Emerging Concepts and Tools in Toxicology

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NESOT Annual Fall Meeting
Friday, October 26, 2018

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Table of Contents

Agenda

Emerging Concepts and Tools in Toxicology

Room: Amphitheatre

8:00-9:00 am	**Check in and Continental Breakfast**
9:00-9:30 am	**Opening Address:** Angela Slitt, President NESOT Scott Fountain, Site Head CRL MA
9:30-10:30	**Student Oral Presentation Competition**
10:30-10:45	**Networking Break / Facility Tour (optional)**
10:45-11:30	**Speaker 1 / Facility Tour (optional): Dr. Julie Gosse, University of Maine** "First Use of Super-Resolution Microscopy in Toxicology: Antimicrobial Agent Triclosan Deforms Mitochondria in Live Cells"
11:30-12:15	**Speaker 2:** Joel Cohen, Senior Toxicologist, Gradient Pharmaceuticals, Inc. "Looking Under the Hood – Expert Review of In Silico Predictions for Pharmaceutical Impurity Safety Assessment"
12:15-1:00	**Lunch and Networking**
1:00-2:00	**Student and Postdoctoral Poster Session**
2:00-2:45	**Speaker 3: Dr. David Sela, Associate Professor of Food Science, University of Massachussetts** Human milk directs the establishment and maintenance of a protective infant gut microbiome
2:45-3:30	**Presentation of Awards**
3:30-4:00	**Closing Remarks:** Angela Slitt, President NESOT

Undergraduate Event

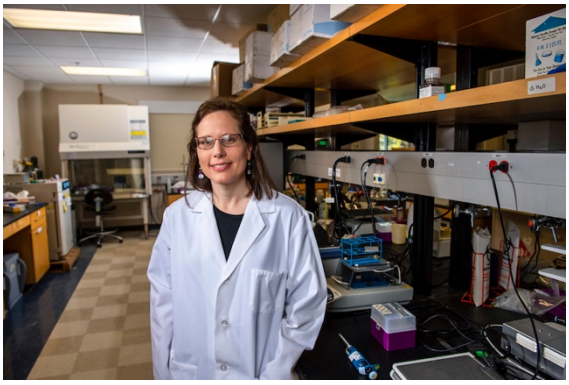
Room: Currie

10:30 – 11:30	**Facility Tour**
12:15-1:00	**Lunch with an Expert**
1:00-2:00	**Student and Postdoctoral Poster Session**
2:00-2:25	**Welcome and introduction to toxicology by Dr. Larissa Williams, Bates College**
2:25-2:50	**Working as a Toxicologist in Industry, Dr, Qihong Huang, Boehringer-Ingelheim**
2:50-3:30	**Active learning activity on toxicogenomics, Dr. Josh Gray, U.S. Coast Guard Academy and Larissa Williams, Bates College**
3:30	**Wrap up and distribution of evaluation on the program**

Guest Speaker #1

Dr. Julie Gosse (Associate Professor - University of Maine)

First Use of Super-resolution Microscopy in Toxicology: Antimicrobial Agent Triclosan Deforms Mitochondria in Live Cells



Biographical Sketch: Dr. Julie A. Gosse is Associate Professor and Graduate Coordinator in the Department of Molecular & Biomedical Sciences at the University of Maine. Her research involves biochemical, molecular, and cellular toxicology; in particular, effects of toxicants on signal transduction pathways and on mitochondria. Dr. Gosse's teaching is focused on biochemistry, physical biochemistry, and mentoring students in laboratory research. She completed her Ph.D. in Biophysical Chemistry at Cornell University and her postdoctoral research in Molecular Toxicology at Dartmouth Medical School.

Abstract: The antimicrobial agent triclosan (TCS) is used in products such as toothpaste and is readily absorbed into oral mucosa and human skin. These and many other tissues contain mast cells, which are involved in numerous physiologies and diseases. Mast cells release chemical mediators through a process termed degranulation, which is inhibited by TCS. Investigation into the underlying mechanisms led to the finding that TCS is a mitochondrial uncoupler at non-cytotoxic, low-micromolar doses in several cell types and live zebrafish. Our aim was to determine the mechanisms underlying TCS disruption of mitochondrial function and of mast cell signaling. We combined super-resolution (fluorescence photoactivation localization) microscopy and multiple fluorescence-based assays to detail triclosan's effects in living mast cells, fibroblasts, and primary human keratinocytes. TCS disrupts mitochondrial nanostructure, causing mitochondria to undergo fission and to form a toroidal, "donut" shape. TCS increases reactive oxygen species production, decreases mitochondrial membrane potential, and disrupts ER and mitochondrial Ca2+ levels, processes that cause mitochondrial fission. TCS is 60× more potent than the banned uncoupler 2,4-dinitrophenol. TCS inhibits mast cell degranulation by decreasing mitochondrial membrane potential, disrupting microtubule polymerization, and inhibiting mitochondrial translocation, which reduces Ca2+ influx into the cell. Our findings provide mechanisms for both triclosan's inhibition of mast cell signaling and its universal disruption of mitochondria. These mechanisms provide partial explanations for triclosan's adverse effects on human reproduction, immunology, and development. This study is, to the best of our knowledge, the first to utilize super-resolution microscopy in the field of toxicology.

Guest Speaker #2

Dr. Joel Cohen (Sr. Scientist, Gradient Pharmaceuticals, Inc.)

Looking Under the Hood - Expert Review of In Silico Predictions for Pharmaceutical Impurity Safety Assessment

Biographical Sketch: Dr. Cohen is a senior toxicologist at the scientific consulting firm Gradient, with specialties in computational toxicology and human health risk assessment. At Gradient, his primary responsibilities include non-clinical safety assessments of medical device and pharmaceutical components, consumer product safety evaluations, and particulate matter inhalation exposure assessment and dose modeling. Before joining Gradient, Joel earned his doctoral degree in Environmental Health at the Harvard School of Public Health. He currently holds a Visiting Scientist appointment at the Harvard T. H. Chan School of Public Health where he researches the applications and human health implications of nanomaterials across the life cycle of nano-enabled products.

Abstract: Alternative testing strategies are seeing increased adoption early on in the drug development process. Mutagenicity and skin sensitization are examples of where reliable *in silico* toxicology screening tools were developed based on well-understood mechanisms of toxicity, and large databases of robust toxicity data. *In silico* screening tools for other health concerns (*e.g*., developmental toxicity) are less robust but are seeing increased attention. However limitations remain, including 1) "out of domain" results (*i.e*. no valid prediction can be made), and 2) potency estimation (or the amount of chemical necessary to cause a toxic effect). In light of these limitations, program output should be reviewed by toxicologists with knowledge of the toxic effect of interest as a final check on model validity. Dr. Cohen will discuss here the role of expert review in dealing with "out of domain" predictions in the context of mutagenicity assessments of pharmaceutical impurities, and evaluating the reliability of in silico predictions for sensitization potency.

Guest Speaker #3

Dr. David Sela (Associate Professor of Food Science, University of Massachusetts)

Human milk directs the establishment and maintenance of a protective infant gut microbiome

Biographical Sketch: Dr. David Sela earned his doctorate in microbiology at UC Davis followed by postdoctoral fellowships at Stanford School of Medicine and the UC Davis Foods for Health Institute. Currently, Dr. Sela holds appointments at the Dept. of Food Science and the Dept. of Microbiology at UMass Amherst. Dr. Sela has an adjunct faculty appointment in the Dept. of Microbiology and Physiological Systems at the UMass Medical School.

Dr. Sela studies molecular constituents secreted in breast milk that interact with commensal bacteria to impact infant health. The Sela Lab routinely uses molecular microbiology and ecology approaches, genomics (comparative and functional), transcriptomics, metagenomics, and attendant informatic/statistical approaches. The overarching mission of Sela Lab is to translate nutrition research to enhance infant health and other stages of human development. In addition to human milk, the Sela Lab broadly studies dietary molecules that interact with members of the gut microbiome with relevancy to host health. This includes plant-derived products as well food-grade antimicrobials that potentially disrupt microbiome structure and function. Dr. Sela is committed to promoting human milk and lactation research through leadership within several scientific communities. Accordingly, Dr. Sela is the past-chair of the Maternal, Perinatal, and Pediatric Research Interest Section (RIS) of the American Society for Nutrition (ASN). Furthermore, Dr. Sela established and chairs a new ASN RIS on Nutritional Microbiology. This RIS is responsible for advancing microbiome, probiotic/prebiotic, and fermented foods research among nutrition scientists. He serves on the executive committee of the International Society for Research in Human Milk and Lactation. Moreover, Dr. Sela is the lead organizer of the next FASEB science research conference on human milk bioactives to be held in July 2019. In addition to human milk and nutrition societies, Dr. Sela is an internationally recognized leader within the bifidobacterial research community. Accordingly, he served on the organizing committee of the international conference on bifidobacteria (Propiobifido 2016). Dr. Sela is a member of the editorial boards for Applied and Environmental Microbiology and Frontiers in Nutrition.

Listing of Posters

#	Title and Authors
1	**Role of Nrf2a in modulating MEHP-induced hepatosteatosis following embryonic exposure in *Danio rerio*** Hadley Moreau,[1,2] Karilyn Sant[1], Larissa M. Williams[2], Alicia Timme-Laragy[1] ; 1. University of Massachusetts Amherst; 2. Bates College
2	**Density of fungal contaminants affects the LD_{50} of inhibitors of fungal growth in biodiesel** Anne McGoldrick, Valerie Hines, Jaimie Chicoine, and Joshua P. Gray; U.S. Coast Guard Academy, New London, CT
3	**CYP1 P450 and EETs signaling regulate Iκβα degradation in TNFα-induced inflammation pathway in asthmatic airway smooth muscle cells** B. Taghizadeh[1], A. Gardner[1]; MCPHS University, MA
4	**EGFR transactivation by Endothelin Receptor involves a Src activated pro-HBEGF cleavage in asthmatic airway smooth muscle (ASM) cells** Prajakta Ambegaokar[1], Alice Gardner[1]; [1]Department of Pharmaceutical Science, MCPHS University, Worcester, USA.
5	**Development of an electrochemiluminescence-based assay to characterize pyroptosis-related proteins in plasma obtained from Parkinson's disease patients** Anderson, F. L[1], Andrew, A. S.[2], von Herrmann, K. M.[1], Bispo, F. P.[3], Young, A. L.[1], Lee, S. L.[2], Havrda, M. C.[1]; [1]Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756; [2]Department of Neurology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756; [3]Department of Biology, Saint Anselm College, Manchester, NH 03102
6	**Role of the transcription factor Nfe2 and pro-oxidant exposure in inner ear development in zebrafish** Anna Bowsher[1], Melanie Mait[1], Larissa M. Williams1; 1Bates College, Biology Department, Lewiston ME 04966
7	**Targeted gene expression assays reveal markedly different gene expression and lipid accumulation profiles for perfluoroalkyl acid (PFAA) mixtures compared to single PFAA treatment in cryopreserved human hepatocytes** Emily Marques[1], Marisa Pfohl[1], Wei Wei[1], Ogochukwu Amaeze[2] and Angela Slitt1; 1 Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI. [2] Department of Clinical Pharmacy & Biopharmacy, Faculty of Pharmacy, University of Lagos, Nigeria
8	**An 'omics approach to unraveling the paradoxical effect of diet on PFOS and PFNA induced non-alcoholic fatty liver disease (NAFLD)** Marisa Pfohl, Hang Ma, Maxine Aubustan, Emily Martell, Navindra Seeram, and Angela Slitt. University of Rhode Island, Kingston, RI
9	**Deficiency of Anti-Inflammatory Protein TNIP1 Has Divergent Effects on Wound Healing** R. Shamilov, and B. Aneskievich. Pharmaceutical Sciences, University of Connecticut, Storrs, CT
10	**Understanding the Role of the Manganese Transporter Slc30a10 in Developing Mice** Heather Conboy, Courtney J. Mercadante, Milankumar Prajapati, Miriam E. Dash, Carolina Herrera, Michael A. Pettiglio, Thomas B. Bartnikas. Brown University
11	**Impact of Acetaminophen-Induce Liver Injury on Expression of Cytochrome P450** Yifan Bao, Pei Wang, Xueyan Shao, Xiaobo Zhong. University of Connecticut
12	**The role of mTOR complex 1 in the induction of long-lasting lipid metabolic reprogramming by flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE-47)** Joseph McGaunn1, Alexander Suvorov1; 1 Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA, USA 01003-9303

