograft models was screened for *in vitro* synergy to the antiproliferative effects of the MEK inhibitor trametinib and the CDK4/6 inhibitor palbociclib. Two models emerged as highly sensitive to the combination treatment strategy, L3.6pl and UM59. *In vivo* studies were subsequently carried out to evaluate the efficacy of this combination in tumor-bearing mice. Mice implanted subcutaneously with L3.6pl or UM59 cells were treated daily by oral gavage for ten days with trametinib (3 mg/kg), palbociclib (100 mg/kg), or the combination of these two agents at these doses. Consistent with *in vitro* synergy observations, both models proved to be exceptional responders *in vivo* to this combination treatment strategy. Analysis of tumors taken from the L3.6pl *in vivo* study showed that animals treated with the combination exhibited a marked decrease in proliferative marker Ki67, and Reverse Phase Protein Array analysis of these tumors offered several leads, including a selective reduction of COX-2 in tumors treated with the combination. These results suggest that the combined inhibition of MEK and CDK4/6 may offer a valuable therapeutic strategy for the treatment of pancreatic adenocarcinoma.

EVALUATING THE INVASIVENESS OF PLEXIFORM NEUROFIBROMA CELL LINES USING A 3D RECONSTITUTED MEMBRANE INVASION ASSAY

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Plexiform neurofibromas (pNFs) are nerve sheath tumors that arise from Schwann cells and are one of the diagnostic criteria in people with Neurofibromatosis Type 1 (NF1). While these tumors are usually benign, they have the ability to transform into malignant tumors. The invasive nature of these cells makes complete surgical resection of these tumors difficult, resulting in tumor regrowth. Traditionally, tissue culture has been performed in a two-dimensional model but a more recent 3D culture system provides an environment that is more representative of *in vivo* conditions. Previous work in our lab has shown that the pNF cells have increased proteolytic activity in 3D culture consisting of reconstituted membrane, rBM (matrigel) supplemented with DQ collagen IV when compared to wild-type Schwann cells (SCs). These results, along with the elongated, tendril-like outgrowth morphology suggests that the pNF cells are more invasive. Our goal is to further evaluate the invasive phenotype of the PN cells using an invasion assay with a Boyden Chamber with an 8 μm pore membrane. The invasion assay utilizes a 3D culture model that the cells invade and are subsequently stained for quantitative analysis. In our experiment we used Matrigel and Collagen 1 as the matrix. Matrigel has major components of collagen IV and laminin which are also present in the microenvironment of SCs. Fibronectin plays a role in adhesion and the directed movement of tumor cells. Previous work has revealed evidence of fibronectin increasing invasive activity in cell types. We hypothesize that the PN cells will exhibit more invasion when compared to the wild-type SCs and this invasion will be further enhanced by the addition of fibronectin to the rBM. After incubation, the cells were stained and the invaded cells were manually counted and expressed as percent matrix invasion as compared to cell migration. The wild-type cells exhibited about 20 percent invasion at concentrations of 0.25 $\mu\text{g}/\text{mL}$ of matrigel and was decreased upon addition of 0.3 $\mu\text{g}/\text{mL}$ of collagen 1. The same amount of invasion was achieved in the pNF cell after less incubation time and a decrease in the amount of cells seeded. This relationship shows an increase in invasive behavior in the pNF cells as well as possible inhibitory effect of collagen 1 on cell movement. Adding fibronectin to the matrigel matrix does show either inhibitory or enhancement of invasion.

SYNAPTOTAGMIN 7 IS NECESSARY FOR A NORMAL SECRETORY RESPONSE TO BASAL CHOLINERGIC STIMULATION IN THE ADRENOMEDULLARY CHROMAFFIN CELL

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Adrenomedullary chromaffin cells respond to splanchnic nerve input by secreting a cocktail of potent hormones and peptides into the blood stream. This secretion requires the fusion of granules containing various hormones and peptides with the plasma membrane and the opening of a fusion pore. Our lab has previously shown that synaptotagmins (Syt) -1 and -7, the calcium sensors for exocytosis, play a major role in fusion pore expansion and cargo release. We showed that the two Syt isoforms are sorted to two distinct populations of granules having drastically different fusion and secretion properties. Granules harboring Syt-1 require a higher stimulus intensity to undergo fusion and exhibit a faster fusion pore expansion and cargo release compared to granules harboring Syt-7. In this study, our goal was to determine the role of each granule population in the secretion profile of chromaffin cells. Using Total Internal Reflection Fluorescence Microscopy in chromaffin cells from wildtype (WT) and Syt-7 KO mice, we measured the rate of fusion pore expansion as well as the rate of cargo release in response to KCl depolarization. We found that the absence of Syt-7 suppresses slow fusion pore expansion and slow cargo release. Moreover, the absence of Syt-7 decreases the probability of dense core granule fusion in response to KCl depolarization. We then hypothesized that the role of Syt-7 granules is to respond to basal cholinergic stimuli whereas Syt-1 granules will preferentially respond to stronger stress signaling stimuli. To explore this hypothesis, we stimulated WT and Syt-7 KO chromaffin cells with acetylcholine (ACh) and pituitary adenylate cyclase activating peptide (PACAP), a stress signal released by the splanchnic nerve terminal. Our data shows that the slow components of NPY release is suppressed in Syt-7 KO cells in response to both ACh and PACAP. Moreover, our data suggest that the Syt-7 KO cells are more likely to secrete NPY in response to ACh + PACAP than to ACh alone. In conclusion, our study shows the importance of the presence of two populations of granules for establishing a secretory profile that can produce different responses to varying strengths of stimuli.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL MET FUSION GENE AMPLIFICATIONS IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and aggressive primary CNS malignant tumor with a 15.2% 2 year survival rate for tumors diagnosed between 2008 and 2012. Amplification of MET proto-oncogene has been identified in 4% of glioblastomas, leading to high expression and ligand independent activation in some cases. Our goal was to develop MET-amplified GBM models to study signaling and investigate response to MET inhibitors as a therapy for GBMs. We identified 2 GBM patients (HF3035 and HF3077) with MET gene amplifications after low-pass whole genome DNA sequencing of 13 cases. Fluorescent in situ hybridization (FISH) analyses confirmed heterogeneous MET amplification in HF3035 and HF3077 tumors, in 63.5% and 83.0% of nuclei, respectively. In vitro neurosphere cultures derived from these tumors showed drastic depletion of MET amplicons, to 15.5% MET in HF3035 (P7) and 1.5% in HF3077 (P11). FISH in the metaphase neurosphere spreads showed that MET amplification was extrachromosomal. Interestingly, MET-amplified neurospheres were strongly selected for after intracranial (IC) implant in immunocompromised mice. HF3035 and HF3077 PDX presented MET amplified in high frequency: 79.5-86.5% for HF3035 and 47-65% for HF3077. Met expression levels by RNAseq were congruent with the oscillating gene amplification pattern. In depth RNA sequencing analysis using PRADA has revealed genomic rearrangements involving MET, yielding three novel MET-CAPZA2 fusion transcripts. For both cell lines exon 1 of CAPZA2 was fused to exon 2 of MET, resulting in full length MET coding region, with altered 5' cis-regulatory sequences. For HF3035 samples, we observed an additional in frame fusion of exon 1 of CAPZA2 to exon 6 of MET, resulting in a truncated MET transcript with 13 codons from CAPZA2. Co-expression of the wild-type and fusion MET transcripts in the tumors and PDXs were validated using PCR. MET and p-MET levels were high throughout the parental and PDX tumors. Capmatinib, which is a selective c-MET inhibitor was administered to the PDXs orally 5days/week. The treatment was effective in improving survival of HF3077 IC PDXs (p=0.028) and decreasing subcutaneous tumor size to 30% of the controls after 2 week treatment (t-test, p=0.017). However, treatment of HF3035 IC PDXs did not significantly improve survival (p=0.313). Kaplan-Meier survival curves were compared by log-rank (Mantel-Cox) test, sig. set at p<0.05. MET and p-MET detection by IHC of control and capmatinib treated xenografts show complete inhibition of p-MET but did not affect MET overexpression in HF3035 PDX.

Our results show that highly amplified regions are susceptible to genomic arrangements and the formation of fusion genes. Under investigation, is the basis for the strong selection for MET expressing cells in vivo and potential novel roles for MET in tumor progression.

EXPRESSION OF MULTIPLE SULFOTRANSFERASE (SULT) 1C4 TRANSCRIPT VARIANTS IN DEVELOPING HUMAN LIVER

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The cytosolic sulfotransferases (SULTs) are conjugating enzymes that catalyze the transfer of a sulfonate group from the cofactor 3'-phosphoadenosine-5'-phosphosulfate to a variety of endogenous and xenobiotic substrates. The SULT1C subfamily includes three human genes, SULT1C2, SULT1C3, and SULT1C4, and relatively little is known about their expression and regulation or the functions of the enzymes. Previous studies have indicated that the human SULT1Cs are preferentially expressed during early life and are capable of metabolizing endogenous molecules such as thyroid and estrogen hormones, suggesting that these enzymes could play physiological roles during development. Several transcript variants (TVs) of SULT1C4 are indexed in GenBank, including the full-length mRNA containing seven exons (TV1, NM_006588), a variant mRNA lacking exons 3 and 4 (TV2, NM_001321770), and two non-coding RNA variants. Using 5'-RACE, RT-PCR, and DNA sequencing, we detected TV1 and TV2, as well as a variant lacking only exon 3 (E3DEL) in Caco-2 colorectal adenocarcinoma cells, HepaRG hepatic cells, and human liver specimens. The aim of the current study was to thoroughly evaluate expression of the SULT1C4 by (1) characterizing expression of individual SULT1C4 TVs in human liver specimens from prenatal, infant, and adult donors, (2) determining whether the individual mRNA variants can be translated into proteins, (3) examine the developmental expression of SULT1C4 protein. Using RT-qPCR assays designed to quantify TV1, TV2, or E3DEL separately, all three variants were found to be primarily expressed in prenatal liver samples. The TV2 transcript lacking two exons was more abundant than the full-length TV1, while E3DEL levels were minimal. In agreement with the RT-qPCR data, RNA-seq analysis of prenatal and pediatric liver specimens showed that TV1 and TV2 were both more abundant during early developmental stages, and that a non-coding transcript variant (ENST00000494122.1) was also expressed in the liver specimens and was predominantly expressed in prenatal liver. TV1 and TV2, but not E3DEL, were detected by western blot when plasmids expressing DDK-tagged TV1 or TV2 were transfected into HEK-293T cells. TV1 protein, which is the only variant that would encode a functional enzyme, was more abundant than that of TV2. Using a library of human liver cytosols isolated from prenatal, infant, and 1-18 years old donors and mass spectrometry analysis, we examined the developmental expression of SULT1C4 protein. Our data indicated that SULT1C4 protein is minimally expressed in the human liver. The physiological significance of this transcript diversity and the role of this enzyme

during development is unclear and will require further investigation. Supported by grants R01 ES022606 and P30 ES020957.

RHO-MEDIATED MRTF AND YAP ACTIVATION CONTRIBUTES TO BRAFI RESISTANCE IN MELANOMA

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Much of the recent focus of melanoma targeted therapy has been on the ERK pathway, which is aberrantly activated in approximately 90% of melanoma tumors. Over half of these express BRAF^{V600E}. Current targeted therapies such as Vemurafenib (BRAF^{V600E} inhibitor), or a combination therapy of BRAF + MEK inhibitors show profound initial effects in a majority of BRAF^{V600E} expressing tumors. However, these responses are often short-lived, and resistance typically develops within months. The goal of this work is to identify pharmacologically targetable resistance mechanisms so that effective combination therapies can be developed.

Using cell line models of acquired BRAFi resistance, we demonstrate that about half of BRAFi-resistant cell lines activate the RhoA pathway. We then demonstrate that ROCK inhibition re-sensitizes drug-resistant melanoma cells to Vemurafenib. Interestingly, only cell lines which activate the RhoA pathway are re-sensitized, highlighting the specificity of this drug combination.

Since only a subset of BRAFi-resistant cell lines activate the RhoA pathway, we then wanted to identify the potential mechanisms which lead to RhoA activation. Using RNA-Seq we identified a robust switch in Sox9/Sox10 expression wherein RhoA^{High} resistant lines have > 1000-fold Sox10 downregulation and 100-1000 fold Sox9 upregulation. This correlation holds true in data from patient tumors. Human melanoma tumors with low Sox10 expression have stronger RhoA activation signatures and elevated Sox9 expression.

In addition to regulating the cytoskeleton, RhoA can also regulate gene transcription through activation of multiple transcriptional co-activators, including MRTF and YAP. MRTF- and YAP-regulated gene transcription. MRTF and YAP are both activated in RhoA^{High} BRAFi-resistant cell lines. Indirectly targeting MRTF with CCG-222740 or YAP with the YES1 inhibitor Dasatinib re-sensitizes BRAFi-resistant melanoma cells to Vemurafenib.

Taken together, these data indicate that the RhoA pathway is activated in about half of BRAFi-resistant human melanoma cells and tumors. Pharmacologically targeting Rho-mediated gene transcription, through inhibition of ROCK, MRTF, or YAP1 may be promising therapeutic strategies to re-sensitize drug-resistant tumors to BRAF inhibitors.

