ABSTRACTS

2026 OVSOT Annual Meeting

November 13, 2026

P&G

Mason, OH

Platform Presentations

Early-Stage Investigator Platform Presentation

Frederick Ekuban, University of Louisville, “Environmental forever chemical exposures alter severity and metabolic signatures of alcohol-associated steatohepatitis via PPARα” (Abstract 001)

PhD Platform Presentations

Henry Stalnaker, University of Toledo, “Identification of Fentanyl Reversal Candidates and Investigation of Noncanonical Molecular Interactions Using a Zebrafish-Based Platform” (Abstract 002)

Zianne Olverson, University of Louisville, “Nicotine and Nicotine-Free Electronic Cigarette Aerosols Elicit Distinct Junctional Zone Responses in Male and Female Placentas” (Abstract 003)

Ehsanul Kabir, Purdue University, “NEIL2 upregulation is associated with an adaptive DNA damage response following acute exposure to electronic cigarette aerosol constituents” (Abstract 004)

Postdoctoral Platform Presentations

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Mariam Habil, University of Louisville, “Primary human hepatocytes as a New Approach Method (NAM) of genotoxicity induced by nitro-polycyclic aromatic hydrocarbons (Nitro-PAHs)” (Abstract 006)

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William Duniver, University of Toledo, “Quantifying Neurodegeneration in Young Rats Following 90-Day Exposure to Hexavalent Chromium in Drinking Water” (Abstract 011)

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Supriya Bhukya, Wright State University, “The impact of platelet-activating factor-receptor signaling in the cytotoxic efficacy of Aurora kinase inhibitors in non-small cell lung cancer” (Abstract 013)

Kirtan Kachhadiya, Wright State University, “Platelet-activating factor-receptor-dependent microvesicle particles modify the cytotoxic efficacy of signal transducer and activator of transcription 3 inhibitors in non-small cell lung cancer” (Abstract 014)

Brandon Capps, University of Louisville, “Effects of Combined Formaldehyde Exposure and Hypertension on Aortic Function in Mice” (Abstract 015)

Presenters by Last Name

Last Name	First Name	Abstract #	Last Name	First Name	Abstract #
Ames	Paxton	040	Kwon	Darbie	051
Annaparthi	Nihal	059	Labde	Kimaya	061
Arora	Harleen Kaur	065	Maines	Garrett	070
Bajad	Tejasvini	069	Mandalapu	Preethika	064
Bhandari	Suvechha	028	McCann	Ren	082
Bhukya	Supriya	013	McNabb	Jasey	009
Boggavarapu	Nishi	010	Minikuri	Siri chandana	055
Bramatti	Isabella	024	Mishra	Ribhav	018
Bryant	Gjhvona	033	Morse	Chase	056
Burciaga	Isaiah	023	Musunuru	Sruthi	062
Butzke de Souza	Nicolli	035	Nail	Alexandra	016
Capps	Brandon	015	Natarajan	Ashwin	074
Chaitanyareddy	Kallu	068	Nguyen	Julie	085
Chintala	Akhila	066	Olverson	Zianne	003
Clark	Jack	081	Padgett	Carson	078
Cochran	Samuel	025	Paridie	Aya	038
Colton	Jana	080	Patel	Dhara	012
Colvin	Victoria	019	Patel	Ragi	052
Crouch	Audra	021	Patel	Garvi	073
Devi	Meenu	063	Pitchai	Arjun	017
Domingos	Rihanna	031	Powell	Allyson	036
Duniver	William	011	Robison	Kiley	048
Duric	Jovana	029	Rodriguez	Gabriel	026
Ekuban	Frederick	001	Rosenzweig	Rachel	049
Ekuban	Abigail	022	Sadoh	Odion	053
Ghising	Syaron	067	Safee	Raihaanah	027
Gibbens	Abigail	041	Sathisaran	Umamaheswari	005
Gootee	Maxwell	071	Savin	Avital	032
Habil	Mariam	006	Scott	Jared	087
Hammonds	Raymond	084	Schott	Evangeline	043
Hanson	Evan	042	Sharma	Mansi	058
Hasan	Syed	030	Simmel	Maxel	054
Hasan	Md Nahid	045	Sridhar	Sainidhi	060
Janzow	Mychaela	050	Stalnaker	Henry	002

Last Name	First Name	Abstract #	Last Name	First Name	Abstract #
Jasper	Genell	037	Swihart	Jenna	044
Jha*	Shamvabi	046	Tandon	Rhea	086
Jogi	Vijayalakshmi	034	Ursell	Samantha	008
Joseph	Ashilyn	077	Weinschutz Mendes	Hellen	007
Joshi	Krishna	057	Wiggam	Addison	083
Kabir	Md Ehsanul	004	Willard	Nicholas	039
Kachhadiya	Kirtan	014	Wirsch	Amanda	076
Kaur	Agampreet	075	Wymer	Emily	072
Ketron	Lila	079	Xu	Jason	020
Khatun	Mst Moriom	047			

*Poster was withdrawn, presenter could not attend meeting

Platform Presentation Abstracts

Abstract 001 – Early-Stage Investigator

Environmental Forever Chemical Exposures Alter Severity and Metabolic Signatures of Alcohol-Associated Steatohepatitis via PPARα

Frederick A. Ekuban[1], Adrian Centers[1], Matthew R. Smith[2], Vatsalya Vatsalya[1], Jasey McNabb[1], Addison Wiggam[1], Kushal Biswas, Dhimiter Bello[3], Jennifer J.Schlezinger[4], Hao Zheng[1], Wanyu Zhang[1], Maiying Kong[1], Dean P. Jones[3], Craig J. McClain[1] and the DASH Consortium, Matthew C. Cave[1]

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Although alcohol use is widespread, only a subset develop advanced liver injury, implicating modifiers beyond ethanol. Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals linked to MASLD and recently detected in US beers above EPA limits (PMID: 40272161), creating exposure pathways for drinkers. We hypothesized that perfluorooctanoic acid (PFOA) disrupts hepatic metabolism and modifies ASH severity via PPARα-dependent mechanisms.

Exposomics and metabolomics (LC-MS² & GC-MS²) were performed in archived plasmas from subjects with ASH (severe=96, moderate=28) and non-drinking controls (n=11). Associations between biomarkers of PFOA exposure, ASH severity, and metabolome were determined using linear regression models. Male C57BL/6J mice on chronic-binge ethanol (EtOH) feeding were co-exposed to PFOA (2 mg/kg) or vehicle for 10 days, followed by a 5 g/kg EtOH binge. Liver disease phenotyping, including plasma/hepatic biochemistry, histology, qPCR, and Western blotting were performed.

Plasma PFOA was elevated in ASH versus controls (P=0.02; 100% detection). MWAS vs PFOA identified 3,318 metabolic features (P<0.05) enriching 32 pathways (fatty acid β-oxidation, glycerophospholipid, alanine/aspartate, urea cycle, glutathione, CYP450); 19 overlapped with the ASH disease-group MWAS, suggesting PFOA contributes to the ASH metabolomic signature. In mice, PFOA worsened EtOH-induced steatosis, hepatic triglycerides, and lipid peroxidation, and activated PPARα targets in both pair-fed and EtOH-fed mice, with concurrent upregulation of nuclear receptors and downstream targets. Plasma ALT and AST were paradoxically reduced in EtOH+PFOA despite intensified tissue injury, marked by ER stress (CHOP ~50-fold), oxidative-stress and pro-inflammatory gene induction, alongside upregulation of ethanol-metabolizing enzymes with markedly lowered blood alcohol (P<0.0001).