#	Title and Authors
13	**Genetic Investigation of Long Non-coding RNAs in HepaRG cells Undergoing Differentiation** SooWan Lee, Bindu Prabhakar, and Theodore P. Rasmussen. Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269
14	**Levels Of Methionine Sulfoxide Associated With An APAP Autoprotection Mouse Model Do Not Confer Cytoprotection Against APAP In Vitro In Hc04 And HepaRG Cell** S. J. Toro1, A. C. Donepudi1, K. H. Liu2, D. P. Jones2, and J. E. Manautou1. 1Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT; and 2Department of Medicine, School of Medicine, Emory University, Atlanta, GA.
15	**Neonatal exposure to brominated flame retardant permanently alters liver lipid metabolism in mice via mTOR-dependent mechanism** Victoria Salemme & Alexander Suvorov; University of Massachusetts, Amherst
16	**Modulation of Peroxisome Proliferator-Activated Receptors gamma (PPARγ) signaling perturbs embryonic pancreas development in the zebrafish, Danio Rerio** Olivia L. Venezia, Karilyn E. Sant, Alicia R. Timme-Laragy University of Massachusetts, Amherst
17	**Nanomaterial Exposure-driven Fibroblast to Myofibroblast Differentiation Is Dependent on Substrate Stiffness** Alysha Simmons1,3, Susan Leggett1, Ian Wong1,2, Agnes Kane1,3; 1Department of Pathobiology, Brown University, 2Department of Engineering, Brown University, 3Department of Pathology and Laboratory Medicine, LMM, Brown University
18	**2,3,7,8 tetrachlorodibenzo-[p]-dioxin exposure and genetic manipulation of the aryl hydrocarbon receptor disrupts forebrain development and axonal targeting** Nathan R. Martin1, Cheryl L. Dunham2, Robert L. Tanguay2, Jessica S. Plavicki1; Pathobiology Graduate Program, Brown University, Providence, RI1 Oregon State University, Corvallis, OR2
19	**Gene expression differences linked to resistance of endocrine therapy in African American and Caucasian patients with breast cancer** Sara Parvin-Nejad, Sana Cheema; George Acquaah-Mensah PhD MCPHS University, Worcester, MA
20	**Correlation of anticancer activity of pelitinib – a tyrosine kinase inhibitor (TKI) – with the expression levels of epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC)** Recheal Kisubika, Donece Hill, Terrick Andey; MCPHS University
21	**Anticancer Drug Repurposing: Efficacy study of etoposide non-small cell lung cancer (NSCLC)** Donece Hill, Recheal Kisubika, Terrick Andey; MCPHS University
22	**Evaluating CD44 receptors in cancer: targeting and toxicity** Dotun Dennis Adegunle, Eugene Boakye Ansah, Danny Nguyen and Robert Campbell, PhD; MCPHS University, Department of Pharmaceutical Sciences, Worcester MA.
23	**Phytotoxic effects of NSAIDs on Pisum sativum lead to animal alternative drug assay** Crystal H Ng, Tyler M Holmes, Matthew D Metcalf; MCPHS University School of Pharmacy Worcester
24	**Study of long-noncoding RNA in cytochrome P450 expression regulation and drug induced liver injury** Liming Chen, Stephanie Piekos, and Xiao-bo Zhong; University of Connecticut
25	**Limited Developmental Neurotoxicity from Neonatal Exposure to Ultrafine Carbon Particles or Diesel Particulate Matter** Keith Morris-Schaffer, Alyssa Merrill, Katherine Conrad, and Deborah Cory-Slechta; University of Rochester Medical Center

#	Title and Authors
26	**Slc30a10-deficient mice, a model of inherited manganese excess, develop hepcidin deficiency and increased manganese absorption** Courtney J. Mercadante, Milankumar Prajapati, Heather Conboy, Miriam E. Dash, Carolina Herrera, Michael A. Pettiglio, Thomas B. Bartnikas; Brown University
27	**The emerging contaminant 3,3' dichlorobiphenyl (PCB-11) impedes Ahr activation and Cyp1a activity to modify embryotoxicity of Ahr ligands in the zebrafish embryo model (*Danio rerio*)** Monika A. Roy, Karilyn E. Sant, Olivia L. Venezia, Alicia R. Timme-Laragy; University of Massachusetts, Amherst
28	**Persistent alteration of hepatic DMEs expression following neonatal activation of PXR** Pei Wang[1], Guming Liu[1], Xiaobo Zhong[2], Lirong Zhang[1] [1]Department of Pharmacology, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, China; [2]Department of Pharmaceutical Sciences, School of Pharmacy; University of Connecticut, CT 06269, USA
29	**Toxicity reducing and mediating strategies for drug-resistant ovarian cancer: Investigating conventional drug delivery and experimental cancer research approaches** Clement, Jenel; Emerson, Josh; Amini, Negar; Campbell, Robert PhD; MCPHS, Worcester, MA
30	**Advances in the treatment of glioblastoma multiforme: evaluation of liposomal drug-delivery systems to mitigate use-limiting toxicities of chemotherapy** Alexander Arau, Ilia Pidroutchniak, Robert Campbell; MCPHS University, Worcester, MA
31	**Mapping Glutathione Utilization in the Developing Zebrafish Embryo** Archit Rastogi1,2, Christopher W Clark2, Alicia R Timme-Laragy2 1. Molecular & Cellular Biology Graduate Program, University of Massachusetts, Amherst – 01003 2. Department of Environmental Health Sciences, University of Massachusetts, Amherst – 01003
32	**Dissolution Kinetics and Mitochondrial Toxicity of 2D Manganese Oxide Nanosheets in Fish Gill Cells** Cynthia L. Browning[1], Evan Gray[2], Allen Green[3], Kyle Gion[3], Robert Hurt[3], Agnes Kane[1]; [1]Pathology & Laboratory Medicine, Brown University, Providence, RI; [2]Department of Civil, Environmental and Construction Engineering, Texas Tech University, Lubbock, TX; [3]School of Engineering Brown University, Providence, RI
33	**Effect of Perfluoroalkyl Substances on the Development of Zebrafish Vasculature and Pericytes** April Rodd and Jessica Plavicki; Brown University
34	**Manganese transport and toxicity in CRISPR-Cas9 mediated SLC30A10 knockout Hep3B cells** Milan Prajapati, Courtney Mercadante, Heather Conboy, Tom Bartnikas Department of Pathology and Laboratory Medicine; Brown University, Providence, RI – 02912
35	**Testicular Toxicity of Sub-chronic Low-dose Methotrexate Exposure in Rat** Hui Li, Tim Nolan, Susan Hall, Enrica Bianchi, Caitlin Hopkins, Samantha Madnick, Angela Stermer, and Kim Boekelheide; Department of Pathology and Laboratory Medicine; Brown University, Providence, RI, 02912. USA
36	**mRNA fragments in rat sperm are biomarkers of testicular injury due to ethylene glycol monomethyl ether exposure** Angela R. Stermer, Lisa Bramer, Susan J. Hall, and Kim Boekelheide; Brown University, Department of Pathology and Laboratory Medicine, Providence, RI 02912

#	Title and Authors
37	**Lack of Multidrug Resistance–Associated Protein 4 (Mrp4) Does Not Alter Susceptibility towards Acetaminophen Toxicity** Ajay C Donepudi[1], Michael J. Goedken[2] and José E Manautou[1]**;** [1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT. [2]Research Pathology Services, Rutgers University, Newark, NJ. University of Connecticut
38	**Maternal Preconception Exposure to PFBS Alters Nutrition and Growth of Offspring** Kate Annunziato, Marjorie Marin, Alicia Timme-Laragy; University of Massachusetts Amherst
39	**Spermatozoal large RNA content as new non-invasive tool to assess male reproductive toxicity in a pre-clinical and clinical setting** E. Bianchi[1,2], M. Sigman[1,2], K. Hwang[1,2], J. M. Braun[3], S. J. Hall[2] and K. Boekelheide[2]; [1]Division of Urology; [2]Department of Pathology & Laboratory Medicine; [3]Department of Epidemiology, School of Public Health Brown University, Providence RI, USA
40	**Effects of selected nootropic supplements on zebrafish *(Danio rerio)* embryogenesis** Paul V. Kaplita, Chuljoong Oh, Luong T. Nguyen and Min J. Kim; MCPHS University

Undergraduate Abstracts

Abstract #1

Role of Nrf2a in modulating MEHP-induced hepatosteatosis following embryonic exposure in *Danio rerio*

Hadley Moreau,[1,2] Karilyn Sant[1], Larissa M. Williams[2], Alicia Timme-Laragy[1]

1. University of Massachusetts Amherst
2. Bates College

Di(2-ethylhexyl) phthalate is a plasticizer and ubiquitous human toxicant. Its bioactive metabolite, mono(2-ethylhexyl) phthalate (MEHP) has been linked to abnormal development, increased oxidative stress, and metabolic syndrome--namely obesity. Nrf2 is a transcription factor that regulates gene expression through Antioxidant Response Elements (AREs) in the promoters of genes and functions as an inducible regulator of oxidative stress. The objective of this study was to investigate the role of Nrf2a in juvenile adiposity following embryonic exposure to MEHP. Zebrafish (*Danio rerio*) wild type (wt) and mutant (m) embryos from a Nrf2a mutant line were exposed to 0 or 200 µg/L MEHP through immersion beginning at 6 hours post fertilization (hpf) and concluding at 120 hpf. At 120 hpf fish were placed in clean system water and maintained to 15 days post fertilization (dpf). At 15 dpf fish were imaged for length and stained with Oil Red O to visualize neural lipid depots. Wild type control fish were significantly longer than mutant control fish. Developmental MEHP exposure significantly increased hepatosteatosis in both wild-type and Nrf2a mutant fish, an effect that was exacerbated in mutant fish. Adipogenesis was moderately increased in fish exposed to MEHP, though this trend was not statistically significant. These data indicate developmental exposure to MEHP may increase risk for hepatosteatosis, and that impaired Nrf2a function may exacerbate this phenotype. This result is consistent with Nrf2's previously reported facilitation of hepatoprotection in adults. Our data suggest that toxicant-induced oxidative stress during embryonic development is a risk factor for hepatosteatosis later in life, and Nrf2 function is important for mitigating this stress and decreasing risk for hepatosteatosis.

Abstract #2

Density of fungal contaminants affects the LD_{50} of inhibitors of fungal growth in biodiesel.

Anne McGoldrick, Valerie Hines, Jaimie Chicoine, and Joshua P. Gray

U.S. Coast Guard Academy, New London, CT

Biodiesel fuel is a renewable fuel currently used as a main alternative to fossil fuels. This fuel is derived from vegetable oil or animal fats, and consists of mono-alkyl esters of long chain fatty acids. Biodiesel is highly susceptible to microbial growth that results in degradation of fuel and fouling. Furthermore, biodiesel is often used as a blend with ultra-low sulfur diesel fuel, which becomes more susceptible to biologically-mediated degradation. One approach to preventing fuel degradation and system fouling is the addition of biocides to inhibit the growth of fungus and bacteria; FQS 1.5 is one such commercially-available biocide used for hydrocarbon fuels and oils. The active ingredients of FQS 1.5 are 5-chloro-2-methyl-4-isothiazol-3-one and 2-methyl-4-isothiazol-3-one. However, even at the prescribed final concentration of 1:10,000, fungal growth can still occur, particularly when the contamination is already established or is present due to a previously-contaminated batch of biodiesel. A variety of fungi and bacteria are contaminants of biodiesel. Each of these may have their own susceptibility to biodiesel preservatives. In this study, we investigated two fungal species previously isolated from the growth of a biodiesel tank in Oklahoma: *Wickerhamomyces* and *Byssochlamys*. These species were then used to test ideal conditions and determine efficacy of traditional versus novel inhibitors. FQS 1.5 inhibited the growth of both species in a dose-dependent manner. However, in some cases growth was delayed rather than prevented altogether, sometimes as long as a few days, before ultimately growing to confluence. This suggests that the inhibitors were metabolized by the fungi, delaying their growth temporarily. To test this hypothesis, we inoculated different starting amounts of each fungi with a fixed concentration of inhibitor to determine if starting fungal density impacted the LD_{50} of the inhibitors. Interestingly, we found that LD_{50} was greatly dependent on starting fungal concentration, with higher concentrations of fungi able to reach confluence relatively quickly. This suggests that treating a contaminated biodiesel container would require that the fungal density be below a critical point, or that excess inhibitor be added to compensate for elevated fungal density. Future studies investigating potential biocides should consider their efficacy at different densities of fungal contamination.

Graduate Student Abstracts

Abstract #3

CYP1 P450 and EETs signaling regulate Iκβα degradation in TNFα-induced inflammation pathway in asthmatic airway smooth muscle cells

B. Taghizadeh[1], A. Gardner[1].

MCPHS University, MA

Rationale: Airway smooth muscle (ASM) haa an inflammatory phenotype. TNF-α regulates numerous proinflammatory genes in ASM via the NF-κB signaling pathway. Epoxyeicosatrienoic acids (EETs) have anti-inflammatory effects We have previously shown that CYP1 isoforms suppress TNFα signaling via an EETs-mediated mechanism. The hypothesis that CYP1 P450 and EETs signaling mediates Iκβα degradation in TNFα-induced inflammation pathway will be tested in human asthmatic ASM cells.
Methods: 1) Western Blot: Asthmatic and normal ASM cells were grown to 80-85% confluency and serum starved for 24h. Cells were tested for expression levels of IkBα and phosphorylated IkBα in the absence or presence of TNFα (10ng/ml; 1h-48 h). Cells were pretreated with 14,15-EETs (1 μM) or the 14,15 EETs antagonist, 14,15-Epoxyeicosa-5(Z)-enoic Acid (EEZ; 10.0μM), or the CYP1 inhibitor 2,3',4,5'-tetramethoxystilbene (TMS;1.0μM) and thereafter treated with TNF-α (10ng/ml; 1h-48 h). IkBα or phospho-IkBα, or ubiquitin antibodies were used. 2) Immunoprecipitation: IkBα or phospho-IkBα antibodies were added to cell lysates (200ug protein) and incubated overnight. Incubation with protein A agarose beads (4 h) followed. Eluted proteins were immunoblotted with ubiquitin antibody.
Results: Post TNFα stimulation IκBα expression and IκBα phosphorylation levels were significantly higher ($p< 0.05$-0.0001) in asthmatic ASM cells compared to normal. Acute exposure to TNFα demonstrated a rapid increase in the phosphorylation status of IkBα ($p< 0.05$-0.0001) which correlated with a rapid decline in IkBα levels (2 to 30 min). 14,15-EETs (1 μM) pretreatment significantly decreased TNF-α-induced IkBα levels and phosphorylation status of IkBα ($p<0.0001$). Antagonism of 14,15-EETs reversed the phosphorylation effect on IkBα ($p<0.01$). CYP 1 inhibition increased IkBα ($p<0.0001$) and phosphorylation levels of IkBα ($p<0.0001$) after acute exposure to TNFα. Pretreatment with EETs decreased ubiquitination compared to TNFα alone, whereas pretreatment with TMS or EETs and EEZ reversed the effect of EETs.
Conclusions: TNFα-mediated IkBα degradation is enhanced in asthmatic ASM. Inhibition of IkBα degradation by a CYP1-mediated EETs mechanism suggests an important strategy to hinder activation of the pro-inflammatory NF-κB pathway in asthmatic ASM.