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INHIBITING THE INTERACTION OF PHOSPHOLIPASE-C- ϵ WITH MUSCLE SPECIFIC-A KINASE ANCHORING PROTEIN- β AS A POTENTIAL CARDIAC HYPERTROPHY TREATMENT

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Phospholipase-C- ϵ (PLC- ϵ) is involved in a plethora of signal transduction pathways. Our group has previously reported that PLC- ϵ -dependent hypertrophy in neonatal rat ventricular myocyte requires PLC- ϵ to be scaffolded to muscle specific A-kinase anchoring protein (mAKAP). This nuclear scaffolding of PLC ϵ generates local signals at the nucleus which regulate hypertrophy and heart failure. Therefore, peptidomimetic targeting of mAKAP- PLC- ϵ interaction to prevent scaffolding of PLC ϵ to the nuclear envelope may provide a promising therapeutic strategy to prevent cardiac hypertrophy. Our group has previously identified the interaction between the RA1 domain of PLC- ϵ and Spectrin Repeat homology domain-1 (SR1) of mAKAP. SR1 consists of three antiparallel α -helices. We designed three peptides corresponding to three antiparallel α -helices of SR1 domain. These peptides were used to compete against mAKAP for PLC- ϵ binding using pull-down experiments with purified proteins. The results showed that Helix-A, but not Helix-C inhibited mAKAP- PLC- ϵ binding. This suggests that Helix-A of SR-1 domain is responsible for the specific binding of mAKAP to PLC- ϵ . As the mAKAP- PLC- ϵ interaction is cardiac myocyte specific, an inhibitory peptide to prevent this interaction may represent a novel peptidomimetic drug for treatment of cardiac hypertrophy.

THE ROLE OF ANKYRIN-G S-PALMITOYLATION IN NEURONAL CELL FUNCTION

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Ankyrin-G is responsible for the formation of neuronal excitable domains. *ANK3*, which encodes the ankyrin-G protein, is a significant risk locus for bipolar disorder, highlighting the need to understand how changes in ankyrin-G localization and function contribute to psychiatric disease. Although ankyrin-G's role in controlling the polarized localization of critical proteins in neuronal microdomains is well studied, it is unknown how neuronal ankyrin-G itself localizes to its specific domains. Previous studies in epithelial cells demonstrated that the canonical 190kDa ankyrin-G isoform is S-palmitoylated by two members of the palmitoyl acyl transferase (zDHHC) family, zDHHC5 and zDHHC8. In cultured hippocampal neurons, a palmitoylation-resistant neuronal ankyrin-G loses its localization at the axon initial segment (AIS) and fails to recruit binding partners necessary for proper AIS formation. This highlights the critical role of S-palmitoylation for ankyrin-G's function. However, whether zDHHC5 and zDHHC8 mediate ankyrin-G localization to excitable domains in neurons is unknown. Interestingly, humans with mutations in chromosomal regions encoding for zDHHC5 and zDHHC8 show increased incidence of psychiatric disease. The gene that encodes zDHHC8 is located in the 22q11.2 locus of chromosome 22, a region associated with a chromosomal 40-gene microdeletion, and is currently the strongest risk loci for schizophrenia. Interestingly, cellular changes observed in the 22q11.2 microdeletion mouse are recapitulated in the *Zdhhc8*KO mouse. However, it is unknown whether these changes in the 22q11.2 mouse are driven, in part, by loss of zDHHC8-mediated ankyrin-G palmitoylation. Using coronal brain sections from a *Zdhhc8* knockout mouse, we provide evidence for the involvement of zDHHC8 in neuronal AnkG localization at the axon initial segment and the Nodes of Ranvier.

A POTENTIAL ROLE FOR SYNAPTOTAGMIN-7 IN PLASTICITY OF THE SYMPATHO-ADRENAL SYNAPSE

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During fasting, the mammalian autonomic nervous system adjusts to maintain homeostasis and prevent hypoglycemia. One effector of this counterregulatory response (CCR) is the sympatho-adrenal synapse (SAS) which releases epinephrine as well as an abundance of other peptides such as neuropeptide Y to prevent a drop in blood glucose levels. Recently, a study reported paired-pulse facilitation (PPF) at the SAS. After 24 hours of fasting, the presynaptic terminal of the splanchnic nerve is strengthened and the probability of neurotransmitter release increases. This leads to a suppression of the PPF at the SAS. The molecular mechanism of PPF and its suppression by fasting remains unknown. One potential candidate to aid in the determination of the mechanistic basis of this phenomenon that we focused on in our lab is Synaptotagmin-7 (Syt-7). Syt-7 is a calcium sensor that plays a major role in neurotransmitter release. A recent study has shown that Syt-7 is essential to PPF in the central nervous system. We hypothesize that Syt-7 may be necessary for PPF in SAS and has direct involvement in the response to fasting and hypoglycemia. Using electrophysiological recordings in the adrenal gland slices of wildtype and Syt-7 knockout animals, we will explore the role of Syt-7 in modulating the strength of SAS in response to fasting for 24 hours. We expect that in physiological conditions, PPF in Syt-7 knockout animals will be decreased or suppressed. This study once completed will showcase the role of Syt-7 in the autonomous nervous system plasticity as well as its potential role in the CCR.

NOVEL HSP90 INHIBITORS TO OVERCOME DRUG RESISTANT MELANOMAS

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Hsp90 is a molecular chaperone whose expression levels are increased 10-fold in malignancies like melanomas. Melanoma treatment is challenging because resistance to therapy, especially BRAF inhibitors like vemurafenib (Ve), occurs in most patients. Since Hsp90 facilitates the activation of kinases involved in resistance pathways, there is high rationale that chaperone inhibition will simultaneously inhibit multiple resistance pathways leading to antitumor efficacy. We hypothesize that our novel C-terminal Hsp90 inhibitors will simultaneously inhibit multiple resistance pathways to rescue drug resistant cell lines. To build a proof-of-concept, we first show in control and Ve resistant (VeR) BRAF wild-type cell lines (Mel 11 and Mel 11 VeR) that in a dose-dependent manner the Hsp90 inhibitor KU757 inhibits cell proliferation irrespective of VeR with low micromolar IC₅₀ values confirmed by MTS assay. Next, a caspase 3/7 reporter assay found that there was an increase of apoptosis in both cell lines. Further analysis of apoptosis by flow cytometry (FC) indicated cell death by necrosis. Analysis of cell cycle modulation using FC showed that the percent of cells in G₀/G₁ decreased in both cell lines with KU757 treatment dose-dependently. In conclusion, preliminary data shows that KU757 is effective in normal and resistant melanoma cell lines inhibiting cell growth and inducing apoptosis. To continue this work cell viability assays are on-going in BRAF mutant cell lines to better illustrate drug resistant melanomas in clinic. Future translational validation *in vivo* will better define the clinical applications of novel C-terminal Hsp90 inhibitors.

ROLE OF PROTEIN DYNAMICS IN SELECTIVITY OF THIADIAZOLIDINONE INHIBITION OF RGS PROTEINS

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Background: Regulator of G-protein Signaling (RGS) proteins play a critical role in GPCR (G-protein Coupled Receptor) signaling by binding to active, GTP-bound $G\alpha$ subunits and accelerating GTP hydrolysis, thus terminating signaling. There are several RGS isoforms with signature tissue distributions, so by targeting RGS proteins in an isoform-specific manner, better tissue specificity of pharmacological effects may be achieved than with global application of a GPCR agonist. Thiadiazolidinones (TDZDs) are a series of compounds that inhibit RGS proteins by covalent modification of cysteine residues. They are very potent against RGS4, but also act less potently on several other cysteine-containing RGS proteins. RGS4, RGS8, and RGS19, three proteins that are inhibited by TDZDs, share a cysteine in the $\alpha 4$ helix of the RGS homology domain. RGS19 has only this cysteine, while RGS4 and RGS8 have additional cysteines, including a shared one on the $\alpha 6$ - $\alpha 7$ interhelical loop. Interestingly, these shared cysteines are not exposed to solvent in crystal structures.

Hypothesis: Differences in potency due to covalent modification of the shared cysteine at the $\alpha 4$ helix is driven by differences in flexibility.

Approach: The effect of modification at the $\alpha 4$ cysteine on protein function can be evaluated by measuring TDZD inhibition of binding between $G\alpha$ and single-cysteine mutants of RGS proteins. In addition, flexibility may be manipulated by mutation of salt-bridge forming residues that differ between RGS isoforms, thereby inducing changes in protein thermal stability and potency of inhibition.

Results: Deuterium exchange and molecular dynamics studies have shown differential flexibility in key regions of these RGS proteins, which may be necessary for TDZD access to buried cysteines. Flexibility is more pronounced in RGS19 than in RGS4 or RGS8. RGS4 is more potently inhibited by CCG-50014 (IC₅₀: 81 nM) than RGS19 (IC₅₀: 1.1 μ M) or RGS8 (IC₅₀: 1.8 μ M). However, when RGS4 or RGS8 are mutated to contain only the $\alpha 4$ helical cysteine, the potency of TDZD inhibition is drastically reduced to below that of RGS19 (RGS4 IC₅₀: 8.5 μ M, RGS8 IC₅₀ >100 μ M). When RGS19 is mutated to introduce a salt-bridge forming residue shared by RGS4 and RGS8, it is both more thermally stable and less potently inhibited than wild-type RGS19.

Conclusion: These results indicate that while additional cysteines in RGS4 may be responsible for its high potency of inhibition by TDZDs, differences in the rate of covalent modification and subsequent potency of inhibition at the conserved $\alpha 4$ cysteine is driven by differences in RGS protein flexibility.

**PROMOTER REGION POLYMORPHISMS IN PDL-1 GENE
ASSOCIATED WITH RISK OF TYPE 1 DIABETES**

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Recently published reports pinpointed a development of insulin-dependent diabetes as a side effect after administering anti PD1 immunotherapy. These reports add more proof to the importance of the PD1-PDL1 pathway in maintaining self-tolerance particularly in the pancreatic beta cells. In this study, we aim to investigate the genetic association between single nucleotide polymorphisms (SNPs) and or haplotypes of the PDL1 gene promoter with the occurrence of insulin-dependent diabetes in T1D patients. The genotype frequencies of 4 SNPs in the promoter region of the PD-L1 gene from insulin-dependent patients were compared to the frequencies occurrence of these SNPs in the 1000 genome database. The PDL1 polymorphism of rs822337 "A/A genotype" was significantly associated with insulin dependent patients when compared to 1000 genomes individuals (OR(95% CI) = 6.56(0.87 291.43), p-value = 0.047). Yet, a haplotype consisting of rs822337, rs73641616, rs73641615 rs822336 found not to be associated with T1D.

SCLEROSTIN, A NOVEL TARGET TO TREAT LOW BONE MASS DISEASES IS UNDER NEGATIVE CONTROL OF PPAR α Amit Chougule^{1,2,3}, Lance Stechschulte^{2,3}, Beata Lecka-Czernik^{1,2,3}¹Department of Physiology and Pharmacology, ²Department of Orthopaedic Surgery, ³Center for Diabetes and Endocrine Research

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By inhibiting Wnt signaling in osteoblasts (OB), sclerostin protein acts as a potent inhibitor of new bone formation. Sclerostin is produced and secreted specifically by osteocytes (OT) which constitute 95% of bone cells and control bone formation and bone resorption. Sclerostin has become a novel target to treat osteoporosis. Different approaches are being taken to develop osteoanabolic therapies including blocking sclerostin activity by using anti-sclerostin Ab (Romosozumab) and manipulation with an expression of *Sost* gene coding sclerostin. We have showed that sclerostin protein is under negative control of PPAR α . The nuclear receptor PPAR α is known as a major regulator of energy production and a pharmacologic target to treat dyslipidemia, however, PPAR α role in maintenance of bone mass has not been studied in details. We analyzed endosteal OB and cortical OT freshly isolated from femora of WT and PPAR α global KO (α KO) mice. OT from α KO have 5-fold increased expression of *Sost* gene, while as expected OB have decreased expression of Wnt signaling markers. Increased *Rankl* expression in OT suggested increased bone resorption in α KO mice. Consistently, there is 2-fold increase in sclerostin protein levels in α KO femur. Bone phenotype and histomorphometry analysis showed that α KO mice have larger bone cavity, thinner cortex, decreased bone formation and increase in marrow fat volume (MFV) which correlates with increased sclerostin levels. α KO mice have thinner trabeculae and increased bone resorption which is consistent with increased *Rankl* expression in OT. Conditioned media (CM) collected from primary cultures of α KO OT have significantly higher sclerostin levels as compared to CM from WT OT. In co-cultures, α KO CM increased expressions of adipogenic markers in recipient WT marrow mesenchymal stem cells (MSC). Depleting sclerostin from α KO CM, decreased adipogenic markers in recipient MSCs, and increased Wnt signaling markers, in MSCs committed to OB lineage. We have identified a PPAR α -specific binding sequence (α PPRE) in the *Sost* promoter located -1.8 kb upstream from the transcription start site. ChIP assay confirmed that PPAR α binds to this α PPRE in basal conditions, and this binding is increased following activation of PPAR α with WY14643 agonist, while it is decreased in the presence of GW6471 antagonist, and is absent when α PPRE is mutated. Increased PPAR α binding correlated with a decreased *Sost* promoter activity as measured in gene reporter assay and decreased mRNA expression. In summary, PPAR α acts as a negative regulator of sclerostin expression in OT, which in α KO mice results in decreased bone formation and increased resorption, decreased bone mass and increase in MFV. These findings position PPAR α as a pharmacological target controlling sclerostin expression in OT.