PFOA was elevated in ASH and associated with broad metabolic disruption. In mice, PFOA exacerbated ethanol-associated hepatic injury while lowering circulating aminotransferases and blood alcohol, suggesting altered lipid and ethanol metabolism may obscure conventional injury biomarkers. These findings support PFOA as a candidate environmental modifier of ASH and warrant further mechanistic investigation.

Abstract 002 – PhD Student

Identification of Fentanyl Reversal Candidates and Investigation of Noncanonical Molecular Interactions Using a Zebrafish-Based Platform

Henry C. Stalnaker[1,3], Millicent T. Akere[1,3], Jovana Duric[2], Abigail N. Collins, William S. Messer, Jr.[1,2], F. Scott Hall[2], Frederick E. Williams[2,3], Alexander S. Wisner[1,3], and Isaac T. Schiefer[1,3]

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Fentanyl overdose remains a major public health challenge, highlighting the need for reversal strategies beyond μ-opioid receptor (MOR) antagonism. This study combined zebrafish phenotypic screening, receptor profiling, and photoaffinity proteomics to identify candidate reversal agents and investigate fentanyl-associated molecular interactions.

Fentanyl-induced locomotor suppression and naloxone sensitivity were characterized in 7-day-post-fertilization zebrafish larvae (*Danio rerio*). Approximately 1,000 compounds were screened at 30 μM alongside fentanyl (30 μM), with reversal assessed by locomotor recovery during a photomotor response assay. Several screening hits shared a common chemical scaffold, prompting resynthesis of a prioritized hit and systematic synthesis of structural analogs to investigate structure–activity relationships. A resynthesized hit and selected analogs underwent Psychoactive Drug Screening Program (PDSP) receptor profiling. In a complementary investigation, the fentanyl-derived photoaffinity probe Photofent was developed to identify fentanyl-associated proteins through competitive proteomics in whole zebrafish larvae.

Fentanyl produced a biphasic concentration–response profile, with naloxone reversing locomotor suppression at lower concentrations but showing reduced efficacy at higher exposures. Screening identified nine candidate reversal agents. Resynthesis confirmed the reversal activity of the prioritized hit, while several structural analogs exhibited reduced locomotor recovery. PDSP profiling of the resynthesized hit showed low inhibition at MOR (18.95%), DOR (5.38%), and KOR (−2.82%), suggesting a potential non-MOR-mediated mechanism. Photofent retained MOR affinity (Ki = 167 nM), produced fentanyl-like behavioral suppression, and enabled concentration- and UV-dependent protein labeling. Competitive proteomics identified 27 proteins whose labeling was displaced by fentanyl but not naloxone, with network analysis implicating metabolic, translational, and cellular stress-response pathways.

Together, these complementary zebrafish studies identified a reproducible fentanyl reversal hit with limited classical opioid receptor-binding activity and revealed candidate fentanyl-associated protein interactions beyond naloxone-sensitive pathways. These findings support further investigation of noncanonical fentanyl mechanisms and alternative reversal strategies.

Abstract 003 – PhD Student

Nicotine and Nicotine-Free Electronic Cigarette Aerosols Elicit Distinct Junctional Zone Responses in Male and Female Placentas

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Up to 15% of pregnant women report electronic cigarettes (e-cig) use during pregnancy. Recent FDA authorization of fruit-flavored e-cigs emphasizes the need to understand gestational exposure risks. We previously showed that gestational exposure to unflavored e-cig aerosol, even without nicotine, reduces uterine artery blood-flow velocity and fetal growth during late gestation. We hypothesize that nicotine-free and nicotine-containing e-cig aerosols elicit placental responses that may contribute to adverse fetal outcomes. These placental responses may reveal mechanisms contributing to adverse maternal and fetal health outcomes.

Pregnant FVB/J mice underwent whole-body exposure to filtered air (FA), aerosolized 50:50 propylene glycol/vegetable glycerin (PV), or PV containing 2% nicotine (PV + Nic), using the InExpose exposure system, 4 h/day, 5 days/week, from embryonic day 0.5 (E0.5), until tissue collection at E14.5 or E17.5. Maternal weight and fetal and placental growth parameters were measured at E14.5, and placental tissues underwent histological and molecular analyses. Male and female E17.5 placental junctional zones underwent RNA sequencing to characterize exposure-associated transcriptional responses within each sex.

At E14.5, maternal weight was lower in the PV group than in FA controls ($P<0.05$, via one-way ANOVA), with no differences in fetal or placental growth parameters detected. This contrasts with the reduced fetal weight we previously reported following PV exposure at E17.5. At E17.5, PV exposure reduced trophoblast giant-cell density and increased Masson's trichrome positivity within the junctional zone ($P<0.05$ and $P<0.005$, respectively; one-way ANOVA), PV + Nic increased whole-placenta hydroxyproline ($P<0.05$, via one-way ANOVA). RNA sequencing identified that both exposures produced more differentially expressed genes in female than in male junctional zones (DESeq2; adjusted $P<0.05$), with downregulation predominating in PV exposure in both sexes. In females, both exposures were associated with lower *Slc7a5* expression and higher *Col3a1* expression, genes involved in nutrient transport and extracellular matrix organization, respectively. PV exposure was also reduced expression of the trophoblast-related gene *Prl3d1*.

Gestational e-cig aerosol exposure alters junctional zone structure and gene expression, with distinct transcriptional patterns across exposures and sexes. These findings implicate altered nutrient transport, extracellular matrix remodeling, and trophoblast signaling as potential mechanisms linking gestational e-cig aerosol exposure to adverse late gestational fetal outcomes.

Abstract 004 – PhD Student

NEIL2 upregulation is associated with an adaptive DNA damage response following acute exposure to electronic cigarette aerosol constituents

Ehsanul Kabir, Benjamin Dierolf, Thivanka Muthumalage

School of Health Sciences, Purdue University, West Lafayette, IN

Background: Exposure to e-cigarette aerosols triggers the production of reactive oxygen species (ROS), which lead to oxidative DNA damage in pulmonary epithelial cells. The base excision repair (BER) pathway can be initiated by DNA glycosylases, and this pathway is crucial to counteract ROS-induced oxidative DNA damage. Endonuclease VIII-like 2 (NEIL2) is a specialized DNA glycosylase that removes oxidized DNA lesions to counteract oxidative DNA damage. However, the regulation of NEIL2 following acute exposure to e-cigarette constituent mixtures remains poorly understood.

Hypothesis: We hypothesized that acute exposure to e-cigarette constituent mixtures upregulates NEIL2 as an adaptive response to oxidative DNA damage in lung epithelial cells.

Methods: Human bronchial epithelial cells (BEAS-2B) were treated with mixtures of e-cigarette constituents including metals and flavoring chemicals. At 24h post-treatment, conditioned media and cells were collected, and cell lysates were prepared. Inflammatory markers (IL-6 and IL-8) were assessed in conditioned media by ELISA. The abundance of BER (NEIL2, PNKP), total H2AX, and the DNA damage-response marker phospho-ATM (p-ATM) were determined by immunoblotting. Then, we exposed male and female C57BL/6J mice (7–20 weeks old) for 2 h to tobacco-flavored e-cigarette aerosols with or without 6 mg/mL nicotine (51 mL puff volume, 3 s puff duration, and 30 s inter-puff interval) in a whole-body chamber. Lung tissue was collected 24 h after exposure, and total RNA was extracted and NEIL2 gene expression was measured in lung tissue by RT-qPCR.