Abstract #4

EGFR transactivation by Endothelin Receptor involves a Src activated pro-HBEGF cleavage in asthmatic airway smooth muscle (ASM) cells

Prajakta Ambegaokar[1], Alice Gardner[1]

[1]Department of Pharmaceutical Science, MCPHS University, Worcester, USA.

Asthma is a chronic airway disease characterized by inflammation of airway passage leading to reversible airflow obstruction in asthmatics. Airway remodeling in asthma causes several significant structural changes in airway and our focus is the phenomenon of airway smooth muscle (ASM) hyperplasia as this irreversible change is a risk for increased morbidity and mortality. Our study focuses on exploring the mechanisms and signaling pathways which could potentially involve increased ASM proliferation in asthmatic airway remodeling. Our study proposes that in asthmatic ASM a potential mitogen such as Endothelin-1 transactivates the Epidermal Growth factor receptor at Tyr 1068 which activates Mitogen activated Protein Kinase (MAPK) signaling cascade leading to ASM cell proliferation. The transactivation is mediated by activated Src, with subsequent activation of metalloproteinases (MMPs) enzymes which further cleave membrane-bound pro-HBEGF to HB-EGF, a soluble protein ligand that binds to the EGFR and phosphorylates it. The investigation of this proposed mechanism is carried out in primary human asthmatic airway smooth muscle cells using western blot and BrdU proliferation assay analysis. The results from Western blot confirmed ET-1 transactivates EGFR at Tyr1068 in asthmatic ASM. The antagonistic effect of BQ123 on Tyr 1068 phosphorylation suggested involvement of ET_A receptor in transactivation of EGFR at Tyr1068.Using the Src inhibitors along with ET_A receptor antagonist, we observed a significant inhibition on Tyr1068 phosphorylation suggesting involvement of Src in this mechanism. To investigate asthmatic ASM proliferation, we used BrdU assay and treated cells with MMP inhibitor (ONO-4817), Src inhibitors and Human EGF Neutralizing rabbit mAb and found a significant reduction in their proliferation potential. To conclude, ET-1 initiates a cross talk with EGFR which is regulated by Src and MMPs suggesting a triple-membrane-passing-signal mechanism operating in asthmatic ASM which may contribute to airway remodeling in asthmatic ASM.

Abstract #5

Development of an electrochemiluminescence-based assay to characterize pyroptosis-related proteins in plasma obtained from Parkinson's disease patients

Anderson, F. L[1], Andrew, A. S.[2], von Herrmann, K. M.[1], Bispo, F. P.[3], Young, A. L.[1], Lee, S. L.[2], Havrda, M. C.[1]

[1]Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756; [2]Department of Neurology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756; [3]Department of Biology, Saint Anselm College, Manchester, NH 03102

Parkinson's disease (PD) is a highly prevalent neurodegenerative disease affecting approximately 5 million people worldwide. Neuroinflammation is a widely recognized aspect of PD; however, the impact of inflammation on PD incidence and progression remains unclear. Exposure to environmental toxicants, including pesticides, heavy metals, and industrial solvents, has been implicated in PD risk, but the molecular basis by which toxicants impact PD progression is not completely characterized. Inflammasomes are pro-inflammatory intracellular protein complexes containing pattern recognition receptors capable of responding to sterile triggers by initiating inflammation and a subcategory of programmed cell death called pyroptosis. Our lab has previously shown that loss of *Nlrp3,* the pattern recognition component of the NLRP3 inflammasome, in mice mitigates the development of PD symptomology resulting from exposure to the pesticide and mitochondrial toxin rotenone. More recently we observed expression of NLRP3 in the mesencephalon in late-stage PD patients. Based on the recognition that inflammasomes are activated in PD and cytosolic proteins are released during the process of pyroptosis, our lab has developed electrochemiluminescence-based immunosorbent assays for the detection of pyroptotic proteins including NLR family pyrin domain containing 3 (NLRP3) and Gasdermin D (GSDMD). Utilizing this method, we have compared NLRP3 and GSDMD protein levels in human plasma samples collected from PD patients and aged-matched controls. The detection of the downstream NLRP3 inflammasome targets, including GSDMD and IL-18, suggest that we may be able to monitor the activity of inflammasomes and evaluate pyroptotic processes in plasma. To complement these biochemical studies we have collected surveys from PD patients and controls detailing potentially inflammatory lifestyle factors, occupational exposures, and medical history information to identify environmental risk factors associated with circulating inflammasome and pyroptosis-associated proteins. Our study will allow us to correlate levels of inflammasome and pyroptotic activity with environmental exposure data to elucidate their relationship to PD diagnosis.

Abstract #6

Role of the transcription factor Nfe2 and pro-oxidant exposure in inner ear development in zebrafish

Authors: Anna Bowsher[1], Melanie Mait[1], Larissa M. Williams[1]

1. Bates College, Biology Department, Lewiston ME 04966

Millions of people worldwide suffer from hearing loss. While several mechanisms have been associated this loss, the role of oxidative stress remains underexplored. Nfe2, a transcription factor in zebrafish and other vertebrates, is localized to the otic vesicle during development and has been shown to mediate oxidative stress. Therefore, it was hypothesized that the absence of Nfe2 would promote otolith deformities, especially upon exposure to pro-oxidant chemicals. This hypothesis was tested by transiently decreasing Nfe2 expression using a morpholino and subsequently measuring otolith and neuromast development. Nfe2 knockdown in the Brn3c transgenic line significantly decreased otolith distance at 24 and 72 hours post fertilization (hpf), increased otic vesical width at 24, 48, 72 and 96hpf, and increased vesicle length at 24, 72, and 96 hpf compared to control ($p<0.05$). However, there was no significant difference in the phenotypes of neuromast cells between wildtype and *nfe2* morphants. Previous studies have shown that the pro-oxidant tBOOH induces changes in wildtype neuromast morphology, but the role of Nfe2 in these changes is unknown. In the wildtype these changes were thought to be due to upregulation of the cilia and flagella associated protein *cfap70* in our *nfe2* knockout model. However, morpholino knockdown of *cfap70* in wildtype and *nfe2* knockout larvae did not produce significant differences in otolith distance, vesicle width or length. These results demonstrate a role for Nfe2 and oxidative stress in inner ear development, but future studies are needed to further elucidate the molecular basis of the response.

Abstract #7

Targeted gene expression assays reveal markedly different gene expression and lipid accumulation profiles for perfluoroalkyl acid (PFAA) mixtures compared to single PFAA treatment in cryopreserved human hepatocytes

Emily Marques[1], Marisa Pfohl[1], Wei Wei[1], Ogochukwu Amaeze[2] and Angela Slitt[1]

[1] Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI. [2] Department of Clinical Pharmacy & Biopharmacy, Faculty of Pharmacy, University of Lagos, Nigeria

Perfluoroalkyl acids (PFAAs) are a family of fully-fluorinated chemicals that are used in fire-fighting foams and as well as many heat-, stain- and water-resistant household and consumer items. These chemicals, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) cause liver steatosis in mouse, rat, and monkey, however it is unclear whether exposure causes steatosis in humans. The goal of this study is to evaluate gene expression and lipid accumulation outcomes in human hepatocytes with different PFAAs and PFAA mixtures based on well-water PFAA ratios in Cape Cod, MA. The overarching hypothesis was that PFAAs have the capacity to induce lipogenic gene expression changes, as well as lipid accumulation based on functional group or chain length and mixtures of PFAAs would have an additive effect. Cryostax 5-donor pool of cryopreserved human hepatocytes were cultured following manufacturer protocols and certified reagents. 24 hrs after plating, cells were treated with various PFAAs and PFAA mixtures at various concentrations (25 μM, 2.5 μM, and 0.25 μM) or 0.1% DMSO vehicle in media. After a 48 hr treatment, gene expression of lipogenic targets related to steatosis were determined using a custom QuantiGene 2.0 plex assay and analyzed using a Bio-Rad Bio-Plex-200 platform. After a 72 hr treatments, cells were stained with DAPI and Nile Red and fluorescence was quantified. Treatment with 12 different PFAAs (3 sulfonates and 9 carboxylates) for 48 hrs caused significant gene expression changes in 6 nuclear factors related to drug metabolism and lipid regulation, as well as induction in 20 drug metabolism, lipid, metabolism, cholesterol metabolism and lipid transport genes. Some PFAAs also induced hepatocyte lipid accumulation, with the shorter chain PFAAs (C4 sulfonate and C4-C7 carboxylates) inducing significant liver lipid accumulation. Hepatic lipid accumulation was not observed in treatments with longer chain PFAAs (C6-C8 sulfonates and C6-C12 carboxylates, except C10). Overall, mixtures of PFAA had remarkable different gene expression profiles and no hepatic lipid accumulation. Further studies will evaluate gene and lipid endpoints of hepatocytes from normal donors and donors with steatosis.

Abstract #8

An 'omics' approach to unraveling the paradoxical effect of diet on PFOS and PFNA induced non-alcoholic fatty liver disease (NAFLD)

Marisa Pfohl, Hang Ma, Maxine Aubustan, Emily Martell, Navindra Seeram, and Angela Slitt.
University of Rhode Island, Kingston, RI

Obesity, diabetes, and insulin resistance are all risk factors associated with the development of hepatic steatosis and non-alcoholic fatty liver disease (NAFLD). It is estimated that 20-30% of the population present with NAFLD in the United States alone. The role of environmental exposures as risk factors for fatty liver disease is not well known. Perfluorooctanesulfonic acid (PFOS) and perfluorononanoic acid (PFNA) are widespread environmental toxicants that persist in over 98% of the general population. The aim of this study was to evaluate whether PFOS or PFNA exposure in combination with a moderately high-fat diet, augmented hepatic lipid content and biomarkers associated with NAFLD. Six-week-old male C57BL/6 mice were fed either a 10% kCal low fat diet (LFD) or 45% kCal high fat diet (HFD), with or without 0.0003% PFOS or PFNA (LFD-PFOS, LFD-PFNA, HFD-PFOS, and HFD-PFNA, respectively) for twelve weeks. The HFD increased liver weight by about 30% and body weight by 50% compared to the LFD controls. Both HFD-PFOS and HFD-PFNA administration significantly increased liver and body weights when compared to the LFD group. PFNA induced significant liver weight increase over both the control groups, as well as PFOS-exposed mice. An untargeted genomic array was used to assess global PFOS/PFNA induced alterations in hepatic transcriptomic profiles. IPA software was used to explore and visualize the impacted lipid associated pathways. A targeted array was used to further explore and confirm the mechanism of PFOS/PFNA induced steatosis within a LFD or HFD diet. Protein level changes were explored using SWATH proteomics. The mechanisms of PFOS and PFNA were compared and the additional impact of diet on these mechanisms was assessed. Both PFOS and PFNA treatment resulted in significantly increased expression of fatty acid uptake genes cluster of differentiation 36 *(CD36)* and solute carrier family 27 member 1 (*Slc27a1)*, while they exerted opposing effects on fatty acid synthesis (*FAS*) expression. PFNA exposure resulted in more profound effects on gene and protein expression overall when compared to PFOS. Diet exerted an additional impact on the mechanisms and potency of PFOS and PFNA in the liver. The data suggests that PFOS and PFNA at an exposure relevant dose (0.0003%) may have an adverse effect on hepatic lipid accumulation when combined with a LFD. The paradoxical impact of diet on the mechanism and potency of PFOS and PFNA is described herein.

Abstract #9

Deficiency of Anti-Inflammatory Protein TNIP1 Has Divergent Effects on Wound Healing

R. Shamilov, and B. Aneskievich

Pharmaceutical Sciences, University of Connecticut, Storrs, CT

TNIP1 protein is a widely expressed, cytoplasmic inhibitor of inflammatory signaling initiated by membrane receptors for pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPS) coming from endogenous and/or environmental sources. TNIP1 deficiency occurs in inflammatory skin diseases such as lupus and psoriasis, possibly sensitizing cells to such stimuli. Recently, we published TNIP1 deficiency in keratinocytes sensitizes them to experimentally-defined PAMPs and DAMPs thus promoting hyper-responsive expression and secretion of inflammatory markers (e.g. IL-8, IL-6 and TNFα). Thus, TNIP1 deficiency with subsequent stimulation establishes intrinsic (gene expression) and extrinsic (cytokine milieu) changes which we predict may modulate global tissue events such as wound healing. In this study, we examined the wound healing parameters of cell survival, migration, and expression of other inflammation-related genes. Specifically, we used our cell culture model of TNIP1 deficient keratinocytes to investigate stimulating toll-like receptor 3 with the agonist poly (I:C), a representative dsRNA PAMP/DAMP. Our studies revealed increased expression of antimicrobial (e.g. S100a family) and wound healing (e.g. IL-8, CCN2) associated genes, suggesting potential benefit of increased inflammatory response from TNIP1 deficiency. Unexpectedly, poly(I:C) challenge of TNIP1 deficient cells restricted re-epithelialization and induced cell death as compared to TNIP1 deficiency alone. Intriguingly, we detected not only increased expression for genes associated with cell death and inflammasome activation (e.g. caspase 1, IL-1β) but strikingly also for A20, a protein that represses cell-death signaling downstream of membrane receptors. Despite this compensatory increase in A20 mRNA, we found evidence of a decrease in its protein activity. Thus, an underling mechanism for the hyper-responsive phenotype of TNIP1 deficient keratinocytes following DAMP/PAMP stimulation involves not only an increase in inflammatory gene expression but also likely includes deficiencies in adequate compensatory responses such A20 activity.