THE EFFECTS OF ER STRESS ON BETA CELL FUNCTION IN PANCREATIC ISLETS

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Type 2 diabetes mellitus (T2DM) is characterized by impaired glucose-stimulated insulin secretion and insulin resistance. Insulin is secreted from pancreatic beta cells in a pulsatile manner in response to a rise in plasma glucose. It is well accepted that maintaining Endoplasmic reticulum (ER) Calcium homeostasis is critical for proper beta cell function, the specific effects of ER stress on beta cell function are incompletely understood. To determine the interrelationship between ER stress and beta cell function, we treated insulin-secreting INS-1 cells or isolated mouse/human islets with tunicamycin (TM; 10 μ g/ml) or DMSO as a control. ER Ca²⁺ was measured using a genetically-encoded FRET probe (D4ER). Treating islets for 16 hours with TM was found to reduce ER Ca²⁺ by 30%. Cytosolic calcium, measured by Fura2am, was found to oscillate in 5mM glucose that is below physiological threshold after 6h of TM treatment in mouse islets. To measure ER stress, levels of xbp1 mRNA splicing and BiP protein were measured by qPCR and immunoblotting. XBP1s levels started to increase after 6 hours of TM treatment in mouse/human islets and INS-1 cells. BiP levels started to increase after 12 hours of TM treatment in islets and in INS-1 cells. Furthermore, PARP cleavage, a marker of the initiation of apoptotic death, was measured by immunoblotting, and 40% of PARP was cleaved in INS-1 cells after 12 hours of TM treatment. One proposed mechanism of ER stress causing change in beta cell function is due to upregulation of Kir2.1 channel, as increased cytosolic calcium sensitivity in 5 mM glucose was inhibited by overnight treatment of Kir2.1 inhibitor ML133. We are continuing to determine the effects of ER stress on beta cell function and specifically insulin secretion and the role of Kir2.1 in beta cell function.

BILIVERDIN REDUCTASE IN THE LIVER TO GUT AXIS

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Biliverdin reductase A (BVRA) is an enzyme that reduces biliverdin to bilirubin, and also has other functions. BVRA has been revealed to be an important player in insulin/IGF-1, IRK/PI3K/MAPK signal transduction pathways as a kinase. Through the kinase cascade, BVRA has been shown to regulate insulin receptor signaling and may play a role in hepatic insulin resistance and whole-body glucose homeostasis. We have previously shown that mice with a liver-specific BVRA knockout (KO) have hepatic insulin resistance and fatty liver, which the latter was most likely due to low levels of the nuclear receptor PPAR α . Recently, fatty liver disease has been directly related to changes in intestinal signaling and the gut microbiome. The function of BVRA in the intestine or how it relates to the gut microbiome is unknown. Through immunostaining, we have identified that BVRA is highly expressed in enterocytes of the small intestine. Interestingly, there was no BVRA expression in goblet cells, which secrete mucous for the intestinal lining. Enterocytes are involved in nutrient absorption. We hypothesize that BVRA in the small intestine may have a signaling function or be involved in the absorption of nutrients. Our studies are to investigate the role of BVRA in the gut for lipid metabolism or if it is essential for nutrient uptake.

POTENTIAL ROLE OF ALTERED GLYCINE METABOLISM IN TYPE II DIABETES

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Type II diabetes (T2D) is characterized by insulin resistance, β -cell dysfunction and impaired glucose tolerance. Multiple large-scale metabolomics studies have demonstrated that diabetes is accompanied by alterations in amino acid metabolism. Interestingly, in both humans and rodents, circulating glycine levels are significantly reduced in obesity, glucose intolerance and T2D. These observations identify glycine as an important biomarker of T2D and raise the possibility that altered glycine metabolism plays an active role in the etiology of the disease. The goals of our study are to determine if altered glycine metabolism plays a functional role in the development of diabetes, and to characterize the regulatory pathways that control plasma glycine levels. Glycine cleavage system (GCS) is the only biochemical route that degrades glycine in human body and loss-of-function mutations of GCS cause hyperglycinemia. Here, we show that GLDC expression is upregulated in livers of insulin resistant mouse models of diabetes. In hepatoma cell lines insulin strongly stimulates transcription of the glycine decarboxylase (GLDC) gene that encodes the rate-limiting component of GCS. We identified the insulin responsive transcription factor SREBP-1c as the primary mediator of this insulin stimulatory effect. Interestingly, we found that altering GLDC expression levels strongly affected intracellular glutathione levels. These observations suggest a working model in which Srebp1c-mediated regulation of GLDC is responsible for the reduction of glycine levels seen in insulin resistant states and may contribute to a compensatory increase in glutathione production as a defense against diabetes-induced oxidative stress.

THE CHOLESTEROL-REGULATORY PROTEIN, ABCG1, CONTROLS SORTING OF SYNAPTOTAGMINS TO DENSE CORE GRANULES IN PANCREATIC BETA CELLS

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Pancreatic beta cells release insulin in response to elevated extracellular glucose. This process is critically dependent on maintaining appropriate levels of cholesterol on intracellular membranes. Indeed, when expression of the cholesterol-regulatory ATP binding cassette protein (ABCG1) is genetically suppressed by siRNA, granule biogenesis and maturation is disrupted. Interestingly, manipulations that suppress expression of ABCG1 also reduce the release competence of insulin granules. In this study, we show that knockdown of ABCG1 is likely to disrupt exocytosis by altering the sorting of a high-affinity calcium sensor for exocytosis, synaptotagmin-7 (Syt-7). Using a beta cell line that stably expresses insulin-GFP and Total Internal Reflection Fluorescence Microscopy (TIRFM) to measure its release, we show insulin release is impaired when cells are mildly depolarized – a condition which requires a higher sensitivity calcium sensor for granule fusion. Insulin secretion after strong membrane depolarization is not different between wild-type cells and cells in which ABCG1 has been knocked-down. Membrane fractionation and immunocytochemistry experiments also demonstrate that amounts of Syt-7 on mature insulin granules is reduced in ABCG1 knockdown cells. This leads us to conclude expression of ABCG1 is important for maintaining cholesterol availability during granule biogenesis in addition to proper sorting of synaptotagmin isoforms to mature granules.

BILIVERDIN REDUCTASE A (BVRA) ATTENUATES THE PROGRESSION OF ALCOHOLIC LIVER DISEASE VIA THE LIVER TO GUT AXIS

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Alcohol abuse and chronic consumption are a significant medical burden in industrialized countries. Long-term alcohol intake is associated with changes in the intestinal microbiota that increase intestinal permeability and the release of deleterious metabolites from the gut. Alcoholic liver disease (ALD) starts with the accumulation of fats in hepatocytes that may progress to alcoholic steatohepatitis (ASH), which can further advance to fibrosis, eventually leading to cirrhosis and hepatocellular carcinoma. Plasma bilirubin levels are increased in alcoholic cirrhotic patients, which is reflective of an injurious effect to hepatocytes as a result of liver damage. In the intestine, microflora convert bilirubin to urobilinoids, which are the predominant bile pigments. We have demonstrated that a knockout of biliverdin reductase A (BVRA) in hepatocytes (hsBVRA KO) of mice, which is the enzyme that converts biliverdin to bilirubin, causes steatosis on a high-fat diet. Recent evidence has shown that bilirubin itself is protective against hepatic lipid accumulation and obesity. We show that BVRA is suppressed in livers of mice fed alcohol. We hypothesized that BVRA is preventive of high alcohol consumption induced steatosis, which protects the liver from damage. Indeed, the hsBVRA KO mice that consume alcohol had exacerbated hepatic steatosis and changes in the gut microbiome. Together, these observations point to an early mechanism whereby high consumption of alcohol may reduce hepatic BVRA and bilirubin excretion to the gut, leading to triglyceride synthesis causing a progression toward ASH. BVRA may be a significant regulator of the gut microbiome. Future studies will investigate BVRA and bilirubin in the regulation of ALD and how they relate to the liver-gut microbiota signaling axis.

TREATMENT WITH A DIRECT THROMBIN INHIBITOR INTERRUPTS THE PROGRESSION OF OBESITY-ASSOCIATED PATHOLOGIES IN MICE FED A HIGH FAT DIET

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Obesity is accompanied by activation of the coagulation cascade in humans and in experimental settings of diet-induced obesity. Genetic and pharmacologic interference with thrombin proteolytic activity has previously been shown to attenuate obesity-associated pathologies, including non-alcoholic fatty liver disease (NAFLD). In this study, we sought to determine if intervention with the thrombin inhibitor, dabigatran etexilate (DE), can slow the progression of NAFLD and obesity when administered after onset of disease. Wild-type C57BL/6J mice were fed a control diet (AIN-93M, 10% calories from soybean oil) or a high fat diet (HFD, 60% calories from lard and soybean oil) for 3 months and then switched to identical diets containing 7.5 mg/g DE (AIN-93M+DE and HFD+DE) for an additional 2 months (intervention groups). Additional groups of mice were fed DE-containing diets for all 5 months (prophylactic groups). Terminal plasma DE levels were comparable (~330 ng/ml) in mice fed AIN-93M+DE and HFD+DE. Therapeutic intervention with DE prevented excess body weight gain, marked by no further increase in body fat mass, in HFD-fed mice. DE treatment elicited a selective reduction in micro-, but not macro-vesicular, hepatocellular steatosis in mice with established HFD-induced obesity. In addition, DE treatment prevented progression of liver injury in HFD-fed mice. HFD-induced hyperglycemia was significantly attenuated in DE-treated mice, suggesting improved insulin sensitivity. DE treatment did not affect these parameters in AIN-93M-fed mice. Inflammatory cytokine analysis revealed a robust increase in plasma KC/Gro, a neutrophil chemokine related to adiposity and insulin resistance, in HFD-fed mice. Notably, both prophylaxis and intervention with DE dramatically reduced plasma KC/Gro levels. Overall, the data suggest that DE can halt the progression of diet-induced obesity and NAFLD in HFD-fed mice. Moreover, the results suggest that thrombin-mediated chemokine induction contributes to HFD induction of obesity and liver disease in mice.

INTERLEUKIN-10 INHIBITS LIVER REPAIR IN EXPERIMENTAL ACETAMINOPHEN-INDUCED LIVER INJURY.

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Overdose of the analgesic drug acetaminophen (APAP) is the leading cause of acute liver failure (ALF) in the United States. APAP-induced liver injury is associated with significant increases in several cytokines, including the anti-inflammatory cytokine interleukin-10 (IL-10). A prior study found that IL-10 knockout mice had increased sensitivity to APAP hepatotoxicity, suggesting that IL-10 reduces liver damage after APAP challenge. However, clinical studies do not imply a protective effect of IL-10. In fact, high IL-10 levels are associated with a poor outcome, indicated by reduced transplant-free survival in patients with ALF. We hypothesized that high IL-10 levels could inhibit liver repair. To determine the role of IL-10 during the repair phase of the APAP-injured liver, wild-type C57Bl/6J mice were given APAP (300 mg/kg) to induce liver injury. Twenty-four hours after APAP challenge, a time point near peak hepatotoxicity, the mice were given recombinant mouse IL-10 (5 µg), to experimentally increase plasma IL-10 levels, or vehicle (saline). Interestingly, IL-10 administration significantly increased hepatocellular necrosis 48 hours after APAP administration, a time typically associated with liver repair. IL-10 administration also reduced leukocyte infiltration within areas of necrosis; however, hepatocyte proliferation, denoted by immunolabeling of PCNA-positive hepatocytes, was unaffected by IL-10 administration. The results indicate that after APAP hepatotoxicity manifests, IL-10 anti-inflammatory activity inhibits repair of the APAP-injured liver. The results suggest an important dichotomy in the role of IL-10 in early APAP hepatotoxicity and late during liver repair.

PARAOXONASE REGULATION OF CARDIOTONIC STEROIDS IN CHRONIC KIDNEY DISEASE

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Introduction: Cardiotonic steroids (CTS) are steroid hormones which are elevated in volume expanded states such as chronic kidney disease (CKD). The 2-pyrone ring structure of CTS is critical for their binding to the Na⁺/K⁺-ATPase and subsequent initiation of pro-inflammatory and pro-fibrotic signaling which can promote cardiac and renal disease. Paraoxonases (PONs) are a family of hydrolytic enzymes which are capable of hydrolyzing chemical structures similar to the 2-pyrone rings found in CTS, however the native physiologic substrate(s) of PON's are unknown.

Hypothesis: We hypothesized that 2-pyrone containing CTS are substrates for PON hydrolytic activity (2-pyronease-like activity) and that this specific activity is decreased in the setting of CKD.

Methods/ Results: We first examined the ability of the CTS to compete with a chemically similar specific fluorogenic substrate of PON's (7-hydroxycoumarin). PON-1 purified from human plasma (both RR and QQ genotype of the PON-1 Q₁₉₂R polymorphism) was reacted with 7-hydroxycoumarin in the presence and absence of equimolar amounts of the CTS telocinobufagin (TCB). PON-1 hydrolytic activity toward 7-hydroxycoumarin was reduced 90% in the presence of TCB (p<0.01 for both PON-1 QQ and RR variants). In order to confirm that this reduction was related to hydrolysis of the TCB, we developed a specific LC-MS assay to measure the 2-pyrone active form of TCB. Incubation of TCB with PON-1 overexpressing HEPG2 cells led to a >65% decrease in the 2-pyrone form of TCB at 24 hours (p=0.0054). Next, we measured circulating PON-1 protein (ELISA) and 2-pyronease-like activity in diabetic nephropathy CKD cohort (n= 249; Stage 2 n=9, Stage 3 n = 103, Stage 4 n= 85 Stage 5 n = 52) vs non-CKD controls (n=15). Interestingly, we found that while circulating PON-1 protein levels were increased significantly ((P≤0.0001) across CKD stages, circulating PON-1 2-pyronease-like activity was decreased significantly (P≤0.0001) across CKD stages (except for stage 2) vs non-CKD controls.