Results: Mixtures of e-cigarette-derived metals and flavoring chemicals, including nickel oxide/ethyl vanillin and chromium oxide/vanillin, significantly induced IL-6 and IL-8 levels in conditioned media and increased NEIL2 and PNKP protein abundance in BEAS-2B cells. Nickel oxide/ethyl vanillin mixture treatment significantly increased p-ATM, and chromium oxide/vanillin mixture treatment significantly decreased total H2AX. In vivo data showed that a single exposure to tobacco-flavored e-cigarette aerosol with or without nicotine significantly increased NEIL2 gene expression in the lung compared with the air-exposed group.

Conclusion: Exposure to e-cigarette constituent mixtures increased inflammation and DNA damage responses and elevated NEIL2 protein abundance in lung epithelial cells after 24 h. A single exposure to tobacco-flavored e-cigarette aerosols significantly increased NEIL2 gene expression in the lung, suggesting an early adaptive response to e-cigarette-induced pulmonary toxicity. Overall, NEIL2 may contribute to the repair of oxidative DNA damage induced by e-cigarette exposure. This study was supported by the National Institutes of Health R00ES033835.

Abstract 005 – Postdoctoral Trainee

Early-Life PFAS Exposure Induces Lipidomic Remodeling and Bioenergetic Dysfunction in Zebrafish

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants associated with developmental and neurotoxic effects. Perfluorobutanoic acid (PFBA; C4) is a short-chain PFAS with lower bioaccumulation potential, while perfluorooctanoic acid (PFOA; C8) is a long-chain PFAS known to bioaccumulate. Their effects on the developing lipidome and cellular bioenergetics remain poorly understood. This study examined lipidomic and functional responses following early-life PFBA and PFOA exposure in zebrafish (*Danio rerio*). Embryos were exposed to 0, 0.04, 0.4, 4, or 40 ppb (µg/L) PFBA or PFOA beginning within 0–2 hours post-fertilization (hpf). Exposure continued through 72 hpf, spanning embryogenesis and early neurogenesis. At 72 hpf, larvae were washed three times with fresh filtered embryo water, transferred to clean filtered embryo water, and maintained until 120 hpf. Lipid extracts from 120 hpf larvae were analyzed using multiple reaction monitoring (MRM)-based lipid profiling. The resulting lipidomic data were further analyzed using MetaboAnalyst and LION enrichment analysis. Neutral lipid storage was measured using BODIPY 493/503 fluorescence z-stack imaging. Lipid peroxidation was assessed using a TBARS-based colorimetric microplate assay with MDA as the readout. Cellular bioenergetics was assessed using BioTracker ATP-LW fluorescence imaging. Functional endpoints were analyzed using one-way ANOVA followed by Fisher's LSD test in SAS 9.4 ($p<0.05$). LION analysis identified concentration-dependent enrichment of glycerophosphocholine, glycerophosphoethanolamine/glycerolipid, and fatty-acid categories following PFBA and PFOA exposure. BODIPY-positive lipid droplets were elevated following PFBA and PFOA exposure. MDA content significantly increased at 4 and 40 ppb PFBA and at 0.4, 4, and 40 ppb PFOA. ATP-associated fluorescence significantly decreased at 4 and 40 ppb PFBA and at 0.4, 4, and 40 ppb PFOA. Together, these findings show that early-life PFAS exposure alters lipid composition and storage, with concurrent oxidative lipid damage and reduced cellular bioenergetic status.

Abstract 006 – Postdoctoral Trainee

Primary human hepatocytes as a New Approach Method (NAM) of genotoxicity induced by nitro-polycyclic aromatic hydrocarbons (Nitro-PAHs)

Mariam R. Habil, Makayla A. Stephens, Luke A. Schroeder, David W. Hein

Department of Pharmacology and Toxicology, University of Louisville

Nitro-PAHs are common environmental pollutants that can be found in various sources such as coal combustion, diesel engines, cigarettes smoke, cooked meat products, and biomass burning. Previous studies reporting the genotoxic effects of nitro-PAHs were inconsistent with most of them done using bacterial or animal models. Recently, the Environmental Protection Agency (EPA) has announced its intention to cease conducting or funding toxicity testing research on mammals by 2035. Use of primary human hepatocytes with high throughput gamma-H2AX in-cell western assay can serve as a New Approach Method (NAM) to determine genotoxicity of nitro-PAHs and human risk extrapolation. The objective of this study was to investigate the genotoxicity of nitro-PAHs and the role of rapid and slow acetylator NAT2 phenotypes on DNA damage following exposure to nitro-PAHs. Cryopreserved human hepatocytes from 6 donors (3 rapid and 3 slow NAT2 acetylators) were treated with 1-nitropyrene (1-NP), 9-nitroanthracene (9-NA), 1,3-dinitropyrene (1,3-DNP) or 1,8-dinitropyrene (1,8-DNP) (0-100 µM) for 24 h. In-cell western assays were used to measure DNA damage response represented by gamma-H2AX (a sensitive marker for double strand DNA breaks). Statistical analyses were done using two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test and non-linear regression model was applied to determine EC_{50}. Rapid NAT2 acetylators showed higher DNA damage response compared to slow NAT2 acetylators ($p< 0.01$ for 1-NP, $p< 0.0001$ for other nitro-PAHs) suggesting higher risk among people with rapid NAT2 activity. 1-NP showed the highest genotoxic potential followed by 1,8-DNP, 1,3-DNP and 9-NA (EC_{50} is 2.786, 6.617, 18.21, 32.13 µM respectively), suggesting that 1-NP and 1,8-DNP possess greater potential hazard at lower exposure level. Our study provides a human model for risk assessment and highlights that interindividual genetic differences of NAT2 alter a population's susceptibility to nitro-PAHs. In addition, 1-NP and 1,8-DNP require close environmental monitoring based on their high genotoxic potential. Future studies will be done to explore the mechanisms involved in nitro-PAHs genotoxicity. Research was supported with NIH grants T32-ES011564, P42-ES023716, P30-ES030283, and P30-GM165331.