Abstract #10

Understanding the Role of the Manganese Transporter Slc30a10 in Developing Mice

Heather Conboy, Courtney J. Mercadante, Milankumar Prajapati, Miriam E. Dash, Carolina Herrera, Michael A. Pettiglio, Thomas B. Bartnikas

Brown University

Manganese (Mn) is an essential trace metal acquired through the diet. It is essential to regulate Mn levels as Mn can be toxic in excess. Mn excess leads to a Parkinson's like disorder which is often seen in industrial workers inhaling Mn-rich particles or fumes. Young individuals are believed to be particularly susceptible to the irreversible damages of Mn excess due to immature regulatory pathways of absorption and excretion. Therefore it is important to better understand early mechanisms of Mn metabolism to prevent Mn toxicity. In 2012, the first case of inherited Mn excess was identified. Patients exhibited systemic Mn excess, liver cirrhosis, a Parkinson's-like disorder, and polycythemia (increased red blood cell counts). Mutations were identified in SLC30A10, a protein previously thought to transport zinc. In this study we evaluate the potential role of SLC30A10 in Mn homeostasis during development. To address this, we generated a global Slc30a10-deficient (Slc30a10KO/KO) mouse line. Slc30a10KO/KO mice are smaller when compared to wildtype littermates, suggestive of early Mn toxicity. Metal analysis indicates postnatal day (PND) 14 Slc30a10KO/KO mice exhibit comparable Mn levels when compared to Slc30a10+/+ mice. However, by PND21 Slc30a10KO/KO mice exhibit a 17-fold increase in liver Mn levels and a 2-3-fold increase in intestinal and brain Mn levels. Interestingly Mn levels in Slc30a10KO/KO mice decrease in the liver by PND28, suggesting the development of other Mn excretion pathways. Slc30a10KO/KO mice also develop polycythemia between PND21 and PND28. 54Mn excretion studies also indicated PND21 Slc30a10KO/KO mice excrete 40% less Mn than wildtype mice. In order to better understand the role of Slc30a10 in development, we also created an Slc30a10GFP/GFP mouse line using CRISPR/Cas9 to determine the localization of Slc30a10. Fluorescent images from Slc30a10GFP/GFP mice indicated Slc30a10 expression along the apical membrane of hepatocytes and enterocytes. Overall, our Slc30a10KO/KO and Slc30a10GFP/GFP mouse line suggest Slc30a10 is essential for early maintenance of Mn homeostasis.

Abstract #11

Impact of Acetaminophen-Induce Liver Injury on Expression of Cytochrome P450

Yifan Bao, Pei Wang, Xueyan Shao, Xiaobo Zhong

University of Connecticut

Drug metabolizing cytochrome P450 enzymes (P450s) are responsible for metabolizing 60-70% of prescription drugs. Recent studies indicate the gene expression in livers can be changed in adult mice during liver injury. Since the majority of these enzymes are expressed in liver, it is important to understand the impact of pathology in livers, such as liver injury induced by acetaminophen, on the expression of Cytochrome P450 enzymes. The aim of this work is to illustrate the impact of liver injury induced by acetaminophen on Cytochrome P450 enzymes in the neonatal age and the adult age, and discuss the potential mechanism. We hypothesized that expression of Cytochrome P450s will be inhibited after liver injury in all these ages. The mice were treated with acetaminophen at dose 200 mg/kg, 300 mg/kg and 400 mg/kg in day 10 and day 60. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were measured as biomarkers for liver injury. Expression of cytochrome P450s, including cyp3a11, cyp2c29, cyp2b10, and cyp2e1 was quantified by RT-PCR. The result indicates the acetaminophen-induced liver injury repress the expression of cyp2c29, cyp2b10 in all three doses, but only represses cyp3a11 and cyp2e1 in 400 mg/kg dose in neonatal mice. In the adult mice, cyp3a11, cyp2c29, and cyp2e1 were found repressed at dose 200 mg/kg and 300 mg/kg, but cyp2b10 were found induced in three doses. In adult female mice, expression of cyp3a11 and cyp2c29 were inhibited at 300 mg/kg and 400 mg/kg dose, and Cyp2b10 were found induced in 400 mg/kg dose. These results indicate that liver injury induced by acetaminophen changes the expression of cytochrome P450s, and the pattern of alterations depend on gender and dose. Future study will focus on the molecular mechanisms of acetaminophen-induced liver injury in the regulation of P450s in different ages of mice.

Abstract #12

The role of mTOR complex 1 in the induction of long-lasting lipid metabolic reprogramming by flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE-47)

Joseph McGaunn[1], Alexander Suvorov[1]

[1]Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA, USA 01003-9303

An abnormal shift in the balance of lipids between the blood and liver can have significant negative effects on overall health. Increased liver uptake of fatty acids from blood results in non-alcoholic fatty liver disease (NAFLD). This is the most common form of liver disease among all age groups, with a 33-88% prevalence. Previous experiments showed that exposure of mouse models to the flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) results in an increase of triglycerides in the liver and a decrease in blood, and additionally showed increased activity of mTOR complex 1 (mTORC1). As a key regulator of metabolism, mTORC1 is a likely regulator of lipid exchange between blood and the liver. We hypothesize that effects of BDE-47 on expression of liver lipid metabolic genes are mTORC1-mediated. In this experiment, wildtype mouse models and models with a conditional hepatocyte-specific knockout of mTORC1 were exposed to vehicle or 1 mg/kg body weight DE-47 from postnatal day (pnd) 1 to pnd 21. Liver tissue was collected on pnd 75. mRNA libraries were prepared from total liver RNA and sequenced using Illumina NextSeq 500. Read alignment was done using TopHat 2 aligner. Aligned reads were assembled to novel transcripts with Cufflinks and differential expression was analyzed using Cuffdiff. Cluster and enrichment analyses were conducted via DAVID functional annotation bioinformatics microarray analysis and Gene Set enrichment analysis (GSEA). DAVID and GSEA results reveal that 1) mTORC1 knockout results in significant changes in hepatocyte gene expression, 2) perinatal exposure to BDE-47 induces long-lasting reprogramming of liver gene expression, and 3) mTORC1 knockout abolishes changes to expression of gene sets and specific lipid metabolic genes (*Acot 1*,*2*, and *3*, *Irs1* and *2*, *Cyp7a1*, *Per1*, *Pik3r1*, *Lpl*, *Fabp5*, and *Aacs*) induced by BDE-47 exposure in wildtype mice.

Abstract #13

Genetic Investigation of Long Non-coding RNAs in HepaRG cells Undergoing Differentiation

SooWan Lee, Bindu Prabhakar, and Theodore P. Rasmussen

Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269

Long non-coding RNAs (lncRNAs) are transcripts with a length of more than 200 nucleotides that do not encode proteins. lncRNAs have received wide attention as key regulators of stem cell proliferation and differentiation, but knowledge of the specific role of lncRNAs in hepatocyte differentiation is still limited. In a previous study, our lab group identified a novel lncRNA, lnc76 which maps to chromosome 11q23.3 in an apolipoprotein (APO) gene cluster. By performing RACE analysis, our group found that lnc76 consists of a 670 base pair polyadenylated lncRNA with two exons and an intron. In HepaRG cells, lnc76 was knocked-down with a doxycycline (dox) inducible lentiviral vector to examine the role of this lncRNA in hepatocyte differentiation. Dox was added 72 hours before harvest or at day 14 when switching to differentiation media. Interestingly, the mRNA expression of all APO genes including APOA1, APOC3, and APOA5 were decreased in the lnc76 knockdown cells and showed more significant down-regulation in dox (day14-33) groups. Moreover, the mRNA levels of cholangiocyte marker, cytokeratin 7 was increased, while the mRNA levels of the mature cholangiocyte marker, cytokeratin 19 (CK 19) was decreased which demonstrated that knockdown of lnc76 causes a failure to differentiate into hepatocyte lineage. In summary, this study explores the requirement for a novel lncRNA, lnc76 in HepaRG cell differentiation, and shows that it is required for the proper production of hepatocytes in these cells.

Abstract #14

Levels Of Methionine Sulfoxide Associated With An APAP Autoprotection Mouse Model Do Not Confer Cytoprotection Against APAP In Vitro In Hc04 And HepaRG Cell

S. J. Toro[1], A. C. Donepudi[1], K. H. Liu[2], D. P. Jones[2], and J. E. Manautou[1]

[1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT; and [2]Department of Medicine, School of Medicine, Emory University, Atlanta, GA.

Drug-induced Liver injury (DILI) is a major impediment in drug development. Acetaminophen (APAP)-induced liver injury is a classic model of DILI. Flavin containing monooxygenase 3 (FMO3) is an enzyme involved in metabolism of several xenobiotics, such as amphetamine and its analogs. In a rodent model of APAP autoprotection, dramatic increases in hepatic Fmo3 mRNA and protein expression have been observed. Fmo3 induction is seen in association with highly increased serum levels of methionine sulfoxide (MSO), an endogenous metabolite of this enzyme. In female mice, pharmacological inhibition of Fmo3 results in greater susceptibility to APAP-induced liver injury compared to males. Moreover, in vitro overexpression of Fmo3 confers partial, yet significant protection from APAP cytotoxicity. Although these studies are suggestive of a potential hepatoprotective role of Fmo3 in APAP hepatotoxicity, the molecular mechanism of this protective function is still unknown. In this study, we investigated the capacity of MSO to protect against APAP cytotoxicity using two immortalized hepatocyte cell lines, HepaRG and HCO4 cells. Additionally, we evaluated the role of MSO in the hepatic differentiation of HepaRG liver progenitor cells. Our results show that MSO treatment by itself at a wide range of concentrations does not produce any cytotoxicity. As expected, APAP treatment produced cytotoxicity in both cell lines in a dose- dependent manner, as evidenced by LDH release into media. However, MSO treatment (pre-as well as co treatment) did not afford any protection against APAP cytotoxicity in either cell line. Furthermore, addition of MSO to both growth and differentiation media did not alter the growth or differentiation rate of HepaRG in to hepatocyte-like cells. Hepatocyte differentiation status was determined by gene expression analysis of prototypical drug metabolizing enzymes such as CYP2E1 and 3A4. Overall, these data indicate that the dramatic increases in serum MSO levels observed in our mouse model of APAP autoprotection, does not appear to be the mechanism by which Fmo3 induction confers protection against APAP toxicity.

Abstract #15

Neonatal exposure to brominated flame retardant permanently alters liver lipid metabolism in mice via mTOR-dependent mechanism

Victoria Salemme & Alexander Suvorov

University of Massachusetts, Amherst

Tight regulation of fatty acids uptake by the liver is the major process that contributes to a healthy balance of lipids between blood and the liver. An increased uptake of fatty acids results in accumulation of triglycerides in hepatocytes and is referred to as hepatic steatosis or non-alcoholic fatty liver disease (NAFLD) - the most common form of chronic liver disease. NAFLD increases the risk of type 2 diabetes, dyslipidemia, hypertension, cardiovascular and kidney disease, liver cirrhosis, hepatocellular carcinoma, and mortality. Thus, improving our understanding of preventable causes of lipid imbalance may have significant consequences for public health. Experimental data from our laboratory demonstrates that exposure to 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), brominated flame retardant prevalent in human samples, during sensitive developmental windows, results in permanent changes in liver lipid metabolism. We hypothesized that these changes are mediated by the mTOR pathway – an intracellular pathway that regulates various aspects of the cell cycle, metabolism, and survival. To test this hypothesis, we generated 2 mouse models with conditional liver-specific knockout of either mTOR complex 1 (mTORC1) or mTOR complex 2 (mTORC2) using Cre/Flox system. Both models as well as wild type animals were exposed to 1 mg/kg body weight of BDE-47 dissolved in corn oil or to vehicle during the neonatal period of development. Triglyceride concentrations were analyzed in blood and liver of these animals at 75 days of age. Our data demonstrate that inactivation of mTORC2 abolishes BDE-47 induced changes in triglyceride concentrations in blood and liver. In experiment with mTORC1 inactivation no changes in triglyceride concentrations were observed following exposure in animals with different genetic backgrounds (both WT and KO) likely due to strain insensitivity BDE-47. Thus, our data on the role of mTORC1 in the mediation of the BDE-47 response is inconclusive. Our evidence suggests that the effect of BDE-47 on liver lipid metabolism is mTORC2 dependent.

Abstract #16

Modulation of Peroxisome Proliferator-Activated Receptors gamma (PPARγ) signaling perturbs embryonic pancreas development in the zebrafish, Danio Rerio

Olivia L. Venezia, Karilyn E. Sant, Alicia R. Timme-Laragy

University of Massachusetts, Amherst

Peroxisome proliferator-activated receptors (PPARs) are essential transcription factors for glucose and lipid homeostasis, as well as critical development processes such as adipocyte formation. Differentiation of preadipocytes into adipocytes is modulated by PPAR gamma (PPARg) - demonstrating the formation of ectopic adipocytes and abnormal birth weight when altered. Toxicants such as phthalates, butylparaben, and PFOS are reported to have affinity for PPARg, and have been previously shown to elicit adverse effects on the developing zebrafish (Danio rerio). This begs the question of how disruption of PPAR activity during development may alter fetal metabolic processes that are carried into adulthood, such as in the pancreas. Here we examine the effects of altered PPARg activity on the developing exocrine and endocrine pancreas in zebrafish embryos. Transgenic fish lines that express GFP in beta cells of the endocrine pancreas (Tg(ins:GFP)) or in ptf1a expressing cells of the exocrine pancreas (Tg(ptf1a:GFP)), were used to assess the impact of PPARg modulation on pancreas development in live zebrafish embryos. Beginning at 24 hours post fertilization, embryos were exposed daily to either Rosiglitazone (selective PPARg agonist) or T0070907 (selective PPARg antagonist) at concentrations of 0 (0.01% v/v DMSO), 0.1, 1, and 10 μM. At 96 hpf, embryos were imaged live with brightfield and fluorescent microscopy, and images were analyzed using morphometrics. Fish length and yolk sac utilization suggests mild developmental delays were associated with 10 μM T0070907 and Rosiglitazone exposures. T0070907 exposure correlates with a decrease in islet area but demonstrates a u-shaped dose curve response in exocrine pancreas length, while Rosiglitazone appears to only alter endocrine pancreas area in a non-dose dependent manner. However, Rosiglitazone results in an increased occurrence of endocrine pancreatic deformities as 21% of 10 μM exposed embryos display a ‘wispy’ or ‘curved’ pancreas, when compared to 0% of controls. In addition, a 7% increase in fragmentation of endocrine pancreas islets was observed in both 1uM and 10uM Rosiglitazone exposed embryos, compared to controls. These findings suggest that pancreas development is sensitive to modulation of PPARg, but it does not fully recapitulate the morphologies observed with previous toxicant studies. This work is being supported by grant funding from F32ES028085 and NIH R01ES025748.