Conclusion: These findings suggest that 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-pyronease-like activity in the setting of CKD.

UPREGULATION OF STROMAL-DERIVED FACTOR-1 BY HYPOXIA REQUIRES HYPOXIA-INDUCIBLE FACTOR-1ALPHA AND TRANSFORMING GROWTH FACTOR-1

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Following both acute and chronic liver injury, expression of the chemokine stromal-derived factor-1 (SDF-1), or CxCl12, is upregulated and plays a vital role in the activation of downstream signaling pathways implicated in either driving hepatic injury progression or leading to its repair. For example, while SDF-1 has been implicated in promoting tumor growth and metastasis in patients with hepatocellular carcinoma, in acute injuries it may play an important role in liver regeneration. This suggests SDF-1 may represent a novel target for therapeutic intervention in both acute and chronic liver diseases. However, the mechanism by which SDF-1 is upregulated following hepatic injury is not fully known. The present study determined whether the transcription factor, hypoxia-inducible factor-1alpha (HIF-1alpha), regulates SDF-1 in primary mouse hepatocytes. To activate HIF-1alpha *in vitro*, primary hepatocytes were incubated for 72 hrs in either room air or hypoxic (1% O₂) conditions. Under hypoxic conditions, SDF-1 mRNA levels were upregulated after 72 hr in WT mouse hepatocytes, an effect that was prevented in hepatocytes isolated from either HIF-1alpha or HIF-1beta knockout mice. We demonstrated previously that upregulation of several genes in hepatocytes during hypoxia requires autocrine release and activation of transforming growth factor-1 (TGF-beta1). To determine if TGF-beta1 is required for the upregulation of SDF-1 during hypoxia, primary mouse hepatocytes were pretreated with a TGF-beta1 receptor antagonist (SB-431542) and placed in hypoxic conditions for 72 hrs. Pretreatment with SB-431542 completely inhibited upregulation of SDF-1 by hypoxia, suggesting TGF-beta is required for upregulation of SDF-1 in hypoxic hepatocytes. Additionally, while treatment of hepatocytes with TGF-beta1 upregulated SDF-1, this effect did not require HIF-1alpha, suggesting that TGF-beta1 is downstream of HIF-1alpha activation. We previously demonstrated that hypoxia upregulates thrombospondin-1 in hepatocytes, which could be responsible for activation of latent TGF-beta1. Upregulation of SDF-1 by hypoxia, however was not different between hepatocytes isolated from wild-type or thrombospondin-1 knockout mice. The results indicate that hypoxia activates HIF-1 α in hepatocytes, which leads to activation of latent-TGF-beta1. TGF-beta1 then acts in an autocrine fashion to upregulate SDF-1. However, the mediator responsible for converting latent TGF-beta to its active form remains unknown.

**CRITICAL ROLE OF PLASMIN IN MACROPHAGE ACTIVATION
DURING LIVER INJURY**

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Activation of hepatic macrophages is critical for liver repair after injury. The mechanism by which liver injury stimulates macrophage activation is not fully understood. We tested the hypothesis that the fibrinolytic enzyme, plasmin, is critical for macrophage activation after liver injury. To test this hypothesis, mice were exposed to a hepatotoxic dose of acetaminophen followed by treatment with tranexamic acid (1200 mg/kg i.p., administered twice daily), a drug that inhibits the conversion of plasminogen to plasmin. Exposure of mice to acetaminophen stimulated rapid macrophage activation, increasing cytokine production and macrophage-mediated phagocytosis of necrotic cells. This activation was reduced by plasmin inhibition, leading to impaired liver repair. Next, we determined whether plasmin directly activates macrophages. Treatment of either bone marrow-derived macrophages or Kupffer cells with plasmin increased expression of the proinflammatory cytokines Cxcl1, Cxcl2, and tumor necrosis factor- α in a JNK1/2 and NF κ B-dependent manner. Studies have indicated a role for high-mobility group B1 protein (HMGB1), a damage-associated molecular pattern molecule, in the activation of macrophages. Therefore, we determined whether HMGB1 affects plasmin-mediated activation of macrophages. While HMGB1 alone at concentrations that are detected in the serum of acetaminophen-treated mice did not increase expression of proinflammatory cytokines in macrophages, it synergistically enhanced plasmin-mediated upregulation of cytokines. Furthermore, necrotic hepatocytes from wild-type mice enhanced plasmin-mediated activation of macrophages, whereas necrotic hepatocytes from hepatocyte-specific HMGB1 knockout mice did not. Collectively, these studies demonstrate that plasmin is an important activator of macrophages after liver injury. Further characterization of this pathway could lead to the development of novel therapies aimed at enhancing macrophage-mediated liver repair in patients suffering from acute liver injury.

THROMBIN-MEDIATED FIBRIN POLYMER FORMATION DOES NOT EXACERBATE EXPERIMENTAL HEPATIC FIBROSIS

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Background. Activation of the blood coagulation cascade is routinely observed in chronic liver diseases that produce hepatic fibrosis. Intravascular fibrin clots and extravascular fibrin deposits that form in the liver as a consequence of coagulation activity are often implicated in the progression of fibrosis. However, evidence supporting a pathological role of fibrin in hepatic fibrosis is indirect and based largely on studies using anticoagulant drugs that inhibit activation of the coagulation protease thrombin, which has many downstream targets including fibrinogen. To date, there is no concrete experimental evidence indicating that fibrin clots promote liver fibrosis. Therefore, the goal of this study was to determine the specific role of fibrin deposits in experimental hepatic fibrosis. **Methods.** To accomplish this, we used mice expressing normal plasma levels of mutant fibrinogen insensitive to thrombin-mediated clot formation (i.e., locked in the monomeric form), termed Fib^{AEK} mice. Female wild-type mice and homozygous Fib^{AEK} mice were challenged with carbon tetrachloride (CCl₄) twice weekly for 4 weeks (1 ml/kg, ip). **Results.** CCl₄ administration caused significant liver injury as indicated by elevated plasma alanine aminotransferase (ALT) activity. Hepatic fibrosis significantly increased in CCl₄-challenged wild-type mice compared to unexposed controls, indicated by increased profibrogenic gene induction and Sirius red staining. Remarkably, hepatic injury and fibrosis were similar in CCl₄-challenged wild-type mice and CCl₄-challenged Fib^{AEK} mice. Similarly, hepatic expression of mRNAs encoding multiple proinflammatory cytokines after CCl₄ administration was unaffected by genotype. Surprisingly, hepatic deposition of fibrin(ogen) was found to occur independently of thrombin-catalyzed polymer formation. **Conclusions.** In conclusion, the results indicate that thrombin-catalyzed fibrin polymer formation does not contribute to the development of CCl₄-induced experimental liver fibrosis. These results support the hypothesis that thrombin contributes to the pathology of liver fibrosis via alternative mechanisms.

A NOVEL CD40-TARGET PEPTIDE INHIBITS PRO-INFLAMMATORY SIGNALING IN RENAL PROXIMAL TUBULE EPITHELIAL CELLS

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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 chemoattractant 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.

INTRAHEPATIC FIBRIN(OGEN) DEPOSITION DRIVES LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN MICE AND HUMANS.

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Platelets rapidly accumulate in the liver remnant following partial hepatectomy (PHx) in rodents and humans, and liver regeneration in rodents is significantly delayed when platelets are depleted or inhibited. However, the exact mechanisms whereby platelets accumulate within the liver and promote regeneration are not completely understood. Recently, fibrin(ogen) deposits were observed in the liver remnant following PHx in mice, but whether fibrinogen plays a direct role in liver regeneration is not known. We tested the hypothesis that platelet-driven liver regeneration after PHx is functionally connected to intrahepatic activation of coagulation and fibrin(ogen) deposition. Two-thirds partial PHx in wild-type mice triggered rapid hepatic accumulation of platelets, which was paralleled by intrahepatic coagulation, evidenced by fibrin(ogen) deposition. PHx-induced platelet accumulation and fibrin(ogen) deposition were reduced in mice with liver-specific tissue factor deficiency, and these changes were coupled to a reduction in liver regeneration assessed by hepatocyte proliferation 3 days after PHx. Thrombin activation of platelets did not contribute to liver regeneration after PHx, as regeneration was unaffected in protease-activated-receptor 4 deficient mice. In contrast, fibrinogen depletion with anicrod significantly reduced hepatic platelet accumulation and hepatocyte proliferation after PHx, indicating that fibrin(ogen) is central to liver regeneration after PHx. Consistent with the regenerative function of fibrin(ogen) documented in this experimental setting, fibrin(ogen) deposition was observed in intraoperative liver biopsies from patients undergoing PHx, with decreased fibrin(ogen) deposition in patients with post-PHx liver dysfunction. Moreover, low postoperative plasma fibrinogen levels were associated with liver dysfunction and mortality in patients undergoing PHx. The results suggest that a tightly interconnected mechanism involving interactions between platelets and hepatic fibrin(ogen) deposition drives liver regeneration after PHx in both mice and humans.

**DETERMINATION OF THE ROLE OF NRF2 AND THE NRF2
ACTIVATOR TBHQ IN PRIMARY HUMAN CD4 T CELL ACTIVATION**Alexandra E. Turley^{1,2}, Cheryl E. Rockwell^{1,2,3}¹Department of Pharmacology and Toxicology²Institute for Integrative Toxicology³Cell and Molecular Biology Program

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Nuclear factor erythroid-derived 2 like-2 (Nrf2) is a transcription factor responsive to cell stressors including reactive oxygen species and electrophilic xenobiotics. Upon activation, Nrf2 up-regulates a battery of detoxification, antioxidant, and metabolizing genes, yielding a cytoprotective response. Nrf2 has been implicated in regulation of the immune system: Nrf2-null mice are more sensitive to inflammatory stimuli, and older female Nrf2-null mice develop a disease similar to lupus. A central component of these responses is the adaptive immune response, which is coordinated and directed by helper (CD4) T cells. In mice, Nrf2 activation skews CD4 T cell differentiation towards a Th2 phenotype, but the role of Nrf2 in T cell activation in primary human CD4 T cells remains undetermined. We have previously shown that the Nrf2 activator tBHQ, a commonly used food additive, inhibits events of T cell activation in primary human CD4 T cells; however, the role of Nrf2 in these events was not determined. To address this gap, we developed a Nrf2 knockdown model in primary human CD4 T cells using siRNA, and tested the effects of tBHQ on T cell activation in this model. Nucleofection with Nrf2 siRNA knocked down Nrf2 protein levels by 85%-90% 12h post transfection, and inhibited induction of Nrf2 target genes in the Nrf2 siRNA transfected cells. tBHQ inhibited T cell activation-induced production of the cytokines IL-2, IFN γ , TNF α , and GM-CSF in a Nrf2-independent manner. Likewise, tBHQ also inhibited induction of the cell surface markers CD25 and CD69 independent of Nrf2. These studies detail the development of a Nrf2 knockdown model in primary human T CD4 cells which can be used to determine the role of Nrf2 in these cells, and demonstrate that the effects of tBHQ on T cell activation are independent of Nrf2 in this model. (Supported by NIH grants ES024966 and ES007255).

IMMUNE-MODULATORY ACTIVITY OF A MIXED LINEAGE KINASE INHIBITOR IN A PRECLINICAL MODEL OF PANCREATIC CANCER

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Pancreatic Ductal Adenocarcinoma (PDAC) is an almost universally fatal form of cancer, with a five year survival rate of less than ten percent. PDAC originates from initiating mutations in the *Kras* oncogene, leading to hyperactivation of the mitogen-activated protein kinase (MAPK) signaling pathway. Inflammation of the pancreas, or pancreatitis, can increase disease risk and accelerate tumor progression. The mixed lineage kinase (MLK) inhibitor CEP-1347, originally intended as a treatment for neurodegenerative disease, could potentially be repurposed to treat PDAC. The therapeutic effects of CEP-1347 were evaluated *in vitro* by assessing its ability to inhibit Nitric Oxide (NO) production in a macrophage-like cell line, and its ability to inhibit both proliferation and the activation of downstream targets of MAPK signaling in a pancreatic cancer cell line. *In vivo*, inflammation was induced in a genetically engineered mouse model with a *Kras* mutation targeted to the pancreas, and these mice were treated with CEP-1347. The development of pancreatitis and PDAC were assessed via flow cytometry, immunohistochemistry, and western blotting. CEP-1347 treatment decreased immune cell infiltration into the pancreas, particularly CD8+ T cells. This decreased the incidence and severity of PDAC, as evidenced by histopathology. Further studies will provide insight into optimal dosing and scheduling, as well as the mechanisms of immune modulation by CEP-1347 which may lead to reduced pancreatic oncogenesis.