Abstract 007 – Postdoctoral Trainee

Lead (Pb) exposure reveals neuroimmune alterations in the developing zebrafish brain

Hellen Weinschutz Mendes[1], Wagner A. Tamagno[1] Jennifer L. Freeman[1], Christina R. Ferreira[2]

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Exposure to the heavy metal Lead (Pb) is a pervasive public health issue with life-long lasting consequences. Dysregulation of neuroinflammation is one of the main mechanisms by which contamination with Pb disrupts the central nervous system (CNS) and exerts its neurotoxic effects. Due to its many advantages, such as the high-throughput tractability of developmental processes, cost-effectiveness, high genetic homology to human genes, and rapid external development, the zebrafish has become an ideal animal model for developmental toxicology and is the animal model used for this study. The purpose of this study is to investigate the effects of developmental Pb exposures on dysregulation of neuroinflammation during development in zebrafish. Developmental exposures consisted of three different Pb concentrations [1, 10, and 100 part per billion (ppb, µg/L)] in comparison to a negative control (0 ppb) and were performed with zebrafish from 1 to 72 hours post fertilization (hpf) encompassing embryogenesis. We then performed assessment of microglia number and spatial distribution with live-image staining at 96 hpf, targeted gene expression analysis of neuroinflammation pathways at 72 and 96 hpf, and lipidomic profiling using multiple reaction monitoring (MRM) at 120 hpf. Analysis using neutral red surface staining on live zebrafish revealed a concentration-dependent increase in the number of microglia cells, which was significant for the 10 and 100 ppb exposed groups ($p<0.05$). Gene expression analysis through qPCR showed significant higher average expression of the genes *tgfb1* and *mpeg* for the 1 and 100 ppb exposed groups, while zebrafish in the 100 ppb exposed group also presented decreased average expression of *apoeb* ($p<0.05$). Lipidomic profiling was conducted on isolated zebrafish larval heads to specifically assess neuroinflammatory alterations rather than systemic inflammation. A total of 92 single lipids were significantly altered among the three exposed groups. Among those, 42 selected lipids, all belonging to the phosphatidylethanolamine (PE) and sphingomyelin (SM) classes, were identified with a log2 fold change greater than 0.5 in at least one Pb treatment group. These alterations were observed mainly in the 1 ppb exposed group. These investigations expand our knowledge on Pb-induced neuroinflammation, suggesting neuroimmune lipid remodeling and identifying specific players in the neuroinflammatory response. This knowledge could be studied and leveraged for therapeutic approaches and public health interventions to ease the burden of Pb-induced neuroinflammation and overall neurotoxicity.

Big Picture Science Abstracts

Abstract 008 – Big Picture Science Undergraduate Student

Evaluation of Neuroinflammation in Rats Following 90 Days of Drinking Water Exposure to Hexavalent Chromium

Samantha Ursell[1], Hamza Jamal[2], Spencer H. Roof[2], Quentin Wise[2], Samuel T. Vielee[3], Johnny Wise[1]

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In 2024, Cr(VI) was identified as the top chemical hazard in U.S. public drinking water. The maximum contaminant level (MCL) for chromium in drinking water set by the World Health Organization (WHO) is 0.05 mg/L, and 0.1 mg/L is the MCL set by the U.S. Environmental Protection Agency (U.S. EPA), with these regulations based on contact dermatitis. Human studies show that Cr(VI) exposure is linked to neurological disorders such as motor neuron disease, polyneuropathy, and acute schizophrenia. There are many key knowledge gaps regarding Cr(VI) neurotoxicity, including where it accumulates in the brain, which brain regions and cell types are vulnerable, and how these effects change depending on the age or sex of the individual.

In 2024, our lab published results reporting behavior effects and regional brain deposition of Cr in female and male rats from three age groups (3-, 7-, or 18-month-old) that were given Cr(VI) in drinking water matching MCLs set by WHO and U.S. EPA. After sacrifice, brains were bisected, with one half for regional metallomics analyses and one half preserved with 4% paraformaldehyde for histology. We reported that Cr(VI) selectively accumulates in the hippocampus (especially in females), and our behavior analyses indicated that hippocampal behaviors were the most impacted. Here, we evaluated Cr(VI)-induced neuroinflammation by assessing morphological changes in hippocampal microglia. We used immunohistochemistry (IHC) staining on hippocampal brain sections with IBA-1, bright-field imaging, and Sholl analysis. Imaging was done using a Keyence BZX-1000 digital microscope to create 10x and 20x stitched images. We then used Sholl analysis to quantify roughly 100 manually selected cells morphological changes to capture activation of microglial.

Currently, we have imaged hippocampal sections of all the age groups of female rats, including 10x and 20x stitched images. Qualitatively, the microglia appear smaller in the middle-aged females as Cr(VI) exposure increases, suggesting there are fewer or shorter branched processes, which is associated with neuroinflammation.

The effects observed following low-dose Cr(VI) exposure raise concerns about its potential neurotoxicity. Because rats are more resilient than humans, these findings suggest that the current MCL may not fully protect against neurological effects. Neuroinflammation is associated with neurological disorders such as Alzheimer's and Parkinson's disease. Our results will help inform how Cr(VI) contributes to neurological diseases and hopefully improve regulations to better protect populations at risk of exposure.

Abstract 009 – Big Picture Science Undergraduate Student

Effects of PFOA and Ethanol Co-Exposure on White Adipose Tissue Function and Metabolic Signaling

Jasey E. McNabb[1], Adrian Centers[1], Paxton M. Ames[1], Abigail Ekuban[1], Oluwanifemi E. Bolatimi[1], Yuan Hua[1], Jianzhu Luo[1], Inas Saad[1], Addison Wiggam[1], Walter H. Watson[1], Banrida Wahlang[1], Dhimiter Bello[2], Jennifer J. Schlezinger[3], Craig J. McClain[1], Matthew C. Cave[1], Frederick A. Ekuban[1]

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Background: Alcohol-associated liver disease (ALD) remains a global health challenge with substantial variability in disease progression, suggesting that additional risk factors may influence disease severity. Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants that exacerbate ethanol-induced liver injury and lipid accumulation, but the role of extrahepatic tissues in these interactions remains unclear.

Hypothesis/Goal: Here, we investigated whether perfluorooctanoic acid (PFOA) alters ethanol-associated metabolic programming in white adipose tissue (WAT) and whether these extrahepatic alterations are associated with hepatic lipid accumulation.

Methods: Sixty male C57BL/6J mice were assigned to four groups (n=15/group): pair-fed vehicle, pair-fed PFOA, ethanol-fed vehicle, or ethanol-fed PFOA. Mice received either an isocaloric control or Lieber-DeCarli ethanol diet, with co-current exposure to daily oral gavage of PFOA (2 mg/kg/day) or vehicle (2% Tween-80) for 10 days. On the final day, ethanol-fed mice received a 5 g/kg ethanol binge and were euthanized 5–6 h later. WAT was evaluated by H&E staining, qPCR to characterize adipose tissue remodeling and metabolic adaptation, along with assessment of hepatic histology, triglyceride accumulation, and gene expression.

Results: PFOA and ethanol co-exposure increased hepatic steatosis and triglyceride accumulation, accompanied by enhanced fatty acid β-oxidation and reduced de novo lipogenic gene expression. In WAT, co-exposure markedly remodeled endocrine and metabolic programs. Ethanol attenuated PFOA-induced adipokine and adipogenic responses, including reduced Adipoq, Retn, and Pparg expression, while altering pathways involved in lipid storage and fatty acid metabolism. Co-exposure also induced a browning/thermogenic signature marked by increased Cited1, Prdm16, and Tmem26 expression.

Conclusions: Co-exposure to PFOA and ethanol alters WAT endocrine, adipogenic, and metabolic signaling, identifying WAT as a key extrahepatic target of PFAS-alcohol interactions. These alterations may promote lipid redistribution to the liver, contributing to hepatic triglyceride accumulation and steatosis. Further studies are required to define the contribution of adipose-liver crosstalk to environmentally modified ALD.