Abstract #17

Nanomaterial Exposure-driven Fibroblast to Myofibroblast Differentiation Is Dependent on Substrate Stiffness

Alysha Simmons[1,3], Susan Leggett[1], Ian Wong[1,2], Agnes Kane[1,3]

[1]Department of Pathobiology, Brown University, [2]Department of Engineering, Brown University, [3]Department of Pathology and Laboratory Medicine, LMM, Brown University

The impact of the lung microenvironment's mechanical properties on fibrogenesis following exposure to engineered nanomaterials (ENMs) is currently underevaluated. Moreover, no *in vitro* screening tool exists to identify ENMs capable of initiating an aberrant fibrotic response in the human lung. We utilize physiologically-relevant, polyacrylamide (PA) substrates on which we model fibroblast exposure to ENMs *in vitro.* We hypothesize that exposure to high aspect ratio, stiff ENMs drives fibroblast to myofibroblast differentiation and that autocrine and paracrine TGF-β1 signaling is responsible for cell differentiation. This model uses human IMR90 cells (normal lung embryonic fibroblasts) exposed to particles/fibers with a variety of physical properties and geometries, including isometric carbon black particles (M120), crocidolite asbestos fibers, Mitsui multi-wall carbon nanotubes (MWCNT, Mitsui-7), and graphene. Fibroblast to myofibroblast differentiation is initiated by nuclear translocation of p-SMAD2/3, followed by upregulation of alpha-smooth muscle actin (α-SMA) expression, a myofibroblast marker. Activation of this pathway is dependent on nanomaterial geometry and stiffness, and the response is more robust on a physiological soft substrate (~2kP). Exposure to MWCNTs, asbestos, and graphene (5μm) induced increased α-SMA protein expression on soft PA gels after 48 hours. In addition, gene expression of α-SMA is upregulated in cells exposed to stiff, fibrous nanomaterials, as well as graphene (5μm). Future work will treat ENM-exposed cells with Pirfenidone, an antifibrotic receptor tyrosine kinase inhibitor that prevents paracrine and autocrine TGF-β1 signaling. We hypothesize that the TGF-β1 pathway is being activated by mechanical interactions with nanomaterials that drives differentiation, leading to development of fibroblastic foci in pulmonary fibrosis. This *in vitro* model can be used as a screening tool to evaluate the potential adverse human health impacts of other ENMs following inhalation and inform safe ENM design. This research is supported by the NIEHS Training Grant T32 ES07272, NIEHS Superfund Research Program P42 ES013660, NIEHS U01ES028184-02, and Unilever.

Abstract #18

2,3,7,8 tetrachlorodibenzo-[p]-dioxin exposure and genetic manipulation of the aryl hydrocarbon receptor disrupts forebrain development and axonal targeting

Nathan R. Martin1, Cheryl L. Dunham2, Robert L. Tanguay2, Jessica S. Plavicki1

Pathobiology Graduate Program, Brown University, Providence, RI1 Oregon State University, Corvallis, OR2

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor with important roles in the development of the nervous, immune, cardiovascular, and reproductive systems. Environmental contaminants such as 2,3,7,8 tetrachlorodibenzo-[p]-dioxin (TCDD, dioxin) activate the AHR and produce transcriptional changes that lead to developmental defects. Epidemiology and developmental toxicology studies have shown that AHR agonist exposure is associated with hyperactivity in humans and zebrafish. Our goal is to determine how AHR activation alters zebrafish brain development and function and, ultimately, leads to hyperactivity. We hypothesize that hyperactivity associated with AHR agonist exposure results from disrupted GABAergic interneuron development and function due to impaired expression of the dlx genes and calcium binding proteins. In the mammalian brain, the homeodomain transcription factors Dlx 1, 2, 5, and 6 play critical roles in the development of inhibitory GABAergic interneurons. Our preliminary data indicate that exposure to the potent AHR agonist TCDD results in the downregulation of dlx 1a, 5a, 6a and multiple calcium binding proteins (parvalbumin 1, 2, 3, and 4) in the developing zebrafish brain. We used fluorescent immunohistochemistry and confocal microscopy to examine morphological changes in the nervous system and found TCDD exposure reduced the size of the forebrain, a region rich with dlx+ GABAergic interneurons. To examine how TCDD exposure affects GABAergic interneuron development, we are exposing a dlx reporter transgenic line to TCDD (1 ppb or 50 ppt). In addition, we generated a UAS line with a fluorescently tagged constitutively-active AHR (caAHR) and used ubiquitous and differentiated neuron-specific Gal4 lines to drive caAHR expression. Neuron-specific AHR activation induced hyperactivity and ubiquitous caAHR activation altered gross brain morphology, axon targeting, and reduced forebrain size. Together, our studies describe novel neural phenotypes associated with TCDD exposure and cell-type specific genetic manipulation of AHR.

Abstract #19

Gene expression differences linked to resistance of endocrine therapy in African American and Caucasian patients with breast cancer

Sara Parvin-Nejad, Sana Cheema, George Acquaah-Mensah PhD

MCPHS University, Worcester, MA

Significant differences in survival and response to current breast cancer therapies exist between African American and Caucasian patients. This study seeks to analyze expression differences of gene sets between these two distinct populations. Using data from The Cancer Genome Atlas, differences in gene expression between patients with breast invasive carcinoma were assessed. The latest RNA-Seq version 2 data was downloaded and normalized in order to identify differentially expressed genes from African-American and Caucasian (non-Hispanic) patients. Of note, carcinogens such as 2, 3, 7, 8-tetrachlorodibenzodioxin (TCDD) and benzo(a)pyrene are known to act as ligands to the aryl hydrocarbon receptor (AHR). This receptor and its dimerization partner, the AHR nuclear receptor (ARNT) are both significantly suppressed among African Americans relative to Caucasians, therefore the next steps sought to probe this distinction further. In order to identify gene sets associated with AHR, the Molecular Signatures Database was searched using AHR as a search term. Subsequently, the data collected was analyzed using Gene Set Enrichment Analysis (GSEA), where the genes were ranked in order to determine phenotypic distinction. Initial analysis identified 688 genes which were differentially expressed between African American and Caucasian patients, with a false discovery rate (FDR) of 5%. The GSEA further revealed that gene sets associated with resistance to endocrine therapy are enriched in samples obtained from Caucasian patients, as compared to those from African Americans with stage 3 breast cancer. Pooled gene expression profiles of responders and non-responders to therapy with endocrine drugs (selective estrogen receptor modulators, selective estrogen receptor down-regulators, gonadotropin releasing hormone agonist, and aromatase inhibitors) were subsequently examined. Analyses of differentially expressed genes between responders and non-responders showed over-representations of pathways including those associated with co-regulators and kinases linked with ERBB2, IGF, VEGF, TGF-β, PI3K/AKT/mTOR, Wnt and oncogenic MAPK signaling. Between and within the races, there were no differentially mutated genes between responders and non-responders. These findings need to be further refined in the pursuit of factors predictive of the response to endocrine drug therapy in breast cancer. Understanding the genetic differences identified between African American and Caucasian patients can help guide patient-centered treatment and potentially avoid development of resistance. Further investigation is being conducted to elucidate patterns in patient response to endocrine therapy, as it relates to the regulation of gene expression.

Abstract #20

Correlation of anticancer activity of pelitinib – a tyrosine kinase inhibitor (TKI) – with the expression levels of epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC)

Recheal Kisubika, Donece Hill, Terrick Andey

MCPHS University

The epidermal growth factor receptor (EGFR) is frequently upregulated in NSCLC. EGFR inhibitors such as pelitinib may be an effective therapy for patients with EGFR advanced non-small cell lung cancer. In this study, we aim to explore how basal levels of EGFR affect pelitinib activity in NSCLC. Basal expression of EGFR protein was investigated in H1299 and H1573 cell lysates using western blot assay. Cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and probed with anti-EGFR primary antibody and anti-rabbit secondary antibody. H1299 and H1573 cell viability assays were performed in a 96-well plate format following treatment for 24, 48 and 72 h with sequential dilutions of pelitinib. Cells viability was measured using the resazurin dye-uptake assay by adding 0.01% of resazurin dye solution to each well of the 96-well plate and measuring the fluorescence of the resulting resorufin produced by live cells. The results were normalized against the corresponding controls and presented as graph of response (percent cell viability) versus concentration. The IC50 of pelitinib was determined using linear regression analysis. Results were obtained using three independent experiments each with six replicates per treatment group and were presented as mean ± SEM. Basal expression levels of EGFR protein were similar in H1299 and H1573 cells. In H1299, a concentration dependent decrease in cell viability with pelitinib and a time-dependent decreases in cell viability at 24, 48 and 72 h (IC50: 78 ± 9 μM, 65 ± 2 μM and 56 ± 2 μM respectively) were observed. In H1573, a concentration-dependent decrease in cell viability and a time-dependent inhibition of viability at 24 and 48 (IC50: 57 ± 5 μM and 46 ± 4 μM respectively) was observed. However, cell viability of H1573 at 72 h (IC50: 59 ± 1 μM) was similar to 24 h. H1573 cells were relatively more sensitive to pelitinib at 24 and 48 h than HI299 cells while both cell lines had similar effects at 72 h. Although similar EGFR expression levels were observed in both unstimulated cells, pelitinib evoked a more potent anticancer activity in H1573 compared H1299 at shorter durations of treatment (i.e. 24 and 48 h). Investigation of EGFR proteins in treated cells versus non-treated controls, and estimation of mutant EGFR fractions will clarify the relationship between pelitinib efficacy and EGFR expression.

Abstract #21

Anticancer Drug Repurposing: Efficacy study of etoposide non-small cell lung cancer (NSCLC)

Donece Hill, Recheal Kisubika, Terrick Andey.

MCPHS University

Current clinical trials are evaluating new therapies and the repurposing of FDA-approved medications for lung cancer treatment. Etoposide is a topoisomerase inhibitor currently approved for the treatment of small cell lung cancer (SCLC) and testicular cancer. It expresses its anticancer activity by inhibiting the cell cycle and therefore causing death of cancer cells. In this study, we explored the effect of dose and time on anticancer activity of etoposide in three non-small cell lung cancer (NSCLC) cells. Efficacy studies with dilutions of etoposide (1000 - 0.1μM) were carried out using the resazurin dye-uptake assay in a panel of NSCLC cells (i.e. H1299, H1573, and H1975) at 24, 48, and 72 h. The assay involved addition of 0.01% resazurin dye to treated cells in a 96-well plate format and reading fluorescence (530 nm excitation and 590 nm emission) after 2 h. The fluorescence intensity of the treatment group was normalized with the corresponding control and presented as concentration-response (cell viability) plots. The inhibitory concentration of etoposide at 50% (IC50), was determined by using linear regression analyses presented as a measure of anticancer potency of etoposide. All experiments were performed in six replicates at least three times and the results presented as mean ± SEM. Etoposide inhibited the viability of H1299, H1573, and H1975 cells in a concentration-dependent manner, with cell viability generally decreasing from 1000μM to 0.1μM. Etoposide also produced a time-dependent inhibition of H1299, H1573, and H1975 cells. In H1299 cells, the time-dependent inhibition was observed at 24, 48, and 72 hours with IC50 of 1526 ± 264 μM, 318 ± 73 μM, and 55 ± 2 μM respectively. Time-dependent inhibition of H1573 cells was also observed at 24, 48, and 72 hours with IC50 of 1022 ± 14 μM, 412 ± 24 μM, and 60 ± 4 μM respectively. In H1975 cells, time-dependent effects of etoposide were observed with IC50 of 2070 ± 115 μM and 591 ± 75 μM at 24 and 48 hours respectively, but not at 72 hours (734 ± 0.2 μM). Etoposide exhibited anticancer activity with an increasing potency observed with longer durations of treatment in all three NSCLC cells. H1299 and H1573 cells showed increased sensitivity to etoposide (96% and 94% respectively) over 72 hours compared to H1573 cells (64%). H1573 cells would benefit from a combination of etoposide with another anticancer agent to increase sensitivity. More studies are needed to understand the full potential of etoposide in treating NSCLC.

Abstract #22

Evaluating CD44 receptors in cancer: targeting and toxicity

Dotun Dennis Adegunle, Eugene Boakye Ansah, Danny Nguyen and Robert Campbell, PhD

MCPHS University, Department of Pharmaceutical Sciences, Worcester MA.

One of the most significant challenges of cancer treatment is severe drug toxicity often due to off-target effects of chemotherapeutic agents even when administered at therapeutic doses. Attempts have been made to improve drug targeting to tumors to reduce off-target effects, improve long-term treatment outcomes and reduce toxicity. One of those attempts have been to evaluate toxicities of targeting CD44, a surface membrane proteoglycan implicated in multidrug resistance and metastasis in various forms of cancer.