ERAP-1 REDUCTION IN NATURAL KILLER (NK) CELLS INDUCES ANTI-TUMOR ACTIVITY

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The immune system has proven to be a vital target for cancer as immune modulatory drugs continue to show promising results. Checkpoint inhibitors such as PD-1 targeted Nivolumab and others have proven effective in some cancers but ineffective in others. Further investigation into new immune targets and development of cancer immunotherapies is necessary for patient populations unaffected by or resistant to currently available immunotherapies. One potential immune target is Endoplasmic Reticulum Aminopeptidase-1 (ERAP-1), which is critical for antigen presentation to immune cells by MHC-1. Our lab has previously shown that ERAP1 plays an important role in natural killer (NK) cell activity. Mice lacking the ERAP1 gene display greater NK cell activity in comparison to wild-type mice. We investigated the role of ERAP1 in NK cell activity further, showing that *in-vivo* ERAP1 regulates the inflammasome responses of NK cells. Recently, a collaborating lab published that thimerosal, an organomercury compound used as a preservative in vaccines, is a potent and selective ERAP1 inhibitor. We investigated thimerosal's potency for ERAP1 inhibition and its ability to induce NK activation *in-vitro*. We determined that thimerosal was able to induce intracellular IFN γ , a marker for NK cell activation, in both mouse and human NK cells. To investigate the role of hyper-activated NK cells as a potential cancer immunotherapy, we induced NK cell activation using thimerosal and measured tumor cell death in presence of the activated NK cells. In comparison to untreated NK cells, thimerosal-treated cells induced a significant increase of cell death in various tumor cell types, both in mouse and human NK cells. This data suggests that ERAP1 targeting within NK cells might represent a novel cancer immunotherapy approach to induce anti-tumor activity of NK cells. Further studies are warranted to elucidate the mechanism of thimerosal-induced NK cell activation and develop non-toxic methods for ERAP1 targeting within NK cells.

THE NRF2-ACTIVATING FOOD ADDITIVE, TBHQ, IMPAIRS BOTH THE PRIMARY AND SECONDARY IMMUNE RESPONSE TO INFLUENZA VIRUS INFECTION

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The influenza virus has been a persistent threat to society on both a global and national level. Within the United States, influenza infections cause tens of millions of medical visits, hundreds of thousands of hospitalizations, and tens of thousands of deaths, annually. In fact, the current death toll is estimated to be upwards of 40,000 deaths per year. Despite increased vaccination efforts, the number and severity of influenza cases have not improved, suggesting a potential disconnect between vaccination efforts and influenza burden. The Nrf2-activating food preservative, tert-butylhydroquinone (tBHQ), might contribute to this discrepancy. tBHQ is widely used to prevent rancidification of fats and oils, and it's estimated that consumers could eat more than 7 mg/kg/day, far above the established ADI of 0.7 mg/kg/day. Notably, our group has shown that activation of Nrf2 by tBHQ impairs CD4 T cell polarization. Specifically, we found that tBHQ inhibits Th1 polarization in favor of Th2 polarization and this occurs in a Nrf2-dependent manner. Importantly, Th1 cells have a prominent role in the generation of a functional memory immune response to influenza. In the context of influenza infection, Th1 cells coordinate an anti-viral immune response by activating macrophages and CD8 T cells in addition to inducing immunoglobulin class-switching in B cells. Consequently, I hypothesized that consumption of tBHQ would impair the memory response to influenza. To test this, I fed wild-type C57Bl/6 mice a control diet or a diet containing 0.001 % tBHQ prior to infection with influenza A. In a primary infection model, gene expression was analyzed at 7 days post-infection. This study showed that consumption of tBHQ significantly reduced mRNA expression of the canonical Th1 cytokines, IFN γ and TNF α , as well as expression of other genes important for antiviral response. In a secondary model of infection, in which the memory immune response plays a vital role, consumption of tBHQ reduced the number of Th1 cells in the lung, as well as the number of CD8 T cells co-expressing IFN γ and T-bet. Together, these data suggest that consumption of low concentrations of tBHQ impairs the primary and memory immune response to influenza infection in mice.

THE EFFECT OF THE COMMON FOOD ADDITIVE TBHQ IN OVA-ELICITED FOOD ALLERGY

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Immune-mediated adverse reactions to food allergens are rising at an alarming rate on a global scale for reasons that are not completely understood. Although many factors such as microbiota, diet, obesity, and environmental chemical exposure have been proposed to contribute to this marked increase, the identification of specific causative factors has remained elusive. Here we tested the hypothesis that a common food additive, tertbutylhydroquinone (tBHQ), at concentrations relevant to human exposure, exacerbates allergic response in ovalbumin (OVA) sensitized mice. Female Balb/c mice (4 weeks old) were maintained on control diet or diet with 0.001% tBHQ for the duration of these studies. The mice were exposed to OVA once per week for 4 weeks during the sensitization phase. Sensitization to OVA was assessed by the rise in OVA-specific IgE and IgG1. Upon oral challenge, mice were monitored for hypothermia shock response (HSR) and mast cell protease (mMCP)-1 response. Although sensitization with OVA elicited a robust OVA-specific IgE antibody response in both the control and tBHQ diet groups, IgE levels were markedly higher in the mice on the tBHQ diet as compared to control diet. Likewise, in response to OVA challenge, a more marked decrease in body temperature was observed in the mice on the tBHQ diet. Furthermore, the mMCP-1 response to OVA challenge was 3-fold greater in the mice on the tBHQ diet as compared to control diet. Taken together, we report low concentrations of the food additive tBHQ promote OVA sensitization and exacerbate anaphylactic response to OVA challenge in a mouse model of food allergy.

THE RXR AGONIST LG100268 ALTERS POLARIZATION OF BONE MARROW-DERIVED MACROPHAGES

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Macrophages and their polarization status are important factors in the tumor microenvironment. Macrophages can change from a pro-inflammatory, tumor-suppressive M1 phenotype to an anti-inflammatory, tumor promoting M2 phenotype as tumors advance. M1/M2 macrophage polarization *in vitro* is phenotypically and functionally plastic in response to various cytokines and the milieu of growth factors. Bone marrow derived macrophages (BMDM) from tibia and femurs of mice were differentiated by GM-CSF or M-CSF to skew them towards M1 or M2 phenotypes, respectively. Retinoids, selective ligands for RXR nuclear receptors, suppress carcinogenesis in a variety of animal models. The retinoid LG100268 inhibits inflammation *in vitro*, but its effects on macrophage polarization are unknown. LPS-stimulated M2 macrophages increased production of TNF- α , IL-1 β and IL-6 mRNA and proteins when treated with LG100268, suggesting redirection to an M1 phenotype. In addition, VEGF α and IL-10 mRNA and protein production were reduced in M2 macrophages treated with LG100268. Nitric Oxide (NO) secretion was induced in LPS-stimulated BMDMs in a dose-dependent manner. There was a significant ($p < 0.05$ vs. control) reduction in NO secretion when the LPS-stimulated M1 and M2 BMDMs were treated with LG100268. This data suggest that LG100268 can repolarize macrophages from an M2 to M1 phenotype. The redirection of macrophage activation and polarization will next be evaluated in animal models of lung and breast cancer.

**THE FOOD ADDITIVE tBHQ INHIBITS NATURAL KILLER CELL
ACTIVATION *IN VITRO***

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Tert-butylhydroquinone (tBHQ) is a commonly used food preservative with known immunomodulatory activity; however, there is little information regarding its role on NK cell activation and function. tBHQ is a known activator of nuclear factor erythroid 2-related factor 2 (Nrf2), which results in induction of cytoprotective genes. Activation of Nrf2 has been shown to modulate immune responses in a number of different models. In addition, studies in our laboratory have shown tBHQ inhibits a number of early events following T cell activation. In the current study, we asked whether activated Natural Killer (NK) cells could be equally impacted by tBHQ since many signaling cascades that control NK cell effector function also contribute to T cell function. Splenocytes were isolated from C57BL/6 female mice and treated with 1 μ M and 5 μ M tBHQ. NK cell function was assessed after activation with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 24 hours. Activation of NK cells in the presence of tBHQ decreased total NK cell count, intracellular IFN γ production and induction of the cell surface markers CD25 and CD69, which are markers of NK cell activation. In addition to NK cell effector function, NK cell maturation was also altered in response to tBHQ. Notably, this is the first study to demonstrate that the Nrf2 activator, tBHQ, negatively impacts effector functions and maturation of NK cells. This study was funded by National Institute of Environmental Health Sciences (ES024966).

LYMPHOCYTE-SPECIFIC PROTEIN TYROSINE KINASE (LCK) IS INVOLVED IN THE ARYL HYDROCARBON RECEPTOR (AHR)-MEDIATED IMPAIRMENT OF IMMUNOGLOBULIN SECRETION IN HUMAN PRIMARY B CELLS

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AHR activation by the high affinity ligand, TCDD, is widely established to suppress the immunoglobulin M (IgM) response in virtually every animal species tested, and extensively investigated in various mouse models. In mice, activation of AHR is known to impair B cell to plasma cell differentiation and IgM synthesis. In contrast to mouse B cells, activation of AHR in human primary B cells impairs immunoglobulin secretion in the absence of suppressing IgM synthesis. In recent studies, we have identified the putative involvement of LCK in impaired immunoglobulin secretion by human B cells. LCK is a well-characterized tyrosine kinase that phosphorylates known critical signaling proteins involved in vesicular secretion by T cells. Specifically, phosphorylation of tyrosine residue 505 inhibits the activity of LCK. By contrast, little is known concerning the role of LCK in human primary B cells. For the first time, our studies show that activation of the AHR by TCDD upregulates LCK protein expression, which then leads to an impairment of IgM secretion. Treatment with a LCK specific inhibitor restores IgM secretion by human primary B cells. Additionally, the presence of AHR antagonist reverses the AHR-mediated increase of LCK and the impairment of IgM secretion. We also observe a significant increase in phosphorylation of Tyr-505 LCK with TCDD treatment, indicating that AHR activation increases the level of inhibitory LCK. Taken together, our studies revealed a novel and species-dependent mechanism involving the AHR-mediated impairment of IgM secretion and an increase in total as well as inhibitory LCK in human but not mouse primary B cells. (Supported in part by NIH ES002520 and ES004911)

PARACRINE CYTOKINE PATHWAYS MEDIATE METASTASIS OF BREAST CANCER TO LYMPHATICS

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Lymphatics, rather than blood vessels, are the primary route for breast cancer metastasis. The presence of breast cancer cells in regional lymphatics, i.e., lymphatic metastasis, is an important prognostic factor for patients. Delineating molecular mechanisms by which the breast cancer cells migrate toward and infiltrate into lymphatics is crucial to designing new therapies to prevent metastatic dissemination. To study tumor:lymphatic interactions, we are using a three-dimensional (3D) heterotypic co-culture model of human breast cancer cells (hBCCs) grown with human microvascular lymphatic endothelial cells (hLECs) in novel chambers that we designed and fabricated. These chambers support growth of the 3D co-cultures, live-cell confocal imaging in real-time and non-invasive collection of conditioned media for secretomic analyses. We use live-cell assays developed in our laboratory for quantitative analysis of temporal and dynamic changes in BCC:LEC interactions in correspondence with changes in their malignant and proteolytic phenotypes. We cultured hLECs in the presence and absence of human MDA-MB-231 (231) triple negative BCCs in 3D cultures for 4 days. In mono-cultures, the 231 cells grow in clusters that exhibit a stellate morphology and hLECs form branching networks with central nodes. We observed that the volumes of 3D structures formed by LECs and 231 cells were significantly greater in co-cultures than in mono-cultures of either cell type. In addition, 231 cells infiltrate into the LEC networks with the infiltration increasing over the 4-day period as assessed by the degree of overlap between 231 cells and hLECs in 3D reconstructions of the co-cultures. Moreover, soluble factors from LECs increase invasive outgrowths of 231 structures. This was demonstrated in 231 cells grown in media conditioned by LECs and in parallel co-cultures of 231 cells and LECs. The induction of invasiveness by LEC conditioned media is reduced by boiling and repeated freeze/thawing, suggesting that the active factor(s) is a protein. Our preliminary results suggest that LECs secrete soluble factors that may be therapeutic targets for reducing invasion of BCCs into lymphatic networks.

**SUPPRESSION OF PLASMACYTOID DENDRITIC CELL ACTIVATION
BY CANNABINOID RECEPTOR 2-SELECTIVE AGONISTS**

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In America, 1.5 million people suffer from a form of lupus and most of them are women. Elevated activation of plasmacytoid dendritic cells (pDC), an innate immune cell capable of robust inflammatory cytokine secretion, contributes to the development of systemic lupus erythematosus (SLE). Due to its potent anti-inflammatory activity, cannabis is gaining acceptance for treatment of SLE and other autoimmune disorders. Δ^9 -Tetrahydrocannabinol (THC), a phytocannabinoid found in cannabis, is believed to modulate immune activation by binding to cannabinoid receptor 2 (CB2) on immune cells. However, THC also elicits potent psychotropic effects by binding to cannabinoid receptor 1 (CB1) on neurons. The goal of this study was to determine whether JWH-015 (015) and JWH-133 (133), both CB2-selective synthetic cannabinoids, can inhibit the secretion of inflammatory cytokines by pDC. To address this, CpG-ODN (CpG), a Toll-like receptor 9 (TLR9) ligand and pathogenic factor in SLE development, was used to stimulate primary human pDC. The effects of cannabinoid treatment on CpG-mediated pDC activation was determined using flow cytometric analysis. We found that all three cannabinoids (THC, 015, and 133) inhibited CpG-induced pDC production of interferon- α (IFN α) and Tumor Necrosis Factor- α (TNF α), key acute-phase inflammatory cytokines. Furthermore, treatment with the cannabinoids (THC, 015 & 133) significantly inhibited the CpG-induced phosphorylation of interferon response factor 7 (IRF-7) and TANK-binding protein 1 (TBK1), key events in the IFN α response. Likewise, treatment with the cannabinoids (THC, 015 & 133) inhibited the CpG-induced phosphorylation of nuclear factor κ B (NF κ B) and the inhibitor of NF κ B kinase subunit- γ (IKK γ), key events in the TNF α response. Additionally, both 015 and 133 significantly suppressed the aforementioned endpoints at lower concentrations than THC. Collectively, these results demonstrate that CB2-selective agonists (015 & 133) can inhibit CpG-induced cytokine response in pDC *in vitro* and suggest a therapeutic potential for these and similar compounds. Specifically, CB2-selective agonists can suppress the pDC-derived inflammatory cytokine responses observed in autoimmune disorders without the psychotropic effects of THC.