Abstract 010 – Big Picture Science Undergraduate Student

Diet induced obesity increases pulmonary linoleic acid metabolism and ozone induced lung injury

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Ground-level ozone (O_3) is a criteria air pollutant known to exacerbate chronic respiratory diseases. Acute ozone exposure induces pulmonary inflammatory response involving neutrophil recruitment, proinflammatory cytokine release, and increases epithelial barrier permeability. The respiratory health effects of ozone disproportionately affect vulnerable populations, including individuals with obesity. However, the mechanisms driving this susceptibility remains unknown. Linoleic acid (LA) is the most abundant n-6 polyunsaturated fatty acid (PUFA) in the food supply and is consumed in excess in the western obesogenic diet. LA can be metabolized into bioactive lipid mediators called oxidized linoleic acid metabolites (OXLAMs), including epoxyoctadecenoic acids (EpOMEs) and dihydroxyoctadecenoic acids (DiHOMEs), which have been shown to increase pulmonary inflammation. Therefore, we hypothesize that increasing dietary LA through a western diet will increase LA accumulation in the lungs and the ensuing production of OXLAMs, which drives obesity-exacerbated O_3 induced lung inflammation.

In a preliminary study, male C57BL/6J mice were fed a western obesogenic diet high in LA (45% milkfat, LA is 13% of the caloric content) and a lean control diet for 15 weeks. Following feeding, obesogenic diet-fed and normal chow-fed mice were exposed to filtered air or 1ppm O_3 for 3 hours. 24 hours post exposure, lung tissue was collected to quantify fatty acids, OXLAMs, and bronchoalveolar lavage fluid (BALF) to assess airspace inflammation and injury. Splenic tissue was also used to measure systemic fatty acids and OXLAMs.

Mice fed the obesogenic diet gained significantly more weight and had elevated systemic LA. Western obesogenic diet-fed mice exposed to O_3 also exhibited increased airspace neutrophilia and total BAL protein, indicating increased lung injury and inflammation. Following O_3 exposure, mice fed the obesogenic diet had increased pulmonary OXLAMs, including 9,10-DiHOME and 12,13-EpOME, compared to control diet-fed mice. Interestingly, these OXLAMs were not increased by obesity or O_3 exposure alone.

Taken together, these data indicate that dietary LA accumulates in the lung and in combination with O_3 exposure, promotes pulmonary OXLAM production that is associated with ozone exacerbated airspace inflammation. Future directions will elaborate on dietary LA controlling mechanisms of resolution by establishing timepoints where ozone induced lung inflammation is resolving.

Abstract 011 – Big Picture Science Undergraduate Student

Quantifying Neurodegeneration in Young Rats Following 90-Day Exposure to Hexavalent Chromium in Drinking Water

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Hexavalent chromium [Cr(VI)] is a widespread U.S. drinking water contaminant and a major public health concern. Although known for its carcinogenic properties, Cr(VI) has an established link to motor neuron disease, polyneuropathy, and acute schizophrenia. Cr(VI)'s capacity to induce neurodegeneration in the brain remains poorly characterized and seldomly accounted for, making it unclear which brain regions are most vulnerable. This study aims to identify and quantify vulnerable brain regions following subchronic, low-dose exposure to Cr(VI) in drinking water to better understand observed behavioral deficits and predict others. Male and female young Sprague-Dawley rats (3 months old at enrollment) were exposed to tap water containing 0, 0.05, or 0.1 mg/L Cr(VI) for 90 days. Brain tissues were collected, bisected into lateral hemispheres with one half for metallomics and one half preserved in 4% paraformaldehyde for histology. Fixed brain hemispheres were sectioned at 35 um, then 8-10 sections per animal were silver-stained (FD NeuroSilver™ Kit II) to enable direct visualization and differentiation of healthy versus degenerating (silver-positive) neurons across an abundance of brain regions. Whole sections were imaged using a Keyence BZX-1000 microscope at 10x stitched of the whole section and 20x for brain regions of interest. Target regions of interest, including the hippocampus, cerebellum, striatum, cingulate cortex, and piriform cortex, were identified using the Paxinos and Watson Rat Brain Atlas (7th Ed). Initial qualitative assessments were conducted to evaluate and map spatial patterns of damage. This qualitative assessment was completed by overlaying a 10x10 grid over a target region, randomly selecting 10% of the grids, and giving a score from 1-5 based on severity of neurodegeneration. The next focal point is to complete a digital image analysis in QuPath using 20x magnification scans to generate precise, quantitative cell counts. Imaging and qualitative analyses revealed marked silver-positive staining in Cr(VI) exposed animals, indicating elevated neurodegeneration compared to age-matched controls. Concentration-associated neuronal damage was observed, with the greatest apparent severity observed in the hippocampus, cerebellum, and striatum. These findings demonstrate that subchronic exposure to environmentally relevant levels of Cr(VI) induced region-specific neurodegeneration in young animals. The localized damage observed in key structural regions provides a biological mechanism for Cr(VI)-induced memory and motor impairments, underscoring the potential neurological risks posed by Cr(VI) in public drinking water systems.

Abstract 012 – Big Picture Science Graduate Student

Mechanisms of platelet-activating factor-receptor (PAFR) signaling in dichloroacetate based combination approaches for lung cancer.

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Introduction: Cancer cells often rely on glycolysis for energy production despite oxygen availability, a phenomenon known as the "Warburg effect." Therefore, targeting key glycolytic regulators, including pyruvate dehydrogenase kinase (PDK), has been explored a potential cancer therapy. In this regard, dichloroacetate (DCA), an antiglycolytic agent, promotes a metabolic shift from glycolysis toward mitochondrial oxidative metabolism and sensitizes cancer cells to cytotoxic agents, promoting oxidative stress and cell death. However, therapeutic resistance often limits the efficacy of combination therapies and highlighting the need to identify counter-regulatory signaling mechanisms that enhance therapeutic responses. Among various signaling cascades, the platelet-activating factor receptor (PAFR) pathway has been implicated in supporting tumor growth and limiting therapeutic efficacy in cancer models, including non-small cell lung cancer (NSCLC).

Aims: Our studies sought to address two major questions: (1) the role of PAFR signaling in the efficacy of DCA and DCA-based combination therapies; and (2) whether targeting the PAFR pathway can enhance the efficacy of these combination therapies.

Hypothesis: PAFR activation may rescue DCA-induces cytotoxicity, while PAFR inhibition may enhance the effects of DCA and DCA-based combination treatments in lung cancer cells.

Methods: We performed a series of assays in NSCLC cell lines treated with DCA, PAFR agonists, and PAFR antagonists, including cell viability, migration, and clonogenic assays.

Results: Our studies demonstrate that DCA inhibits NSCLC cell survival and migration in a dose- and time-dependent manner. PAFR activation attenuates, whereas PAFR antagonism enhances, the anti-migratory and anti-clonogenic effects of DCA. Further studies investigating the effects of PAFR signaling on DCA combinations with MEK and EGFR inhibitors, as well as the underlying mechanisms, are ongoing.

Conclusion: Collectively, our findings demonstrate that PAFR signaling modulates the effects of DCA, combination treatments and may represent a potential target for enhancing the efficacy of DCA-based combination therapies in NSCLC.