Methods: Electronic searches were conducted using databases such as EMBASE, Pubmed and clinicaltrials.gov. The search criteria were narrowed to include information regarding pre-clinical and clinical trials involving cluster of differentiation protein 44 (CD44), cancer and toxicity. These search terms were used based on their relevance to our team research study.

Results: In a clinical trial, Riechelmann et al. demonstrated that the adverse effects associated with anti-CD44v6 bivatuzumab mertansine included gastrointestinal, neurological, and dermal events. Tijink et al. used bivatuzumab to target CD44-positive incurable squamous cell carcinoma and had one drug-related fatality and various grades of skin reactions. For patients treated with hyaluronic acid-CD44 incorporated with irinotecan, Gibbs et al. showed that 8% of patients experienced grade III/IV diarrhea and 17% experienced neutropenia in Phase I. In another phase I clinical trial, Gomez-Roca et al. used RG7356 – humanized monoclonal antibody that binds to all CD44 isoforms and showed 2 cases of grade 2 headache, 1 case of grade 4 febrile neutropenia. Kesharwani et al showed the incorporation of hyaluronic acid (a natural ligand of CD44) conjugated to nanomicelles significantly increased targeting in vitro.

Conclusion: Targeting CD44 receptors is a promising approach to circumvent multidrug resistance. The use of nanoparticles has been explored as an approach to increase specific targeting of CD44 expressed by tumors. Future directions include the development of liposomal formulations with therapeutic chemical and biological agents directed against CD44 to improve drug targeting while reducing toxicity.

Abstract #23

Phytotoxic effects of NSAIDs on *Pisum sativum* lead to animal alternative drug assay

Crystal H Ng, Tyler M Holmes, Matthew D Metcalf

MCPHS University School of Pharmacy Worcester

Animal drug assays are the gold standard proof of clinical safety and efficacy prior to human trials. However, ethical concerns surround animal use, and IACUC protocols state attempts should be made to develop non-animal based assays. To that end herein is presented the development of an alternative assay using plants for the acetic acid antiwrithing assay. NSAIDS, a drug class developed from the plant hormone class - auxins, are hypothesized to act as auxins in plants. Based on this auxin/NSAID hypothesis, an assay was developed to detect the phytotoxic effects of NSAIDs in *Pisum sativum*, creating an alternative NSAID drug assay. *Pisum sativum* seeds were germinated in distilled water, sprouted for 72 hours in sphagnum moss, and initial growth of root and shoot length were measured. The seedlings were then exposed to varying concentrations of drug in a liquid growth solution for 72 hours and growth characteristics were re-measured. Dose response curves were generated using the percent change in growth. NSAIDs revealed phytotoxicological effects on root growth in a dose dependent manner in *Pisum sativum* sprouts. Effects were similar to auxin plant hormones, indole-3-butyric acid (IBA) and 2-napyhtlacetic acid (2NA), but significantly different in gibberellic acid (GA3), and acetaminophen (APAP). For root growth, IBA had an LD50 of 1.7μM (95%CI 1.1-2.8, $p<0.0001$) and 2NA had an LD50 of 2.1μM (1.5-2.9, $p<0.0001$). In NSAIDs for root growth, naproxen had an LD50 of 23.1μM (16.6-32.2, $p<0.0001$), diclofenac had an LD50 of 36.5μM (24.7-53.8, $p<0.0001$), and acetylsalicylic acid (ASA) had an LD50 of 7.8μM (4.9-12.8, $p<0.0001$). For shoot growth, IAA, 2NA, naproxen, diclofenac, and ASA, inhibited growth ($p<0.0001$); GA3 enhanced growth ($p=0.0002$); and APAP had no effect on growth ($p>0.90$). In root and shoot growth, the NSAIDs produced similar effects to the auxins and dissimilar effects to GA3 and APAP. This data supports the hypothesis of auxin-like phytotoxic effects on *Pisum sativum* growth characteristics and the assay can serve as an animal replacement assay.

Abstract #24

Study of long-noncoding RNA in cytochrome P450 expression regulation and drug induced liver injury

Liming Chen, Stephanie Piekos, and Xiao-bo Zhong

University of Connecticut

Cytochrome P450 (P450) enzymes are responsible for biotransformation of xenobiotics, including environmental toxicants and drugs. Expression of P450s can directly affect drug metabolism, resulting in various outcome in either therapeutic efficacy or adverse effects. Nuclear receptors are a class of transcription factors that can regulate expression of P450s at both basal and drug-induced levels. Some long non-coding RNAs (lncRNAs) near a transcription factor are found to participate in the regulatory functions of the transcription factors in either promotion or repression manner. The aim of this study is to determine whether lncRNAs are involved in transcription regulation of P450 enzymes through transcription factors and downstream drug toxicity. Hepatocyte nuclear factor 1α (HNF1α), hepatocyte nuclear factor 4α (HNF4α), and two lncRNAs, HNF1α-antisense 1 (HNF1α-AS1) and HNF4α-antisense 1 (HNF4α-AS1) were examined in the current research. Small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) were applied to knock down the transcription factors and lncRNAs in HepaRG cells. Expression of the transcription factors, lncRNAs, and P450s was measured by RT-PCR and Western blots. Knocking down of HNF1α or HNF4α affected basal expression of numerous P450s as well as other transcription factors. Moreover, HNF4α knockdown was able to decrease HNF1α expression and showed more potent effect than HNF1α knockdown. Knocking down of HNF1α-AS1 showed a similar regulation trend on the P450s compared to HNF1α or HNF4α knockdown, indicating involvement of this lncRNA in the regulation of P450s. However, knocking down of HNF4α-AS1 showed an opposite regulation trend compared to HNF1α or HNF4α knockdown, indicating that HNF4α-AS1 may play an inhibitory role in the HNF4α function. Further, knockdown of HNF1α-AS1 and HNF4α-AS1 also lead to altered cell viability after acetaminophen treatment. Altogether, out study showed that lncRNAs are involved in P450 expression regulation and affected drug toxicity in HepaRG cells.

Abstract #25

Limited Developmental Neurotoxicity from Neonatal Exposure to Ultrafine Carbon Particles or Diesel Particulate Matter

Keith Morris-Schaffer, Alyssa Merrill, Katherine Conrad, and Deborah Cory-Slechta

University of Rochester Medical Center

Epidemiological studies have shown exposure to anthropogenic fine particulate matter is associated with adverse neurodevelopmental outcomes in children. Complementary studies using rodent models have shown that developmental exposure to ambient nanoscale particulate matter can lead to sex-specific neurotoxicity and learning deficits. However it is still unclear about the direct sources and particulate matter constituents that contribute to these deleterious outcomes. To better evaluate potential particulate matter contributors to developmental neurotoxicity, two studies were conducted to assess the potential developmental effects of pure ultrafine carbon particles (UFCP: median aerodynamic diameter: <40 nm) and nanoscale diesel particles (median aerodynamic diameter: <100 nm) on brain pathology and behavior in C57Bl/6 mice. The UFCP aerosol was generated from a spark-discharge setup and the diesel particles were generated from ultrasonic nebulization of dissolved National Institute of Standards and Technology Standard Reference Material 1650b (SRM 1650b). Separate inhalation exposure of each material with neonatal mice occurred on postnatal days 4-7 and 10-13 for 4hr/day, 4 days/week at a mass concentration of 50 µg/m3 for UFCP and 100 µg/m3 for SRM 1650b. Assessments of central nervous system pathology 24 hours following exposure showed no gross inflammation or injury following pure ultrafine carbon exposure, while SRM 1650b did increase glial fibrillary acidic protein (GFAP) immunodensity in the corpus callosum and cortex, suggestive of inflammation. To assess learning deficits, behavior on a fixed-interval schedule of reinforcement, a paradigm that involves temporal learning and is historically effective at detecting the protracted effects of low-dose neurotoxicants such as lead, was utilized in exposed adult . No significant treatment-related learning differences were found in the adult mice. The lack of extended effect from the developmental particulate matter exposures, even at relatively high mass concentrations, suggests neither ultrafine elemental carbon nor diesel particle exposure alone are sufficient contributors to adverse developmental neurotoxicity. Further research on more reactive constituents of particulate matter including volatile organic species, reactive metals, and gases need to be done to better clarify specific toxic contributors.

Abstract #26

Slc30a10-deficient mice, a model of inherited manganese excess, develop hepcidin deficiency and increased manganese absorption

Courtney J. Mercadante, Milankumar Prajapati, Heather Conboy, Miriam E. Dash, Carolina Herrera, Michael A. Pettiglio, Thomas B. Bartnikas

Brown University

Manganese (Mn) is an essential metal and nutrient yet toxic in excess. There are several populations at risk of Mn toxicity, most notably miners and welders exposed to Mn-rich fumes and particulates. In 2012, the first known inherited disease of Mn excess was reported in patients with mutations in SLC30A10, a Mn efflux transporter highly expressed in the liver, brain, and duodenum and hypothesized to be essential for Mn excretion. Characterized by increased blood Mn levels, dystonia, polycythemia (increased red blood cell counts), and liver cirrhosis, SLC30A10 deficiency is a novel disease that offers a unique opportunity to investigate systemic Mn regulation. Our overall goal is to establish the role of SLC30A10 in mammalian Mn homeostasis by characterizing global and tissue-specific mouse models of Slc30a10 deficiency (KO). Since Slc30a10 is a Mn efflux transporter highly expressed in the liver, it is hypothesized that tissue Mn excess is a result of impaired hepatobiliary Mn excretion. Data from both global and hepatocyte-specific KO mice support this. However, global KO mice also have aberrant Mn absorption, which is likely to exacerbate tissue Mn overload and disease severity. We hypothesize that excess liver Mn acts as a “hypoxia mimetic” to induce HIF signaling and downstream targets such as erythropoietin (Epo) and hepcidin. Epo regulates the production of red blood cells and is aberrantly expressed in the liver of our global KO mice, likely causing polycythemia. Elevated Epo levels have been associated with hepcidin deficiency. Hepcidin is a hormone produced by the liver that inhibits dietary metal absorption. Our global KO mice are hepcidin-deficient and both iron and manganese absorption are increased following gavage. Absorption rates may also be directly influenced by Slc30a10 expression in the gut. Characterization of an intestinal-specific KO mouse reveals minimal tissue Mn excess, however deficiency in both hepatocyte and intestinal Slc30a10 has a synergistic effect in mice, suggesting that both tissues contribute greatly to Mn homeostasis. Understanding these mechanisms of Mn homeostasis is important for developing pharmacological treatment for both inherited and acquired Mn toxicity.

Abstract #27

The emerging contaminant 3,3' dichlorobiphenyl (PCB-11) impedes Ahr activation and Cyp1a activity to modify embryotoxicity of Ahr ligands in the zebrafish embryo model (*Danio rerio*)

Monika A. Roy, Karilyn E. Sant, Olivia L. Venezia, Alicia R. Timme-Laragy

University of Massachusetts, Amherst

3,3'-Dichlorobiphenyl (PCB-11) is a non-legacy PCB congener widely detected in environmental samples and has been detected in human serum, but its toxicity potential is poorly understood. We assessed its embryotoxicity and interactions with the aryl hydrocarbon receptor (Ahr) pathway in developing zebrafish (*Danio rerio*). Zebrafish embryos were exposed to 45 µg/L, 450 µg/L, or 4,500 µg/L PCB-11 from 24-96 hours post fertilization (hpf), when they were assessed for gross morphology and Cyp1a activity using the *in vivo* EROD bioassay. Ahr pathway interactions were probed by co-exposing zebrafish to the Ahr agonists PCB-126 and the model PAH beta-naphthoflavone (BNF). Liver development was assessed using the *Tg(gut:GFP)* zebrafish line. Zebrafish exposed to 4,500 µg/L PCB-11 were also collected at 96 hpf for qRT-PCR, RNAseq, and histology. Zebrafish exposure to PCB-11 alone mildly affected EROD activity but did not affect gross morphology. However, 4,500 µg/L PCB-11 alone altered the transcription of xenobiotic metabolism and liver development genes, impeded liver development, and increased vacuole formation. In co-exposures, 4,500 µg/L PCB-11 prevented deformities caused by PCB-126 but exacerbated deformities in co-exposures with BNF. The 4,500 µg/L PCB-11 concentration tested in zebrafish can affect liver development, act as both a partial agonist/antagonist of the Ahr pathway, and act as an antagonist of Cyp1a activity to modify the toxicity of compounds that interact with the Ahr pathway.

Abstract #28

Persistent alteration of hepatic DMEs expression following neonatal activation of PXR

Pei Wang[1], Guming Liu[1], Xiaobo Zhong[2], Lirong Zhang[1]
[1]Department of Pharmacology, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, China; [2]Department of Pharmaceutical Sciences, School of Pharmacy

University of Connecticut, CT 06269, USA

Pregnane X receptor (PXR), which can be activated by xenobiotic chemicals including paediatric drugs, plays a key role in the regulation of drug metabolism enzymes (DMEs). The induction of DMEs due to the activation of PXR may reduce therapeutic efficacy or cause toxicity. The goal of this work was to demonstrate the impacts of neonatal PXR agonists exposure on DMEs expression in adulthood. PCN (pregnenolone-16a-carbonit, Toronto Research Chemicals Inc., Cat# P712240) was used as a PXR agonist. Dose of PCN (0, 50, 100, 150, 200 $mg \cdot kg^{-1} \cdot day^{-1}$, constitutive 4 days) and age of treatment (day 5, 10, 15, 25) were studied. All mice were sacrificed at day 60 after birth and liver samples were collected for detecting the expression of PXR target genes. Compared with vehicle group, the significant inductions of CYP2B10, CYP3A11 and PXR were observed in high dose groups (150, 200 $mg \cdot kg^{-1} \cdot day^{-1}$, 5-8 days after birth) both in male and female mice (n = 4-9/group, $P < 0.05$). Furthermore, high dose groups (200 $mg \cdot kg^{-1} \cdot day^{-1}$, 5-8 days after birth) were found to have higher mRNA expression levels of CYP2A4, UGT1A1, ABCC4, and OATPLA4 in female mice, while PAPSS2 in male mice (n = 4-9/group, $P < 0.05$). Interestingly, a decreased mRNA expression of SULT2A1was identified in 200(5-8d) groups (n = 4-9/group, $P < 0.05$). Consistent with these results, the protein expression of CYP3A11 was only increased in 200(5-8d) groups compared with the vehicle groups (n = 3/group, $P < 0.05$). Importantly, the persistent impacts on DMEs only occurred in day 5 and day 25 treatment groups, not day 10 and day 15 groups (n = 4/group). Taken together, our data suggest that the persistent alterations of hepatic DMEs following neonatal PXR activation are dependent on the drug dose and treatment exposure time at early age.