THE DELTA OPIOID RECEPTOR POSITIVE ALLOSTERIC MODULATOR BMS 986187 IS A G PROTEIN BIASED ALLOSTERIC AGONIST

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The delta opioid receptor (DOPr) has gained attention in recent years as agonists have been shown to promote analgesia and relieve depression. Additionally, evidence suggests that targeting this system results in less respiratory depression and abuse liability than the commonly targeted mu opioid receptor. Unfortunately, the clinical utility of DOPr agonists is limited as they lead to the rapid development of tolerance and many have proconvulsant properties. Evidence suggests that biased ligands may be capable of activating the downstream effectors associated with the positive effects of DOPr agonists, while mitigating the negative ones. BMS 986187 is a positive allosteric modulator (PAM) of the DOPr with apparent direct agonist properties, acting as an “ago-PAM”. Interacting with an allosteric site on DOPr, ago-PAM’s could promote biased receptor signaling leading to reduced on-target side effects. Based on preliminary data, this study set out to determine if BMS 986187 is acting as a biased ago-PAM. The results confirm that BMS 986187 is an ago-PAM at the DOPr with biased agonism favoring G protein activation over β -arrestin-mediated pathways, including receptor phosphorylation and internalization, resulting in reduced receptor desensitization. This is the first evidence of biased agonism mediated through direct binding to a unique allosteric site on the DOPr without occupancy of the orthosteric site. Our data suggests targeting the allosteric site on the DOPr, or indeed any GPCR, may be a novel way to promote signaling bias and thereby potentially produce a more targeted pharmacology.

**A LOSS-OF-FUNCTION VARIANT IN ANK3 FROM A FAMILY WITH
BIPOLAR DISORDER
CAUSES ALTERED FOREBRAIN CIRCUITRY**

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Bipolar disorder is a highly prevalent brain disease that affects approximately 1-2% of the general population worldwide. ANK3, which encodes the ankyrin-G protein, is one of the most significant genes linked to bipolar disorder through genome-wide association studies (GWAS); however, the functional effects of bipolar disorder-associated ANK3 variants on brain circuitry are not known. Previous work in cultured neurons has shown that ankyrin-G plays a key role in the regulation of GABAergic synapses on the axon initial segment and somatodendritic domain of pyramidal neurons where it interacts directly with the GABAA receptor associated protein (GABARAP) to stabilize GABAA receptors. Here, we generated a knockin mouse model expressing Ank3 W1989R to understand how this loss-of-function variant affects neuronal circuitry in vivo. Coronal brain sections from homozygous Ank3 W1989R mice showed a striking reduction in forebrain GABAergic synapses. In addition, whole-cell patch clamp recordings of miniature inhibitory postsynaptic current (mIPSCs) revealed a decrease in both the frequency and amplitude of GABA-mediated currents. Ank3 W1989R mice also displayed smaller kainate-induced gamma oscillations, suggesting disruptions in network synchronization. Moreover, Ank3 W1989R pyramidal neurons demonstrated reduced dendritic spine density and shorter axon initial segments likely as compensatory mechanisms to attempt to maintain homeostasis of neuronal excitability. Finally, we identified this variant, ANK3 W1989R, in a family with bipolar disorder, suggesting a potential role of this variant in disease. Our results highlight the importance of ankyrin-G in regulating forebrain circuitry and provide novel insights into how ANK3 loss-of-function variants may contribute to bipolar disorder in human patients.

**EVALUATING THE EFFECTS OF ANK3 VARIANTS IDENTIFIED IN AN
EPILEPTIC ENCEPHALOPATHY PATIENT COHORT**

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Epileptic encephalopathies (EE) are severe epilepsies that manifest in infancy or early childhood. Patients with EE display intractable seizures, severe neurological deficits, and have a high risk of Sudden Unexpected Death in Epilepsy (SUDEP). Ongoing investigations into the mechanisms underlying the disorder have largely focused on altered expression and distribution of voltage-gated ion channels, especially sodium (NaV) and potassium (KV) channels. Recently, results from a whole exome sequencing of EE patients in China found variants of unknown significance in a novel candidate, ANK3, implicating the gene as a potential target. ANK3 encodes ankyrin-G (AnkG), a critical cytoskeletal protein that organizes the axon initial segment (AIS) through binding β 4 spectrin and neurofascin-186 and anchors NaV and KV channels to intracellular microtubule bundles. Therefore, AnkG is required for the initiation and propagation of action potentials facilitating neurotransmission. Previous studies in which ANK3 expression was reduced or deleted reveal altered AIS morphology and decreased β 4 spectrin, neurofascin-186, and NaV and KV levels. Furthermore, our lab and others have demonstrated the role of AnkG in maintaining dendritic structure and in clustering of excitatory and inhibitory postsynaptic receptors, AMPA and GABA, respectively. What remains unclear is how the ANK3 mutations are associated with EE. We propose that ANK3 mutations linked to EE alter AIS assembly well as impair normal localization of associated ion channels and receptors, thereby altering neuronal excitability and resulting in epilepsy. Using neuronal cultures that express the ANK3 EE variants, we propose to evaluate: 1) the effect of the mutations on AIS assembly and expression of known AnkG associated proteins and receptors; and 2) the functional consequences of the mutations on neuronal excitability. Results obtained from the proposed research will guide studies using neurons derived from EE patient pluripotent stem cells in order to develop novel therapeutic interventions.

THE USE OF A MOUSE MODEL IN UNDERSTANDING GNAO1 ASSOCIATED MUTATIONS

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Recently patients presenting with epilepsy and/or movement disorders have been found to have mutations in *GNAO1*. The gene product, Gai/o protein couples to GPCRs and prevents Adenylyl cyclase activity to produce cAMP which plays a role in many different pathways. Gai/o protein also plays a role in neurotransmitter levels, through its regulation of cAMP concentrations. Patients are presenting with different de novo mutations within the gene. Existing studies have shown a phenotype genotype correlation linking LOF mutations with epilepsy and GOF mutations with movement disorders in children and infants with *GNAO1* mutations. In order to better understand this correlation, and possible mechanisms, here we use a pre-existing human engineered GOF, RGS insensitive mutation, G184S in a mouse model. The model was evaluated on different behavioral tests; Open Field, RotaRod, Digigait and Grip Strength. We report significant phenotypic evidence of movement abnormalities within the model. Further, this research showed a sex difference more prominent in our female mice which is consistent with the delineation affecting female patients. To begin to understand a possible mechanism and as Gao protein is most predominately expressed in the brain, we evaluated cAMP and neurotransmitter levels within the brain hemispheres the G184S mice. However, no significant differences were shown potentially implicating more localized differences within the brain, or different mechanisms unrelated to cAMP and neurotransmitters levels all together. Additional studies will be done looking at regions of the brain associated to movement including; striatum cortex, hippocampus and cerebellum. Lastly, we are evaluating the use of a dopamine depleting agent, Tetrabenazine to alleviate movement disorders. In the clinic TBZ has shown possible benefits for the patients.

**DETERMINING THE EFFECT OF TAU IN RESPONSE TO TRAUMATIC
BRAIN INJURY**

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Traumatic brain injury (TBI), defined as damage to the brain resulting from an external mechanical force, can lead to impairment of cognitive and physical function. TBI is an emerging health epidemic with ~2.5 million incidents severe enough to cause hospitalization or death. This study utilizes *Drosophila melanogaster*, a highly tractable genetic model organism for studying human diseases, to investigate TBI outcome. Flies subjected to rapid acceleration and impact exhibit TBI related symptoms consistent with other mammalian and human studies. The primary effect of TBI is axonal damage, which when coupled with brain injury triggers a cascade of events increasing phosphorylation of tau, a protein involved in microtubule stability. Tau phosphorylation plays physiological and pathological roles in the cell. However, aberrant tau phosphorylation causes tau filament formation, disruption of microtubule binding and in some instances increases cell death. This study hypothesizes that changes in tau activity have an impact in response to TBI and that the absence of tau will help alleviate TBI-related toxicity. This study makes use of transgenic tau lines to assess the baseline activity, the impact of overexpression of mutant tau and to determine if absence of tau alleviates the toxicity resulting from TBI. We have observed increased tau expression and locomotion impairments post-TBI. Our results establish that changes in tau expression play an important role in the outcome of TBI and we further propose to study how the genetic background and sex differences affect this outcome.

SEX DIFFERENCES IN HIPPOCAMPAL PHYSIOLOGY: CIRCUIT-SPECIFIC MECHANISMS UNDERLYING STRESS SUSCEPTIBILITY

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Depressive syndromes are a major cause of morbidity, and often arise in response to life stress. Aside from its social and financial burden, a striking characteristic of depression is that it affects women nearly twice as often as men. The impact of depression and the disparity in numbers of affected men and women have been recognized for some time, but the molecular underpinnings of the disease remain unknown. This knowledge gap is critical, as treatments remain ineffective in many patients and no sex-specific therapies are known. Glutamatergic pyramidal neurons that project from the ventral hippocampus (vHPC) to the nucleus accumbens (NAc) are mediators of stress responses, but little is known of the regulation of this circuit at the level of cell function or gene expression. This circuit has gained recent attention in mood disorder research as it has been shown that increased activity in these neurons promotes susceptibility to chronic social defeat stress (CSDS), a validated mouse model of depression. Using whole-cell slice electrophysiology, we show that vHPC-NAc neurons from female mice have heightened excitability compared to those from male mice. Additionally, we show that vHPC-NAc neurons from orchietomized male mice have similar activity to those from wild-type female mice, suggesting a role for androgens in the regulation of excitability in this circuit. These findings may begin to explain the apparent differences in the number of males and females diagnosed with depression, as the increased activity observed in vHPC-NAc neurons of female mice may indicate increased susceptibility of females to stress. Our group has also shown that the transcription factor Δ FosB is required for hippocampal learning, and its expression is induced in the vHPC by stress or antidepressant treatment. Because of its clear role in resilience in other brain regions, Δ FosB is an exciting prospective target for the differential regulation of vHPC-NAc neuronal activity in male and female mice at baseline as well as in response to stress. To this end, we show that general inhibition of Δ FosB function throughout the vHPC (but not dHPC) promotes susceptibility to subchronic stress, that overexpression of Δ FosB in vHPC reduces cell excitability, and that reduction of FosB gene expression in vHPC-NAc neurons increases cell excitability. We thus hypothesize that stress-induced Δ FosB in vHPC-NAc neurons mediates changes in the function of these neurons and regulates gene expression to promote stress resilience in male and female mice.

A MOUSE MODEL OF GNAO1 ASSOCIATED MOVEMENT DISORDER

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Rationale: The heterotrimeric protein G_o , whose α subunit is encoded by GNAO1, regulates ion channel function, neurotransmitter release, and neurite outgrowth. Mutations in GNAO1 have been identified in children with either epileptic encephalopathy (EIEE17) or neurodevelopment disorder with involuntary movements (NEDIM). The mechanism underlying the complex clinical spectrum of these GNAO1 encephalopathies is poorly understood. Previously, we discovered a genotype--phenotype correlation on patients mutations with an in vitro functional assay. De novo GNAO1 mutations have both GOF and LOF biochemical function with the former associated with seizures and the latter with movement disorder. We also reported a *Gnao1* GOF knock--in mutant (*Gnao1* G203R/+) with a mild seizure phenotype in C57Bl/6J mice. In the current study, we assessed behavioral characteristics and electrophysiology properties and morphological changes in the cerebellum of *Gnao1* mutant mouse models to explore the pathophysiology of GNAO1--associated movement disorders and to further validate our model associating GNAO1 GOF mutations with movement disorder.

Methods: *Gnao1* G203R/(GOF) and *Gnao1* --/+ (HetKO) animals of both sexes (age 8 to 14 weeks) were analyzed. Spontaneous inhibitory (sIPSCs) and excitatory postsynaptic currents (sEPSCs) in Purkinje cells in cerebellar slices were measured using whole cell patch--clamp recording techniques. Morphology was assessed with Nissl staining. Motor capabilities were assessed using a battery of behavioral tests.

Results: Compared to *Gnao1* WT mice, GOF mice showed a number of behavioral abnormalities related to movement including open field, rotarod, stride length, paw angle variability, and grip strength. HetKO mice were relatively normal in motor functions. Cerebellar slices from GOF mice showed reduced sIPSC frequency but relatively normal EPSCs. Cerebella from GOF mice also had reduced lobule number but molecular layer thickness was unchanged.