Abstract 013 – Big Picture Science Graduate Student

The impact of platelet-activating factor-receptor signaling in the cytotoxic efficacy of Aurora kinase inhibitors in non-small cell lung cancer

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Introduction: Non-small cell lung cancer (NSCLC) remains a difficult-to-treat malignancy, in part because of therapeutic resistance. The Aurora kinase inhibitors alisertib and barasertib have shown promising preclinical activity but limited clinical efficacy, suggesting the involvement of counter-regulatory pathways. Studies, including ours have demonstrated that Platelet-activating factor receptor (PAFR) signaling supports tumor growth, and drives acid sphingomyelinase (aSMase)-dependent release of microvesicle particles (MVPs) implicated in drug resistance.

Hypothesis: PAFR activation can attenuate the cytotoxic effects of alisertib and barasertib through an aSMase-mediated MVP release.

Aims: To determine: 1) the effects of PAFR activation and antagonism on alisertib and barasertib-induced MVP release. 2) whether these agents induce MVP release through an oxidative stress-dependent PAFR-aSMase pathway. 3) Evaluate the functional significance of drug-induced MVPs on NSCLC cell migration.

Methods: NSCLC cells were treated with alisertib or barasertib with or without PAFR agonist/antagonist or aSMase inhibitor, antioxidant N-acetyl cysteine (NAC) or a pancaspase inhibitor. The effects of these agents were assessed using cell survival by viability assay, cell migration by wound-healing assay, and MVPs by NanoSight Tracking analysis. The functional analysis of drug-induced MVPs was analyzed by “added-back” studies by cell migration.

Results: Alisertib and barasertib inhibited NSCLC cell survival and migration in a dose- and time-dependent manner. PAFR activation attenuated, whereas PAFR antagonism enhanced their anti-migratory effects. Both agents induced MVP release through an oxidative stress-dependent PAFR-aSMase pathway, inhibited by PAFR antagonism, aSMase inhibition, and N-acetyl cysteine, but not by pancaspase inhibition, indicating that MVP release is independent of apoptosis. Adding drug-induced MVPs back to untreated NSCLC cells increased cell migration, similar to PAFR activation.

Conclusion: These findings identify a novel PAFR-aSMase-MVP pathway that limits the efficacy of Aurora kinase inhibitors, with MVPs acting as early mediators of therapeutic resistance. Targeting this pathway provides a rationale for enhancing therapeutic responses in NSCLC.

Abstract 014 – Big Picture Science Graduate Student

Platelet-activating factor-receptor-dependent microvesicle particles modify the cytotoxic efficacy of signal transducer and activator of transcription 3 inhibitors in non-small cell lung cancer

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Background: Despite advances in current therapeutic approaches, non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related deaths in the United States, highlighting the involvement of counter-regulatory oncogenic pathways in therapeutic resistance. One such pathway involves signal transducer and activator of transcription 3 (STAT3), which is frequently hyperactivated and promotes carcinogenesis through crosstalk with other signaling pathways, including platelet-activating factor-receptor (PAFR) signaling. Our recent studies have demonstrated that nano-sized subcellular microvesicle particles (MVPs), released in response to stressors, including therapeutic agents, are dependent on PAFR and the enzyme acid sphingomyelinase (aSMase). The ability of MVPs to carry bioactive components, including PAF agonists, into the local environment and systemic circulation enables them to serve as potent mediators of cell-to-cell communication, contributing to drug resistance. Although *in vitro* and preclinical studies have demonstrated promising anticancer activity of small-molecule STAT3 inhibitors, the role of PAFR in regulating their efficacy remains unclear. We hypothesized that PAFR-dependent MVP release attenuates the cytotoxic efficacy of STAT3 small-molecule inhibitors in NSCLC.

Methods: A549 and H1299 NSCLC cell lines were treated with the STAT3 inhibitors C188-9 and Stattic, with or without inhibitors of PAFR, aSMase, NF-κB, MAPK, apoptosis, or antioxidant. MVPs were isolated and analyzed using nanoparticle tracking analysis (NTA). The functional significance of Stattic-induced MVPs was assessed using a wound-healing assay.

Results: C188-9 and Stattic induced MVP release in a dose-dependent manner, which was attenuated by inhibition of PAFR, aSMase, NF-κB, or MAPK. Pretreatment with the antioxidant N-acetyl cysteine (NAC), but not a pan-caspase inhibitor, also blocked MVP release, indicating that MVP release is associated with oxidative stress, and independent of apoptosis. Importantly, add-back of Stattic-induced MVPs to untreated A549 cells increased cell migration, similar to PAFR activation, supporting a role for MVPs as early mediators of resistance to Stattic.

Conclusions: Collectively, these findings demonstrate that PAFR-dependent MVP release contributes to reduced efficacy of STAT3 inhibitors and identify the PAFR-aSMase axis as a potential therapeutic target for enhancing the efficacy of STAT3 inhibitors in NSCLC.

Abstract 015 – Big Picture Science Graduate Student

Effects of Combined Formaldehyde Exposure and Hypertension on Aortic Function in Mice

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Formaldehyde (FA) is a reactive chemical present in both environmental and occupational settings. Short-term FA exposure reduced vascular function and increased oxidative stress in young healthy women, yet the effects of repeated FA exposure on vascular function remains to be determined. Hypertension impacts about half of U.S. adults, yet its impact on effects of FA exposure are unstudied. Thus, we hypothesized that FA exposure would harm endothelium-dependent aortic relaxation – an effect likely worsened in the presence of hypertension.

To test this hypothesis, aortic function was assessed after a 3-week FA exposure and after a two-week recovery in normotensive and hypertensive female mice. Female C57BL/6J mice were implanted with osmotic minipumps that released either saline or angiotensin II (ANG II; 2.5 mg/kg/d). After 1 week, mice were exposed to either air or FA (5 ppm, 6 h/d) for 21 days. Daily body weight was tracked, and blood pressure was measured regularly with a noninvasive tail cuff. Aortic rings were evaluated by myography ex vivo immediately after exposure or after two-week recovery. Concentration-response curves were created using phenylephrine (PE) to evaluate contraction, acetylcholine (ACh) for endothelium-dependent relaxation, and sodium nitroprusside (SNP) for endothelium-independent relaxation. The relaxation efficacy of ACh and SNP was the % relaxation of maximum contractions to PE and PE+L-NAME, respectively. Sensitivity (EC50, the concentration producing 50% maximal response) was calculated from response curves normalized to 100% response and expressed as pEC50.

Immediately after FA exposure, maximal ACh-mediated relaxation was 82.9±1.7% (n=5) in normotensive mice and 80.7±3.9% (n=7) in hypertensive mice (P=0.616). SNP-mediated relaxation was 98.7±0.8% (n=5, normotensive) and 96.1±1.5% (n=6, hypertensive; P=0.162), with pEC50 values of 8.09±0.07 and 7.48±0.13, respectively (P=0.0045). After recovery, ACh-mediated relaxation in normotensive mice was 84.9±3.9% (n=6) following FA exposure and 80.2±4.9% (n=5) following air exposure (P=0.482). In hypertensive mice, values were 76.4±3.0% (n=4, FA) and 55.0±7.4% (n=5, air; P=0.040). Recovery SNP pEC50 values were 7.82±0.13 (FA) and 7.75±0.04 (air) in normotensive mice (P=0.598), and 7.29±0.19 (FA) and 6.99±0.13 (air) in hypertensive mice (P=0.234). Values shown are mean ± SEM.

Following recovery, FA-exposed hypertensive mice exhibited greater endothelium-dependent aortic relaxation than air-exposed hypertensive mice, contrary to the hypothesized worsening of endothelial dysfunction.