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Corresponding author: Lirong Zhang, E-mail: zhanglirongzzu@126.com, Phone: +8637167781855.

Abstract #29

Toxicity reducing and mediating strategies for drug-resistant ovarian cancer: Investigating conventional drug delivery and experimental cancer research approaches

Clement, Jenel; Emerson, Josh; Amini, Negar; Campbell, Robert PhD

MCPHS, Worcester, MA

Toxic effects of chemotherapeutic agents present many challenges to the treatment of ovarian cancer, such as multidrug resistance and side effects. Non-specific delivery of chemotherapeutic agents results in high amounts of cellular uptake in healthy cells. We provide an overview of nanovesicles and other strategies used to minimize toxicity while maximizing drug uptake by ovarian tumors. Methods: A primary and secondary literature search was conducted for toxicity and multidrug resistance in treatment of ovarian cancer. Databases PubMed, Ovid, Scopus, and ClinicalTrials.gov were queried. Sources used include research published within the last 15 years. Our student-faculty research team has been actively involved in the early development of nanoliposome formulations to improve drug delivery. Results: Our review revealed a wealth of exciting areas of cancer research which aim to increase selectivity for nefarious cells and curb toxicity in healthy tissue, such as a drug delivery systems consisting of cell lipid extracts derived from tumor cells to enhance selective targeting regardless of tumor type. The addition of the chemosensitizing agent, curcumin, inhibited p-glycoprotein expression and increased tumor cell toxicity in A2780 adriamycin-resistant ovarian cancer cells in vivo. Additional studies provide evidence for magnetic nanoparticles combined with external magnet application. Other studies provide support for tumor sonication (at 28 kiloHertz) as a way to increase ovarian tumor drug uptake. The approach promoted site-specific drug release at the tumor site in vivo. Conclusions: The use of alginate nano-droplets or cell lipid extract liposomes has been shown to bypass tumor defenses, while simultaneously lessening systemic toxicity. Augmenting therapy with physical methods like sonication and magnetic drug targeting show increases in site selectivity, promote drug release, and disrupt physical barriers. Treating multidrug-resistant ovarian cancer will require a multi-pronged approach including drugs administered in combination, targeted delivery strategies, and novel mechanical technologies.

Abstract #30

Advances in the treatment of glioblastoma multiforme: evaluation of liposomal drug-delivery systems to mitigate use-limiting toxicities of chemotherapy

Alexander Arau, Ilia Pidroutchniak, Robert Campbell

MCPHS University, Worcester, MA

Purpose: Glioblastoma multiforme (GBM) is the most prevalent malignancy of the central nervous system. Challenge in developing effective chemotherapeutic agents is due to mechanisms of drug resistance combined with the inability to adequately penetrate the blood-brain barrier. As a result, high-dose chemotherapy is required to reach cytotoxic levels within tumor cells, but is opposed by dose-dependent toxicities which limit use of many chemotherapeutic agents in the treatment of GBM. Drug incorporation into nanoliposomes is a novel approach used to maintain cytotoxic accumulation of drug within tumor cells, while mitigating the use-limiting sequelae possessed by many traditional chemotherapies. The purpose of this evaluation is to assess safety and efficacy of liposomal delivery systems compared to current standard of care with temozolomide (TMZ) alone in treatment of GBM.
Methods: Following a review of scientific literature from tertiary databases, clinical and preclinical studies were identified and evaluated on their pertinence to the current management of GBM, relevance to overcoming current treatment barriers with TMZ, and which included both a safety and efficacy component in their treatment outcome analysis.
Results: Sang-Soo et al., evaluated TMZ-loaded liposomes composed of anti-transferrin antibody fragments (scL-TMZ) in mice. The scL-TMZ formulation delayed tumor growth in mice in addition to decreasing off-target toxic effects as compared to free-TMZ. Kato et al., and Tsujiuchi et al., co-administered TMZ with liposomal MGMT-siRNA, an inhibitor of the primary mechanism of TMZ resistance. The combination of combination improved antitumor activity in mice while maintaining a similar toxicity profile when compared to TMZ monotherapy.
Conclusion: To overcome use-limiting toxicities conferred by the low selectivity of freely circulating agents, the use of drug-delivery nanosystems represent a promising approach for improving selective targeting and accumulation of cytotoxic drugs to solid tumors. Subsequent clinical investigations are needed to evaluate experimental nanosystems to improve the current management of GBM.

Abstract #31

Mapping Glutathione Utilization in the Developing Zebrafish Embryo

Archit Rastogi[1,2], Christopher W Clark[2], Alicia R Timme-Laragy[2] [1]. Molecular & Cellular Biology Graduate Program

University of Massachusetts, Amherst – 01003 2. Department of Environmental Health Sciences, University of Massachusetts, Amherst – 01003

Glutathione (GSH), the most abundant endogenous redox buffer in vertebrates, plays key roles in organogenesis and embryonic development. Toxic insults during development are often neutralized by glutathionylation and excretion. However, scant information exists regarding organ-specific GSH utilization during development. Monochlorobimane (MCB), a dye conjugated with GSH by glutathione-s-transferase (GST) to form a fluorescent adduct, was used to visualize organ-specific GSH utilization in live developing zebrafish (Danio rerio) embryos at timepoints used in the OECD fish embryo acute toxicity test. Zebrafish embryos were incubated in 20 μM MCB for 1 hour and imaged on an epifluorescence microscope under the DAPI channel. All images were background corrected; unstained controls for every treatment were used to correct autofluorescence. At 24 hours post-fertilization (hpf), GSH conjugation with MCB was highest in the heart, brain and yolk, increasing 10 fold in the gut and otolith at 48 hpf. By 72 hpf, MCB staining was 18-fold higher in the heart and gut, and at 96hpf it was brightest in the gut, liver and gill arches. The sensitivity and specificity of MCB staining was tested with known GSH modulators. A 10-minute treatment at 48 hpf with 750 μM tert-butyl hydroperoxide, caused organ-specific reductions in MCB staining, with the heart & brain losing 30% fluorescence, and, the gut & myotome losing 50% (n=32). A 24 hour treatment from 24-48 hpf with the GSH precursor N-Acetylcysteine (NAC) resulted in significantly increased fluorescence, with the brain showing a 640% increase and the heart showing a 350% increase (n=31), these increases were abolished upon co-treatment with buthionine sulfoximine (n=28), an inhibitor of the enzyme that utilizes NAC to synthesize GSH. Ethacrynic acid, a highly specific GST inhibitor caused a timedependent decrease in fluorescence; a 60 minute 100 μM treatment caused a 20% reduction (n=23) and a 48h 100 μM treatment caused a 40% reduction (n=21). MCB staining was then applied to test for GSH disruptions caused by the environmental toxicant PFOS; a 32 μM treatment from 3 hpf to 48 hpf caused a 40% reduction in yolk fluorescence, but the other structures remained largely unaffected (n=18). MCB staining is a robust, sensitive method for detection of spatio-temporal changes in GSH utilization in the zebrafish embryo, and, can be applied to identify sensitive target tissues of toxicants. This work was supported by R01ES025748.

Postdoc Abstracts

Abstract #32

Dissolution Kinetics and Mitochondrial Toxicity of 2D Manganese Oxide Nanosheets in Fish Gill Cells.

Cynthia L. Browning[1], Evan Gray[2], Allen Green[3], Kyle Gion[3], Robert Hurt[3], Agnes Kane[1]
[1]Pathology & Laboratory Medicine, Brown University, Providence, RI; [2]Department of Civil, Environmental and Construction Engineering, Texas Tech University, Lubbock, TX; [3]School of Engineering

Brown University, Providence, RI

The development and production of engineered metal oxide nanomaterials continues to increase exponentially, increasing the risk of their release into the aquatic environment. The practice of “safety by design” aims to reduce the toxicological impact of engineered nanomaterials by assessing their toxicity during development. Here, we employed this approach utilizing an *in vitro* fish gill cell line model to represent a target tissue of freshwater fish likely to be exposed to nanomaterials in the aquatic environment. Using this cell line, we investigated the toxicity of novel 2D manganese oxide (MnO_2) nanosheets under development for use as catalysts and in supercapacitors or batteries. Acellular filtration studies showed MnO_2 nanosheets undergo reductive dissolution in the presence of biological reducing agents such as glutathione. In fish gill cells, MnO_2 nanosheets decreased glutathione levels. We then compared the cytotoxicity of the MnO_2 nanosheets to that of soluble $MnCl_2$ and insoluble MnO_2 particles. Cytotoxicity of Mn compounds increased with solubility (soluble Mn > MnO_2 nanosheets > MnO_2 particles). Since manganese is known to induce toxicity by dysregulating mitochondria, we investigated the effect of the 2D nanosheets on mitochondrial morphology and membrane potential. Our results show that mitochondrial morphology and membrane potential are disrupted by MnO_2 nanosheets at sub-cytotoxic concentrations. Together, these data suggest MnO_2 nanosheets release Mn ions through reductive dissolution, inducing both cytotoxicity and sub-cytotoxic effects on the mitochondria. Finally, we utilize this 2D nanomaterial as an example in our proposed screening strategy whereby physical characterization and the stability of 2D nanomaterials can be used to streamline the toxicity testing process. This research is supported by NIEHS Superfund Research Program P42 ES013660 and NIH F32 Training Grant ES00727225.

Abstract #33

Effect of Perfluoroalkyl Substances on the Development of Zebrafish Vasculature and Pericytes

April Rodd and Jessica Plavicki

Brown University

During early development, small perturbations can alter the formation and function of tissues to cause adverse effects that last well into adulthood. Environmental contaminants are a potential source for these developmental disruptions, and understanding a toxicant's impact during early life exposure is critical to elucidating its impact in exposed communities. Per- and poly-fluoroalkyl substances (PFAS), found at high concentration in certain firefighting foams and contaminated groundwater, have recently received increased attention as a widespread chemical of emerging concern. Previous work *in vitro* shows the toxicity of PFAS towards endothelial cells, but much remains unknown on how these chemicals affect vascular development. We are using zebrafish as a model organism to assess how PFAS alters the development and function of the vasculature, particularly in the brain. Using transgenic tools, we followed the movement of endothelial cells and pericytes during the first week of development to measure changes in structure, developmental timing, and function. These two cell types are critical to the function and maturation of the blood brain barrier and disruption of their health or development could have important implications for brain development and behavior. Early results with perfluorooctanesulfonic acid (PFOS) show limited morphological changes in the growth and development of the vasculature, but significantly fewer pericytes were seen in the brain compared to vehicle-treated controls after 4 days of exposure to high PFOS concentrations. Future work will investigate the functional impact of this pericyte depletion, compare the peripheral changes to those in the neurovasculature, and determine the critical exposure windows and concentrations to cause this adverse effect.

Abstract #34

Manganese transport and toxicity in CRISPR-Cas9 mediated SLC30A10 knockout Hep3B cells

Milan Prajapati, Courtney Mercadante, Heather Conboy, Tom Bartnikas
Department of Pathology and Laboratory Medicine

Brown University, Providence, RI – 02912.

Manganese (Mn), an essential metal, can be toxic at elevated levels. Mn toxicity can arise from occupational/environmental exposure, parenteral nutrition, hepatic conditions and certain genetic risks. Earlier this decade, such genetic risk was reported in the patients due to mutations in SLC30A10, a Mn efflux transporter, leading to aberrant systemic Mn levels. Recent studies of genetically altered mouse model offered a better understanding of its importance in Mn homeostasis. To explore the function of SLC30A10 *in-vitro*, the current study employs a SLC30A10 knockout Hep3B cell line (KO) designed using CRISPR-Cas9 gene editing tool. The mutations (in-del) leading to loss-of-function were confirmed by Sanger sequencing of target DNA. Nucleotide sequences showed a 5-BPs deletion and a T insertion in exon 1 of SLC30A10 gene confirming the viable clone as a compound heterozygous (biallelic). qPCR analyses showed ~70% reduction in *SLC30A10* expression of the engineered cells without any evident alteration in morphology or cell-growth pattern. Intracellular Mn concentrations measured using atomic absorption spectroscopy (AAS) indicated 1.7-fold and 3.6-fold increases in KO cells (118 ±36 and 293 ±71 µg Mn/g protein) compared to WT cells (69 ±14 and 81 ±11 µg Mn/g protein) upon 24-hour exposure to 0.5 and 1 mM $MnCl_2$ respectively. Cytotoxicity studies carried out using a series of concentrations of $MnCl_2$ indicated the KO cells were susceptible to a short (24 hours) and a long-term (7 days) $MnCl_2$ exposure when compared to wild-types. Such a difference may be attributed by intracellular Mn accumulation measured using AAS. Results from cytotoxicity studies carried out using series of concentrations of $ZnSO_4$ and $CuCl_2$ were largely similar when compared to wild-type cells, suggesting the KO cells are susceptible to Mn toxicity, not to Zn and Cu. Transport studies carried out using radio-labeled ^{54}Mn indicated a statistically significant reduction of ^{54}Mn import and export by KO cells when compared to wild-types. While the impaired ^{54}Mn export was hypothesized, the impaired ^{54}Mn import in these KO cells is currently under investigation. Such impaired import may result from suppressor mutation/s leading to synthetic rescue, off-target effects, *in-vitro* variation in localization of SLC30A10 due to cell polarity. Results from these experiments provide insights into the functional role of SLC30A10 *in-vitro*.