Conclusions: The *Gnao1* GOF mutant mice, as with human GNAO1 GOF patients show a pronounced movement disorder. This may be related to altered inhibitory signaling in the cerebellum.

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DEVELOPMENT OF A CELL ADHESION ASSAY FOR SCN1B-LINKED DRAVET SYNDROME VARIANTS

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Dravet Syndrome (DS) is a devastating pediatric epileptic encephalopathy (EE) that typically presents in the first year of life. Patients with DS have a variety of comorbidities such as developmental delay, high risk of sudden unexpected death in epilepsy (SUDEP), ataxia, and cognitive impairment. In greater than 80% of cases, DS is linked to variants in voltage-gated sodium channel genes (VGSCs). VGSCs are responsible for the initiation and propagation of action potentials in excitable cells. VGSCs are composed of a pore-forming α subunit and one or two non-pore forming β subunits. Variants in the gene which encodes the $\beta 1$ subunit, SCN1B, have been linked to DS and Scn1b null mice model DS. $\beta 1$ subunits are multifunctional proteins that participate in modulating the gating and kinetics of the ion channel pore, cell adhesion, cell signaling, and neuronal outgrowth and fasciculation in vivo. The presence of an immunoglobulin (Ig) loop domain in the structure of all five β subunits demonstrated their potential as cell adhesion molecules (CAMs). $\beta 1$ subunits participate in both trans-homophilic and heterophilic adhesion in vitro. In cerebellar granule neurons, $\beta 1$ - $\beta 1$ trans-homophilic cell adhesion drives neurite outgrowth and Scn1b deletion in mice leads to aberrant pathfinding and neuronal fasciculation in vivo. Most DS-linked variants in SCN1B are located in the Ig loop, suggesting that $\beta 1$ CAM activity may be clinically relevant in the mechanism of DS. To study the cell adhesion activity of DS-linked SCN1B variants, we aim to develop an in vitro cell adhesion assay using an immortalized T-cell line, Jurkats. Jurkat cells grow in suspension and express low levels of endogenous CAMs, making them an ideal mammalian cell line to study CAM activity. Understanding CAM activity in DS-linked SCN1B variants may provide additional mechanistic understanding of SCN1B-linked DS and suggest potential avenues for therapeutic intervention for this intractable

**OPIOID ANALGESICS WITH REDUCED SIDE EFFECTS:
MERGING DELTA-OPIOID RECEPTOR INHIBITION WITH
MU-OPIOID RECEPTOR ACTIVATION**

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Opioid analgesics are routinely and effectively used for the treatment of moderate to severe pain. However, there are very serious problems associated with the use of these medications. Acute effects like constipation and respiratory depression, as well as effects that develop after prolonged use, such as analgesic tolerance and physical dependence, hinder their clinical utility. Opioid addiction is rampant in the United States; every day more than 100 people die from an opioid overdose. Innovative approaches are needed in the short-term to prevent opioid overdoses and improve treatment of opioid-use disorders and also long-term strategies that target the root of the problem, the ineffective management of chronic pain. Previous studies in animal models showed that inhibition of the delta-opioid receptor (DOR), or knockout of the DOR gene, reduced the development of morphine tolerance and dependence. These studies prompted the hypothesis that peptide and small molecule compounds that are DOR antagonists and simultaneously mu-opioid receptor (MOR) agonists (“mixed-efficacy opioids”) could be developed as analgesics that show improved clinical utility compared to traditional opioid painkillers. In collaboration with medicinal chemists in the lab or Dr. Henry Mosberg in the College of Pharmacy, we have designed and synthesized opioid compounds with this improved pharmacological profile. By performing radioligand competition binding assays to calculate binding affinity [K_i] and [^{35}S]-GTP γ S binding assays to determine potency [EC_{50}] and efficacy [% maximal binding compared to standard agonist] I assisted in a long-standing effort to pharmacologically characterize a vast library of newly synthesized opioid compounds. We identified several promising compounds that bind with nanomolar affinity to both MOR and DOR, display the MOR agonist/DOR antagonist profile and in vivo intraperitoneal administration produced robust acute antinociceptive activity. Furthermore, after chronic administration, mice showed significant reductions in the development of tolerance and dependence. Mixed-efficacy opioids show promise in providing effective treatment of chronic pain while mitigating the risks of opioid misuse and addiction.

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IMPORTANCE OF PROTEIN CONTEXT IN SPINOCEREBELLAR ATAXIA 3

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Spinocerebellar Ataxia Type 3 (SCA3; also known as Machado-Joseph Disease) is a member of the family of polyglutamine (polyQ) neurodegenerative disorders that includes Huntington's Disease (HD) and several other SCAs. SCA3, the most common dominant ataxia in the world, is caused by polyQ tract expansion in the protein, ataxin-3. How SCA3 occurs and how to treat it remain unresolved issues. The primary culprit of toxicity in all polyQ diseases is the glutamine repeat: its abnormal expansion leads to neuronal dysfunction and death. Yet, there is indisputable evidence that the manner in which polyQ-dependent toxicity presents—areas impacted, cellular processes perturbed—is predicated in large part by regions outside of the polyQ tract, i.e., protein context. This has been made clear by studies in HD and SCA1. For SCA3, the role of ataxin-3 protein context has not garnered much attention. Defining the role of non-polyQ regions of ataxin-3 in the toxicity of its expanded glutamine tract will increase our understanding of the biology of disease in SCA3. While working to understand the toxicity of ataxin-3 in vivo, we found that two of its non-polyQ regions greatly modulate ataxin-3-dependent degeneration. One area controls interaction with the proteasome-associated protein, Rad23; the other with the AAA ATPase, VCP. Each interaction has a specific and clear effect on the toxicity of ataxin-3. My PhD studies will expand on the current understanding of protein context in SCA3 by providing a detailed comparison of the importance of various ataxin-3 domains on its toxicity, and by examining the VCP-ataxin-3 interaction as a potential therapeutic route for this disease. In this study, we will use *Drosophila melanogaster* models of SCA3 to determine if VCP over-expression impacts degeneration caused by ataxin-3's expanded glutamine tract. The effects on degeneration will be measured through longevity and motility assays alongside western blotting to analyze changes in the level of aggregated ataxin-3. We will also investigate how expression of a VCP-mimic protein impacts ataxin-3 based degeneration. Collectively, my PhD studies will help explain how the interaction of non-polyQ regions of ataxin-3 with specific proteins impacts its toxicity.

SEX DIFFERENCES IN INTRINSIC PROPERTIES OF DEVELOPMENTALLY DEFINED MEDIAL AMYGDALA NEURONAL SUB-POPULATIONS

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Developmental programs establish brain circuitry controlling critical social and non-social behaviors. The medial amygdala (MeA) acts as a central command station for processing critical social behaviors, like territorial aggression or mating, which are expressed differently in males and females. While the MeA has been identified as a sexually dimorphic brain region, its functional connectivity and how this differs between sexes is poorly understood. Our previous studies revealed that the embryonically expressed transcription factors Dbx1, Foxp2 and OTP define MeA progenitor pools destined to generate different subclasses of mature MeA output neurons (Hirata et al., *Nature Neuroscience*, 2009; Lischinsky et al., *eLife*, 2017). Moreover, these populations appear to have different roles in processing olfactory-based cues that trigger innate behavioral responses and in a sex specific manner. Here, we characterized the intrinsic electrophysiological profiles of these developmentally defined MeA subclasses to determine sex specific physiological basis of behavioral processing. To accomplish this, we conducted whole-cell patch-clamp electrophysiology to characterize the spiking patterns of Dbx1 and Foxp2-derived MeA neurons. We observed striking differences in the spiking patterns of across lineages, and also differences between the spiking patterns across sex within each lineage. Dbx1-derived neurons have lower capacitance than the Foxp2-derived, while females and males differ in their action potential adaptation properties throughout lineages. At the network level, we also found lineage specific differences in synaptic properties, suggesting differential inputs to these lineages. Thus, these results uncover lineage and sex specific differences in the computational functions of MeA circuitry, and provide a physiological basis for how the male and female brain may differentially process specific subsets of social and non-social environmental cues that trigger innate behavioral responses.

THE NON-SELECTIVE OPIOID DIPRENORPHINE PRODUCES DELTA-OPIOID RECEPTOR-MEDIATED RAPID ANTIDEPRESSANT-LIKE EFFECTS IN MICE

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Major depressive disorder (MDD) is the most common mood disorder worldwide with a lifetime prevalence of ~15%. However, current treatments are limited by a delayed onset of action and minimal efficacy in some patients. Preclinical evidence indicates delta-opioid receptor (DOR) agonists and kappa-opioid receptor (KOR) antagonists are novel targets in treatment resistant patients. [³H]-diprenorphine saturation binding experiments in membranes from CHO cells expressing the mu-opioid receptor (MOR); ($K_{D\mu} = 0.31 \pm 0.04$ nM); DOR ($K_{D\delta} = 1.1 \pm 0.16$ nM); and KOR ($K_{DK} = 0.36 \pm 0.09$ nM) revealed non-selective affinity, as expected. *In vitro* [³⁵S]GTP γ S assays in the same cell lines demonstrated diprenorphine is a DOR and KOR partial agonist ($EC_{50\delta} = 4.1 \pm 2.0$ nM, $E_{MAX\delta} = 40 \pm 5.0$ %; $EC_{50\kappa} = 0.71 \pm 0.21$ nM, $E_{MAX\kappa} = 41 \pm 5.3$ %); it is also a potent MOR antagonist against DAMGO ($K_B = 0.18 \pm 0.05$ nM). *In vivo* diprenorphine produced anti-depressive-like effects in the tail suspension test and the novelty-induced hypophagia test that were blocked by the DOR-selective antagonist naltrindole. While classical DOR agonists, such as SNC80, produce seizure activity, diprenorphine did not produce seizures and blocked SNC80 mediated seizures indicating that higher DOR efficacy is needed to produce seizures than anti-depressant-like effects. Alternatively, the DOR/KOR partial agonist and MOR antagonist profile of diprenorphine may mitigate against seizures. Future directions include the development of diprenorphine analogues to determine if modulating DOR/KOR agonist activity improves diprenorphine anti-depressant-like efficacy. Supported by R01 DA07315 and 035316.

EVIDENCE OF HEPATOTOXICITY FOLLOWING CHRONIC LOW DOSE EXPOSURE TO MICROCYSTIN-LR IN A MURINE MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Microcystins are a class of hepatotoxic cyclic heptapeptides produced by cyanobacteria. These toxins frequently contaminate freshwater sources (including municipal and agricultural sources) and pose a serious and growing global public health risk. While the Lake Erie cyanobacteria bloom in 2014 gained considerable notoriety, these blooms occur frequently not only in every single region of the US, but have been reported with increased frequency in almost every country world-wide. 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). We gavaged male *Lepr^{db}/J* mice with 50µg/kg or 100µg/kg microcystin-LR (MC-LR, one of the most common microcystin congeners) or vehicle every 48 hours for 4 weeks (n=12-16 mice/group). This treatment strategy yielded MC-LR exposures below the published NOAEL levels reported by Fawell et al (Hum Exp Toxicol. 1999). During the course of the treatment, we observed a non-statistically significant trend in decreased survival with control group showing 100% survival whereas the 50µg/kg and 100µg/kg group showed 93% and 85% survival respectively. To determine if MC-LR aggravated the lipid accumulation in the livers of the NAFLD mice, we performed Oil Red O staining on liver tissue sections. Quantitative histopathologic analysis of the stained sections indicated that while macrovesicular steatosis did not increase significantly in the MC-LR treated groups, there was a significant ($p<0.01$) increase in microvesicular steatosis in a dose-dependent manner. Next we investigated the changes in genetic markers of major drug-induced hepatopathology using quantitative PCR (qPCR). Here we observed that treatment with MC-LR (both 50 and 100 µg/Kg doses) 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 LR results in significant hepatic injury in NAFLD.

LEAD-INDUCED TRANSGENERATIONAL NEUROBEHAVIORAL TOXICITY AND TRANSCRIPTOMIC CHANGES IN ZEBRAFISH BRAINS

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Lead (Pb^{2+}) is a major public health hazard for urban children and has profound developmental and behavioral implications into adulthood across generations. The significance of adverse health outcomes due to lead exposure is exemplified by the recent discovery of lead in municipal water systems, notably in Flint, MI. For this study, zebrafish embryos (<2 hours post fertilization; EK strain) were exposed for 24 hours to waterborne Pb^{2+} at a concentration of 0, 1, or 10 μM Pb^{2+} . Zebrafish (*Danio rerio*) are an ideal model for translational and transgenerational research due to their short generation time, sequenced genome, and analogy to human development and disease. The exposed F_0 generation was raised to adulthood, underwent neurobehavioral analysis and was spawned to produce the F_1 generation, which was subsequently spawned to produce the F_2 generation which also underwent neurobehavioral analysis. RNA was extracted from the control and 10 μM Pb^{2+} -lineage F_2 generation (n=11 for each group). We prepared 3' mRNA-Seq libraries (QuantSeq) that were sequenced and the transcript expression was quantified. Selected genes were also evaluated by qPCR. In the behavioral assays, we found that both the F_0 and F_2 generations had significant learning deficits and behavioral changes. In the F_2 generation brain tissue, 367 genes were found to be significantly differentially expressed between the control and the F_2 offspring of the lead exposed fish. A subset of these altered genes are known to be associated with learning and memory and also have been reported to be affected by lead exposure. Genes associated with the endocrine system and hormone regulation also had differential expression. Pathway analysis revealed altered genes in pathways involved in neurological disorders, nervous system development and function, steroid metabolism, learning, memory and behavior. These data will inform future investigations to elucidate the mechanism of adult-onset and transgenerational health effects of lead exposure.