Poster Presentation Abstracts

Abstract 016 – Early-Stage Investigator

Defining the Genomic Landscape of Human Breast Epithelial Cells Malignantly Transformed by Chronic Cadmium Exposure

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Background: Rapid industrialization has markedly increased human exposure to toxic heavy metals, including cadmium (Cd), a group 1 human carcinogen. Cd exposure increases breast cancer risk in women, and Cd levels are elevated in breast tumors. Human breast epithelial cells are malignantly transformed by chronic Cd exposure (2.5 μM, 40 wks). Mechanistically, Cd increases DNA double-strand break (DSB) burden while suppressing DNA damage response signaling, including ATM and BRCA1 activation required for homologous recombination repair. Previous studies have shown that when homologous recombination repair is disrupted, cells increasingly rely on more error-prone DSB repair pathways, promoting genomic instability and carcinogenesis. Reliance on error-prone DSB repair processes can leave characteristic patterns of genomic alterations in surviving cell populations, providing a record of error-prone DSB repair mechanisms utilized during carcinogenesis.

Hypothesis: Chronic Cd exposure promotes human breast epithelial cell malignant transformation by impairing homologous recombination.

Methods: To address our hypothesis, genomic long-read sequencing (PacBio) was performed on passage-matched MCF10A cells, comparing unexposed cells (0 μM, 40 wks) to those malignantly transformed by long-term Cd exposure (2.5 μM, 40 wks). Applied Spectral Imaging (ASI) was used for karyotyping to assess chromosomal instability. Somatic and structural variants were identified relative to the hg38 human reference genome, compared between exposure conditions, and evaluated by chromosomal distribution and genomic context.

Results: Our analysis revealed variants inherent to MCF10A cells and condition-specific alterations. Unexpectedly, Cd-associated mutational signatures included those associated with mismatch repair, nucleotide excision repair, and base excision repair deficiencies. Cd- associated structural alterations were disproportionately enriched on chromosome 19, and many localized within repetitive genomic regions. Deletion junction homology revealed a greater proportion of junctions consistent with impaired homologous recombination.

Conclusions: Our study is the first to define mutational signatures associated with chronic heavy metal exposure during carcinogenesis. Cd-transformed cells exhibit a distinct repair- deficient mutational profile that does not recapitulate canonical hereditary BRCA1-associated homologous recombination deficiency.

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Abstract 017 – Postdoctoral Trainee

Targeting Lipid Metabolism Mitigates the AgNP-induced Pulmonary Inflammation in a Metabolic Syndrome Mouse Model

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Evaluations of nanoparticle safety have concentrated on healthy models; however, epidemiological studies demonstrate individuals with metabolic diseases are more vulnerable to particulate air pollution. Our previous research identified specific alterations in lipid mediators involved in inflammatory regulation in metabolic syndrome (MetS) mice, as well as an increase in lung inflammation following exposure to silver nanoparticles (AgNPs). This exacerbated response was associated with decreases in pulmonary levels of specific specialized pro-resolving mediators (SPMs) and elevated pulmonary gene expression levels of epoxide hydrolase 2 (Ephx2), which encodes soluble epoxide hydrolase (sEH). she hydrolyzes anti-inflammatory epoxy fatty acids, facilitating their clearance. Inhibiting sEH may be beneficial for resolving inflammation by enhancing lipid mediators responsible for resolution signaling. We hypothesize that modulating resolution lipid mediator availability may address susceptibility. To test this hypothesis two cohorts of mice were either administered a sEH inhibitor, 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), to improve the bioavailability of epoxyeicosatrienoic acids (EETs) SPMs prior to AgNP exposure or treated with specific SPMs following AgNP exposure. To examine this hypothesis, C57BL/6J healthy and MetS mouse models were exposed to either a vehicle (control) or 50 µg of 20 nm AgNPs via oropharyngeal aspiration. Mice in the sEH inhibitor cohort were treated with 0.15% DMSO (vehicle/control) or TPPU (10mg/L) via drinking water ad libitum for one week prior to AgNP exposure to inhibit sEH. Mice in the SPM treatment cohort were treated 24 h post-exposure with either a vehicle (control) or 400 ng of RvE1, PD1, or MaR1 via oropharyngeal aspiration. MetS mice treated with TPPU prior to AgNP exposure demonstrated decreased neutrophilia infiltration. This decrease in neutrophils was associated with lower expression levels of pro- inflammatory cytokine genes. In the DMSO-treated AgNP-exposed mice, the expression of the resolution inflammatory cytokine gene IL-10 was reduced while TPPU treatment increased IL- 10 levels in both mouse models. SPM treatments differentially reduced neutrophil infiltration and proinflammatory cytokine levels in MetS mice following AgNP exposure. Specifically, Protectin D1 (PD1) and Maresin 1 (MAR1) promote resolution via inflammatory cytokine IL-10. Overall, enhanced inflammatory responses induced by particulates due to MetS may be treated via modulation of lipid metabolism to enhance resolution signaling, thereby benefiting susceptible subpopulations.

Abstract 018 – Postdoctoral Trainee

Proteomics and Metabolomics Reveals Persistent Changes after a Transient Developmental Methylmercury (MeHg) Exposure to a Human Stem Cell-Based Cortical Model

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Early developmental exposure to methylmercury (MeHg) is linked with life-long persistent neurological anomalies. Reports have linked these persistent effects with changes in cellular epigenetics, but an understanding of the role of the proteome and metabolome in causing or contributing to the persistent effects remains poorly understood. Here using an *in vitro* brain developmental model of hiPSC-derived cortical cells, we hypothesize that a transient developmental exposure to environmentally relevant concentrations of MeHg (1,10 and 100 nM) would cause persistent proteomic and metabolomic changes in immature neurons well beyond the exposure window. During the study, cells were exposed for 6 days over the early neurodevelopmental phase to MeHg concentrations, followed by a ~30-day post-exposure period and then the cells were analyzed using proteomics, metabolomics and immunofluorescence. Our study found that even though MeHg exposure had ceased, changes in cellular proteome were still detected. Specifically with proteins and functionally associated pathways linked to cell proliferation, mitochondrial function, and proteostasis. Untargeted metabolomics for the same conditions and in a different experiment observed changes in cellular metabolites and metabolomic pathways related to amino acid metabolism. Integrating proteomics and metabolomics findings identified proteins and metabolites that are co-associated under conditions of MeHg persistent effects. Autophagy identified as one of the potential mechanisms linking proteomics and metabolomics was validated with immunofluorescence-based experiments and with markers like p62 and ubiquitin. The autophagy analysis showed that MeHg exposed cells are highly prone to develop autophagy disruptions and protein aggregation. Overall, our work showed that MeHg persistent effects are present at the proteome and metabolome level following a transient neurodevelopmental exposure, thus providing insight into the development of neurological anomalies in the maturing brain due to early-life toxicant exposures.