Abstract #35

Testicular Toxicity of Sub-chronic Low-dose Methotrexate Exposure in Rat

Hui Li, Tim Nolan, Susan Hall, Enrica Bianchi, Caitlin Hopkins, Samantha Madnick, Angela Stermer, and Kim Boekelheide
Department of Pathology and Laboratory Medicine

Brown University, Providence, RI, 02912. USA

Methotrexate (MTX) is a widely prescribed drug to treat neoplastic, autoimmune and inflammatory diseases. MTX antagonizes the folate pathway and leads to the inhibition of DNA and RNA synthesis, causing cytotoxicity and genotoxicity in various organ systems. In particular, testicular injury has been observed with MTX treatment in both preclinical models and clinically. The present study established a low-dose subchronic MTX exposure model in the rat with the ultimate goal of identifying distinct sperm RNA expression patterns associated with testicular injury for the purpose of creating novel biomarker panels for testicular toxicity assessment.

MTX was administrated intraperitoneally (IP) at the dose of 2, 5 and 10mg/kg to adult male Fisher rats (n=10) weekly for 13 weeks. The animals were sacrificed one week after the last MTX injection. To characterize the testicular injuries, testes/epididymal weights, sperm count, sperm motility and testes/epididymal histology were assessed. Moreover, total RNA was isolated from epididymal sperms and RNA yield per sperm was calculated by normalizing the total RNA yield to sperm count.

Sperm count and motility were similar among the control and treated groups. MTX treatment at 10mg/kg significantly decreased testes weight. There was a clear dose-related effect of MTX exposure on testicular histopathology, including a loss of spermatocytes in late stages of the seminiferous epithelial cycle and a loss of round spermatids in early stages of the cycle, resulting in seminiferous epithelial thinning and decreased seminiferous tubule diameter. Neither retained spermatid heads (RSH) nor Sertoli cell vacuoles were a prominent feature, only being observed in a minority of the high dose rats. Epididymal round cells consistent with sloughed germ cells were dose-dependently increased. The RNA yield per sperm were not altered by MTX treatment.

These data showed that a 13-week exposure to low-dose MTX caused testicular damage indicated by low testes weight and histopathological abnormalities.

Keywords: Testicular toxicity, Methotrexate, Histopathology

Abstract #36

mRNA fragments in rat sperm are biomarkers of testicular injury due to ethylene glycol monomethyl ether exposure

Angela R. Stermer, Lisa Bramer, Susan J. Hall, and Kim Boekelheide

Brown University, Department of Pathology and Laboratory Medicine, Providence, RI 02912.

Male reproductive toxicity poses a regulatory challenge in environmental, occupational, and pharmaceutical exposures due to the lack of simple robust analytical methods. RNA in sperm is reflective of the developmental process of the sperm, and changes in RNA composition could indicate toxic exposure. In order to identify highly predictive small RNA biomarkers of exposure in sperm, we used a combination of differential expression and LASSO regression after exposure to a known testicular toxicant, ethylene glycol monomethyl ether (EGME). Adult rats were exposed to 0, 50, 60 and 75 mg/kg EGME for 5 days then measuring small RNAs in sperm by next-generation sequencing (small RNA-seq) 5 weeks after exposure. There was a significant dose-dependent increase in mRNA fragments. Differential expression showed 11,000 mRNA fragments that are significantly changed with treatment (q-value <0.05). Then, the normalized counts of these significant mRNA fragments were used to perform a LASSO regression to identify genes that can accurately predict each level of treatment. The LASSO regression was run in a bootstrap manner, recursively selecting groups of mRNA fragments that gave predictive probabilities above 0.9. We focused only on mRNA fragments that were selected in every bootstrap iteration, to ensure we were looking at highly reproducible biomarkers. Interestingly, there were more identified biomarkers with increasing treatment. There were 547 mRNA fragments that accurately predicted 50 mg/kg (from control), 2744 mRNA fragments that were identified to predict 60mg/kg and 7963 mRNA fragments that were identified to predict 75mg/kg treatment levels. 91% of the genes selected in the lowest dose were also selected in the highest dose, suggesting there was high homology across treatments. To narrow these genes down to a panel of biomarkers, the common genes to all treatments were subset by the selection group to see if there were any highly selected groups of genes. These mRNA fragments are highly predictive biomarkers of EGME in sperm.

Abstract #37

Lack of Multidrug Resistance–Associated Protein 4 (Mrp4) Does Not Alter Susceptibility towards Acetaminophen Toxicity

Ajay C Donepudi[1], Michael J. Goedken[2] and José E Manautou[1]
[1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT. [2]Research Pathology Services, Rutgers University, Newark, NJ.

University of Connecticut

Acetaminophen (APAP) is the most frequent cause of drug-induced liver injury in humans and a common chemical model to investigate genetic determinants of susceptibility to drug-induced liver injury (DILI). Previous studies performed in our laboratory identified the efflux transporter multidrug resistance-associated protein 4 (Mrp4) as an inducible gene in liver following toxic APAP exposure in both humans and rodents. In mice, prevention of hepatic Mrp4 induction following APAP administration increases susceptibility towards APAP hepatotoxicity. Collectively, these findings suggest that Mrp4 plays an important role in tolerance towards APAP-induced liver injury. To further study the role of Mrp4 in APAP-induced hepatotoxicity, we challenged 10-12 weeks old male wild type (WT, C57BL/6J) and Mrp4 knock out ($Mrp4^{-/-}$) mice with either APAP (400 mg/Kg in saline, *i.p.*) or vehicle. Following treatment, plasma and liver samples were collected at 12, 24 and 48 hr and liver injury was assessed by plasma alanine aminotransferase (ALT) activity and histopathological examination. No significant differences in plasma ALT levels or histological scores were observed between $Mrp4^{-/-}$ and WT mice, except at 12 hr where $Mrp4^{-/-}$ mice exhibited decreased ALT levels and hepatic necrosis compared to WT mice. Gene expression analysis indicates that lack of Mrp4 is associated with decreased expression of hepatic glutathione metabolism and drug metabolizing genes, such as glutamate-cysteine ligase modifier subunit (Gclm), Cyp3a11 and Mrp2 under basal conditions. These differences in gene expression were not observed in $Mrp4^{-/-}$ mice at any time point post APAP administration. In contrast to the gene expression data, $Mrp4^{-/-}$ mice had increased hepatic non-protein sulfhydryl (NPSH) content at 12 and 24 hr after APAP treatment. Although significant decreases in endpoints of liver injury were detected early after APAP treatment in $Mrp4^{-/-}$ mice, these changes were not sustained at later time points. In conclusion, our data indicate that lack of Mrp4 in mice does not alter susceptibility to APAP toxicity.

Abstract #38

Maternal Preconception Exposure to PFBS Alters Nutrition and Growth of Offspring

Kate Annunziato, Marjorie Marin, Alicia Timme-Laragy,

University of Massachusetts Amherst

Perfluorobutane sulfonate, PFBS, is a perfluoroalkylated substance and a shortened (4 carbon) alternative of PFOS, perfluorooctane sulfonate (8 carbon). PFBS has a shorter half-life and lower reported toxicity than PFOS. Exposures in zebrafish embryos have shown it to affect pancreatic islet morphology and other developmental endpoints, but the critical exposure window prior to conception has not yet been examined. Using the zebrafish model (*Danio rerio*), this pilot study aims to investigate the effects of a maternal, preconception exposure to PFBS on reproductive and developmental endpoints. Three adult female fish, *Tg(ins-GFP),* were stripped to remove existing eggs and exposed to 0.25 µg/mL PFBS or 0.01% DMSO for 1 week during a cycle of oocyte maturation. Following exposures, female fish were bred daily with unexposed male fish for a period of two weeks. Embryos were imaged daily through 120 hours post fertilization, hpf, via live brightfield and fluorescence microscopy, and total larval length, yolk sac area, and pancreatic beta cell area were measured. Additionally, pooled samples of 25 embryos (3 hpf) or larvae (120 hpf) were assessed for total protein, triglycerides, cholesterol, and glucose using commercially available kits. At 3 hpf, there was a significant 7% reduction in yolk sac area in the embryos collected from the 0.25 µg/mL PFBS-exposed maternal group compared to controls. These embryos also had a significant 50% decrease in cholesterol. At 24 hpf, embryos from PFBS-exposed females had significantly decreased yolk sac area (8%), which persisted at 5 dpf (11% reduction). In addition, at 5 dpf, the 0.25 µg/mL PFBS larvae had a significant 13% increase in pancreatic beta cell area that corresponded with a trend towards lower larval glucose content, as would be expected with increased islet size and capacity. This study demonstrates that embryos are developmentally impacted by maternal exposures to PFBS during oocyte maturation. The exposure in female fish may impact the nutrients loaded into the egg during this process, which is evident in the effects on cholesterol and yolk sac area in the early developmental stages. This nutritional deficit, which is possibly coupled with loading of toxicant into the eggs, may explain the persistence of effects at 5 dpf. Together this suggests that there may be alterations in oocyte maturation and metabolic homeostasis in larvae associated with maternal preconception exposure to PFBS. This work was supported by R01ES028201.

Other Abstracts – By Faculty and/or Research Staff

Abstract #39

Spermatozoal large RNA content as new non-invasive tool to assess male reproductive toxicity in a pre-clinical and clinical setting.

E. Bianchi[1,2], M. Sigman[1,2], K. Hwang[1,2], J. M. Braun[3], S. J. Hall[2] and K. Boekelheide[2]

[1] Division of Urology; [2] Department of Pathology & Laboratory Medicine; [3] Department of Epidemiology, School of Public Health

Brown University, Providence RI, USA.

Assessing male reproductive toxicity of environmental and therapeutic agents relies on testis and epididymis histopathology in a pre-clinical setting while in humans assessment depends on semen and serum hormone analysis, both of which are poor indicators of sperm health and reproductive potential. Therefore, there is an urgent need to identify a novel, non-invasive and reliable approach to monitor environmental and therapeutic agents' exposures effects on male reproductive health. The present study was designed to investigated whether spermatozoal large and small RNA content can discriminate "healthy" from abnormal sperm and to determine how spermatozoal RNA varies with behavioral/lifestyle stressor effects on male fertility. Semen specimens were collected from men aged between 18 to 55 years undergoing male factor infertility evaluation, from VA medical center patients treated for autoimmune arthritis with methotrexate and known fertile patients presenting to Brown Urology for vasectomy. Semen samples were assessed according to World Health Organization (WHO) 2010 criteria. Sperm large and small RNAs were extracted after somatic cells were lysed, and the association of spermatozoal large or small RNA content with semen quality and behavioral/lifestyle factors was evaluated using a generalized additive model, Student's t-test and one-way ANOVA. Our findings demonstrated that sperm total count was inversely associated with spermatozoal large RNA content while there was a non-linear association to sperm small RNA content. Sperm motility was inversely associated with spermatozoal large and small RNA contents while large RNA content per sperm was significantly increased in semen samples showing abnormal numbers of round cells. Alcohol consumption was strongly associated with increased large RNA amount per sperm. In addition, patients treated with a known testicular toxicant, methotrexate, showed 25 times more spermatozoal large RNA content than the control fertile group. In conclusion, the present study demonstrated that spermatozoal large RNA content has the potential to predict sperm abnormalities and male reproductive risk following environmental and pharmaceutical chemical exposure.

Abstract #40

Effects of selected nootropic supplements on zebrafish *(Danio rerio)* embryogenesis

Paul V. Kaplita, Chuljoong Oh, Luong T. Nguyen and Min J. Kim.

MCPHS University

Numerous nootropic or neuro-nutrient supplements are reported to temporarily boost memory, learning, and mental function by supporting neurogenesis, neurotransmitter synthesis, cerebral vasodilation, and brain metabolism, and by reducing oxidative stress. These supplements are advertised as treatments to slow or prevent the decline of cognitive skills in neurodegenerative diseases like dementia or Alzheimer's disease, enhance memory and cognition in healthy adults, and improve learning and mental concentration in school-age children. Some nootropic agents have been used for thousands of years as components in Ayurvedic or traditional Chinese medicine. Although many of these nootropics are considered GRAS (generally regarded as safe) by the FDA, reliable information about safety in children or women who are pregnant or breast-feeding are lacking. The objective of this project was to visually observe the effects of four selected nootropics (bacoside A/B, vinpocetine, huperzine-A and L-theanine) on the embryogenesis of zebrafish. To investigate any potential developmental toxicity, fertilized zebrafish eggs were exposed to increasing concentrations (0.01 – 100 □M) of these selected nootropics. Physical morphology, development, and mortality of the developing embryos were recorded up to 72 hours post fertilization (hpf) with a stereomicroscope equipped with a digital camera and compared to untreated controls. Exposure to vinpocetine subtly slowed embryogenesis of the zebrafish eggs in a concentration-dependent fashion. Malformations were observed in the developing embryos, as well. Vinpocetine also appeared to decrease the movements of embryos within their chorions. Control eggs and embryos developed at the expected pace, as did the eggs that were exposed to the test concentrations of huperzine A, bacopin A/B, and L-theanine. These results suggest that one (vinpocetine), but not all, of the tested nootropic supplements may be toxic to the developing zebrafish embryos. Future studies are necessary to confirm the results and extend the conclusions to safe pharmacotherapeutic uses in humans.

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