SUBLETHAL TCDD EXPOSURE DURING ZEBRAFISH DEVELOPMENT PRODUCES MULTIGENERATIONAL TESTICULAR ABNORMALITIES IN HISTOLOGY AND GENE EXPRESSION

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The industrial by-product TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent environmental toxicant and endocrine-disrupting chemical (EDC) with known teratogenic effects on humans, rodents and fish. Developmental exposure to some EDCs, including TCDD, is linked to the occurrence of adult-onset and multigenerational disease. Our lab uses zebrafish (*Danio rerio*) as a model to study these effects due to their short generation time, transparency in early development, and ease of developmental exposure. Previous work in this lab has shown that both structural and reproductive abnormalities (spinal deformities, sex ratio skewed toward female fish, body plan/gonad mismatch, and decreased fertility) were observed in young zebrafish exposed to TCDD. Reproductive abnormalities observed in subsequent unexposed generations (F_1 and F_2) were male-mediated, which suggested heritability through the male germline. We analyzed the testicular tissue of TCDD-exposed male zebrafish from all three generations, looking for changes in histology and gene expression that could account for decreased reproductive capacity. For histological analysis, spermatogenic cells were categorized by differentiation stage and quantified within seminiferous tubules. Statistical analysis (Student's t-test) demonstrated significant differences in certain spermatogenic cell types between exposed and control groups in the F_0 and F_1 generations, indicating delayed spermiation in exposed males and descendants. Analysis of exposed testes revealed multigenerational gene expression changes in pathways implicated in reproduction and infertility, including testis development and spermatogenesis, lipid metabolism and steroidogenesis, citric acid cycle, peroxisome, and aryl hydrocarbon receptor (AhR) xenobiotic response pathways. Overall, we found that differential expression of reproductive genes and reduced capacity of sperm cells to mature could account for the reproductive defects previously seen in TCDD-exposed male zebrafish and their descendants.

**VISCERAL ADIPOSITY-STIMULATED GENOTOXICITY AND
MALIGNANT TRANSFORMATION OF EPITHELIAL CELLS**

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Obesity is a global epidemic with a predicted rate of 42% in the USA by 2050. Epidemiological studies show that obesity is a risk factor for developing cancer however; the molecular mechanism has not been fully elucidated. Our published data demonstrate that fibroblast growth factor-2 (FGF2) released from fat cells (adipocytes) in the visceral adipose tissue (VAT) induces transformation/tumorigenicity in skin and mammary epithelial cells. Specifically, FGF2 released from VAT stimulates epithelial cell growth in soft agar by inducing the proto-oncogene c-Myc. Growth in soft agar is a measure of transformation/tumorigenicity; neither transformation nor c-Myc induction in epithelial cells was reversible. c-Myc overexpression can initiate a process of genetic instability linked to tumor initiation. Our discovery of this novel direct path of VAT-stimulated tumorigenesis adds mechanistic insight to our earlier discovery that VAT secretions promote UVR-induced non-melanoma skin cancer. The objective of our current study was to determine the mechanism by which FGF2 stimulates malignant transformation. We hypothesized that FGF2 from VAT induces c-Myc and subsequent genomic instability in epithelial cells leading to increased carcinogenesis. To test hypothesis we generated a filtered conditioned-medium from human VAT, treated MCF-10A (mammary epithelial) and JB6 P⁺ (skin epithelial) cells and measured several downstream mediators of FGF2 and activation of FGFR-1 (FGF2 receptor). Following VAT treatment, epithelial cells demonstrated induced c-Myc protein expression along with ROS accumulation, elevated -H2AX foci, and increased micronucleus (MN) formation. We found that inhibition of c-Myc attenuated VAT-induced neoplastic transformation of MCF-10A and JB6 P⁺ cells, while constitutive activation of c-Myc-induced spontaneous neoplastic transformation of JB6 P⁺ cells. Collectively, our data suggested FGF2 released from VAT interacts with FGFR-1 and activates c-Myc. The role of c-Myc in the formation of MN and DNA damage is under investigation. Determining the impact of excess VAT on cancer will lead to strategies to help prevent adiposity-associated cancers and identify individuals at risk for disease or individuals that may be susceptible to compounded genotoxicity due to DNA damaging environmental exposures.

ASSESSMENT OF THE ANTIOXIDANT COMPOUNDS IN PROTECTION OF SPINAL CORD ASTROCYTES FROM METHYLMERCURY TOXICITY

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Loss of redox homeostasis is one of the cellular mechanisms that is involved in methylmercury (MeHg) induced motor neuron (MN) degeneration. An increase of reactive oxygen species and perturbation of the antioxidant pathways has been indicated in MN and astrocyte studies. While astrocytes are the first cells in the central nervous system exposed to MeHg due to their role in blood brain barrier, neurons are more susceptible to MeHg. During oxidative stress, glutathione (GSH) supplied from astrocytes is imperative for neurons to be protected against oxidative damage. We hypothesized that the loss of redox homeostasis in spinal cord astrocytes (SCAs) could contribute to spinal cord MN death in MeHg exposure. Therefore, if we supply SCAs with antioxidant compounds such as triterpenoid derivatives, edaravone or N-acetyl cysteine (NAC) to maintain the antioxidant level in SCAs, the GSH level may be sufficient to supply to MN and result in a reduction of MN degeneration from MeHg-induced toxicity. To test the efficacy of antioxidant compounds in SCAs protection, the SCAs viability were assessed as a function of time of MeHg exposure. Pretreatment of SCAs with triterpenoid derivatives, 1 μ M and 10 μ M CDDO-IM or CDDO-ME or free radical scavenger edaravone for 2h prior to MeHg exposure did not protect SCAs from degeneration. When SCAs were pretreated with 10mM NAC 2h prior to MeHg exposure, the viability of SCAs remained at the same level as the controls (DMSO+H₂O and NAC+H₂O) across 24h exposure and was significantly higher than MeHg treatment alone through 28h. This study suggests MeHg perturbed the GSH level in SCAs and supplementation of GSH substrate precursor could protect SCAs from MeHg induced toxicity. This research is supported by NIH grant R01 ES024064.

EFFECTS OF ACUTE METHYLMERCURY-INDUCED CALCIUM CHANGES IN THE VENTRAL LUMBAR SPINAL CORD REGION OF THE C57BL6J MOUSE

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Studies have demonstrated that methylmercury (MeHg) produces motor neuron cell death at the lumbar spinal cord region. In many cell types, a key MeHg neurotoxicity marker is dysregulation of intracellular calcium (Ca^{2+})_i homeostasis. Spinal cord alpha motor neurons (α MN) send excitatory signaling onto Renshaw interneurons, which in turn send inhibitory neurotransmission back to the same α MNs, ultimately modulating their signaling. The effects of Ca^{2+} _i concentration ($[Ca^{2+}]_i$) during MeHg exposure at the lumbar ventral spinal recurrent inhibition have never been studied. The aim of this project is to determine the effects of acute MeHg-mediated $[Ca^{2+}]_i$ during the excitatory and inhibitory signaling of recurrent inhibition between MNs and Renshaw cells. Lumbar sections of an adult C57BL6J mouse were exposed to 20 μ M MeHg during 15 min through a real-time perfusion system. Ca^{2+} changes were recorded using Fluo4-AM at 15 min of MeHg exposure. The role of the acetylcholine (ACh), glycine and GABA_A receptors, cysteine-containing ligand gated channels, mediators of recurrent inhibition, was determined using a pharmacology approach. ACh receptor antagonists: Mecamylamine (MEC), Dihydro- β -erythroidine hydrobromide (DH β E) and glycine and GABA_A receptors antagonists: Strychnine and Bicuculline (BCC), were used as a pretreatment and during MeHg treatment. It is hypothesized that MeHg neurotoxicity produces a differential disruption of Ca^{2+} homeostasis in both cells types that should potentially lead to hyperexcitability of the Renshaw area. Results show (N = 11) that MeHg treatment alone significantly increases $[Ca^{2+}]_i$ at 15 min and 1 hr (0.27 and 0.21 relative fluorescence, respectively). Presence of MEC, DH β E, Strychnine and BCC during MeHg treatment significantly decreases ventral $[Ca^{2+}]_i$ (0.21, 0.18, 0.19 and 0.14, relative fluorescence, respectively) from baseline. Determining the role of MeHg-induced disruption in Ca^{2+} homeostasis through these receptors could potentially elucidate the possible mechanisms of MeHg-mediated $[Ca^{2+}]_i$ neurotoxicity during recurrent inhibition.

TRANSCRIPTIONAL REGULATION OF GATA3 IS DEPENDENT ON NRF2

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Tert-butylhydroquinone (tBHQ) is a food preservative and known activator of the transcription factor, nuclear factor erythroid 2-like 2 (Nrf2), which functions to activate cytoprotective genes in response to cellular stress. When exposed to stressors, Nrf2 translocates to the nucleus and binds to an antioxidant response element (ARE) to induce expression of target genes. We have previously shown that exposure to tBHQ directly influences immune function through polarization of murine CD4⁺ T cells towards a Th2 phenotype. Immune skewing was evidenced by increased Th2 cytokine (IL-4, IL-5 and IL-13) protein production, as well as increased DNA binding capacity of the master regulator of Th2 helper cell differentiation, GATA3 in a Nrf2-dependent manner. However, the mechanism(s) by which increased GATA3 DNA binding occurs, remains unknown. This study addresses this gap, through *ex vivo* analysis of GATA3 expression in response to tBHQ exposure in mouse CD4⁺ T cells. CD4⁺ T cells from wild-type (WT) and Nrf2-null (KO) mice were magnetically isolated and activated with vehicle or tBHQ, followed by T cell specific stimulation for 96 hours. A concentration-dependent increase in GATA3 gene expression was noted with exposure to tBHQ in WT, but not KO mice, indicating Nrf2-dependent transcriptional regulation. In addition, we identified potential antioxidant response elements within a GATA3 intron as well as in the promoter of the GATA3 gene further suggesting transcriptional modulation. Our data provide insight into the molecular regulation of GATA3 by Nrf2, and provide additional evidence for the role of Nrf2 in modulating adaptive immune cell function. This study was supported by National Institute of Environmental Health Sciences (ES024966).

ASSESSMENT OF MICROCYSTIN INDUCED HEPATOXICITY IN THE SETTING OF PREEXISTING LIVER DISEASE

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Introduction: Microcystin-LR (MC-LR) is a liver toxin produced by freshwater cyanobacteria, also known as blue-green algae, that has become an alarming public health concern in the Great Lakes region and world wide. Guidelines for safe exposure levels of MC-LR were established based on healthy liver studies and fail to account for the large population of humans with pre-existing liver diseases. Current clinical parameters to measure liver damage include the measurement of liver enzymes, most commonly alanine transaminase (ALT) and alkaline phosphatase (ALP). While these enzyme levels have been used to assess MC-LR induced hepatotoxicity in healthy settings, their suitability in the setting of pre-existing liver disease, such as Non-alcoholic Fatty Liver Disease (NAFLD), is unknown.

Hypothesis: We tested the hypothesis that ALT and ALP are unsuitable biomarkers for the diagnosis and assessment of MC-LR hepatotoxicity in the setting of pre-existing liver disease.

Methods/Results: Leprd/J mice (a model of NAFLD) were orally gavaged with vehicle, 50ug/kg or 100ug/kg MC-LR every 48 hours for 4 weeks. While serum ALT levels were increased in a non-specific fashion, serum ALP levels were only increased in the MC-LR treated groups ($p < 0.05$ for both 50 and 100ug/kg treated mice). Interestingly, hepatic gene expression of ALT and ALP was significantly decreased in the MC-LR treated mice (ALT decreased by $>30\%$ in the 100ug/kg treated mice, $p < 0.05$, while ALP decreased by $>40\%$ in both the 50 and 100ug/kg treated mice, $p < 0.01$ for both). To further examine the regulation of these enzymes in a defined setting, HepG2 human liver cells were cultured in either normal glucose cell culture media (1 g/L glucose to simulate normoglycemia) or high glucose media (4.5 g/L to simulate hyperglycemia) and treated with vehicle or MC-LR (10nM, 100nM, and 1000nM) for 24 hours. Under these conditions, hyperglycemia increased ALP activity in HepG2 cells compared to normoglycemia cultured cells ($>45\%$ increase, $p < 0.002$). Interestingly, while significant decreases in ALT expression were seen across all doses of MC-LR under normoglycemia ($>35\%$ decreases for 10 nM, 100nM, and 1000 nM, $p < 0.05$), in hyperglycemic conditions ALT expression was only significantly decreased after exposure to 100 nM ($p < 0.05$) or 1000 nM ($p < 0.01$) MC-LR.

Conclusion: Our results suggest that ALT and ALP are inadequate biomarkers of MC-LR induced liver injury in the setting of pre-existing liver disease and that additional work is needed to determine suitable diagnostic measures of MC-LR induced liver injury in this setting.