Abstract 019 – Postdoctoral Trainee

Cessation of Mn Exposure Exhibits Varying Degrees of Cellular Recovery and Latency Between Sub-Chronic and Chronic Low-Dose Mn Exposure in SHSY5Y Cells

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Chronic manganese (Mn) exposure is known to cause Parkinsonian-like symptoms in humans, and cessation of Mn exposure has been linked to delayed onset of neurological dysfunction. Previous studies have shown cessation after a chronic low-dose Mn exposure to induce both persistent and unique latent effects in cortical neurons derived from human induced pluripotent stem cells (iPSCs). However, conducting further studies in iPSCs can be difficult due to lengthy differentiation and culture times to reach a mature neuronal stage. We hypothesized that similar results would be seen in a human neuronal cell line model to reduce time and cost for future studies on chronic Mn exposure with cessation. In the present study, we utilized the SHSY5Y human neuroblastoma cell line differentiated with retinoic acid (RA) to study the effects of sub-chronic (10-day) and chronic (29-day or 30-day) low-dose Mn exposure (0.05 or 0.5 μM) with and without cessation (1-day or 10-day) using bulk RNA- sequencing. Chronic exposure resulted in more differentially expressed genes (DEGs) compared to sub-chronic. Sub-chronic exposure with 10-day cessation resulted in near-complete cellular recovery. Chronic exposure with 10-day cessation resulted in both persistent and unique latent DEGs similar to results in iPSCs. Chronic exposure with 1-day cessation greatly resembled chronic exposures with no cessation. These results demonstrate that cessation of Mn exposure can result in persistent and latent/novel transcriptional regulation compared to chronic exposures and further suggests that the length of Mn exposure can impact the cellular response to cessation. Additionally, we show that RA-differentiated SHSY5Y cells can be utilized to study the effects of chronic Mn exposure with and without cessation with similar results to the more complex iPSC models.

Abstract 020 – Postdoctoral Trainee

ICP-MS: what is it, what feature it has and what is our new insight/application?

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In this presentation, we will discuss the common techniques, including Inductively Coupled Plasma Mass Spectrometry (ICP-MS), ICP Optical Emission Spectrometry (ICP-OES), and Atomic Absorption Spectroscopy (AAS). Among these, ICP-MS offers exceptional sensitivity, reaching parts-per-trillion (ppt) levels for many elements, while rapidly measuring multiple metals in a single sample. Its broad elemental coverage, wide dynamic range, relatively straightforward sample preparation, and isotope-specific measurement make it especially powerful for diverse research and analytical applications. The high sensitivity and multi-element capability of ICP-MS make it particularly valuable for analyzing limited and archived biological specimens. Our recent study to compare measurements of 25 metals in 30 paired frozen and formalin-fixed paraffin-embedded (FFPE) human liver tissues using ICP-MS, which has addressed whether metal levels and interindividual variability are preserved after FFPE processing. The new insights are that cadmium (Cd), iron (Fe), and zinc (Zn) showed both linear and rank-based correlations between frozen and FFPE tissues, while selenium (Se) showed a moderate relationship. This study suggests that archived FFPE tissues may provide a valuable resource for retrospective ICP-MS-based metallomics and disease research.

Abstract 021 – Postdoctoral Trainee

Establishing *in vivo* and *in vitro* approaches to investigate urobiome metabolism of environmental chemicals and bladder cancer development

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Interactions between host cells, environmental chemicals, and microbes play a critical role in bladder health and bladder diseases, including bladder cancer (BC). Polycyclic aromatic hydrocarbons (PAHs) are environmental chemicals linked to bladder cancer that are a byproduct of incomplete combustion and are prevalent in charred foods, automobile exhaust, and cigarette smoke, with diet and tobacco smoke serving as primary exposure routes. Benzo[a]pyrene, a prototypical PAH, can be metabolized into secondary metabolites that induce pro-inflammatory, mutagenic, and carcinogenic responses in host tissues. While human cells are known to metabolize BaP, it remains unclear whether bladder-associated microbes can metabolize BaP into reactive intermediates and contribute to inflammatory, mutagenic, or carcinogenic effects in bladder tissues. Importantly, previous studies have shown compositional differences between the urinary microbiome of healthy individuals and those with BC, suggesting potential microbial contributions to disease pathogenesis. Finally, it remains unknown if urinary microbiomes remain stable or dysbiotic after long-term exposures to BaP. To address these gaps, we combined ***in vivo* and *in vitro* approaches** to investigate microbial contributions to BaP metabolism and its impact on bladder health. *In vivo*, mice (N = 36) were gavaged daily with BaP for four weeks, and fecal and urine samples were collected weekly for targeted metabolomics, 16S rRNA amplicon sequencing, and metagenomic sequencing. In a parallel *in vitro* study, a bladder-associated microbial isolate *Pseudomonas aeruginosa* was **co-cultured in the presence of but sans contact with urothelial cells (HBLAK) in a trans-well system, enabling BaP diffusion between compartments**. HBLAK cells exposed to BaP with or without *Pseudomonas* showed increased cytotoxicity in a dose-dependent manner. Cytotoxicity of HBLAK cells increased from 5.5% with BaP alone to 17% when BaP was combined with *Pseudomonas aeruginosa.* Spent media will be analyzed for BaP and its metabolites via targeted metabolomics. In the *in vivo* mouse study, we anticipate distinct microbial community differences between treatment groups, characterized by an increased abundance of taxa involved in BaP and secondary metabolite metabolism. This methodological framework enables controlled assessment of host-microbe-chemical interactions and PAH metabolism across biological system, providing a foundation for elucidating microbial contributions to bladder cancer development.

Abstract 022 – Postdoctoral Trainee

Sex-Dependent Effects of Early-Adulthood Trichloroethylene Exposure on Metabolic Dysfunction Induced by Later-Life High-Fat Feeding in Humanized PPARα Mice

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Background: Active military personnel are frequently exposed to various chemicals and toxicants during their service, including trichloroethylene (TCE), a solvent primarily used in dry cleaning. Between 1953 and 1987, TCE contaminated the drinking water at USMC Base Camp Lejeune, North Carolina. Previous studies have suggested a link between chronic solvent exposure and hepatic steatosis, with effects that may persist even after exposure ends. Additionally, the "two-hit" model—where solvent exposure is followed by a high-fat diet (HFD)—has been shown to exacerbate high-fat diet-induced metabolic dysfunction-associated steatotic liver disease (MASLD). Sex is a modifying factor in the development and progression of MASLD. Metabolites of TCE activate the transcription factor PPARα, a major regulator of lipid metabolism in the liver; however, there are known differences between mouse and human PPARα.

Hypothesis: TCE exposure in early adulthood exacerbates high-fat diet-induced metabolic dysfunction and liver injury later in life, in a sex-dependent manner, using a humanized PPARα mouse model.

Methods: Male and female humanized PPARα C57BL/6J mice aged 10-12 weeks were fed a low-fat diet (LFD; 10% fat) and exposed to either 5 mg/mL of TCE or 1% ethoxylated castor oil (used to emulsify TCE) in drinking water for 12 weeks. This was followed by 8 weeks of either a high-fat diet (HFD; 42% fat) or LFD feeding. Body weight and water consumption were measured each week. EchoMRI was performed at the end of both the 12 weeks of TCE exposure, then at the end of the 8 weeks of HFD or LFD feeding. A glucose tolerance test (GTT) was performed at week 19. At the end of week 20, mice were euthanized, and plasma and tissue samples were collected for downstream analyses. Statistical comparisons were performed using two-way ANOVA, followed by Tukey's post hoc test, with a significance threshold of $p \leq 0.05$.

Results: Both male and female mice fed a HFD with or without exposure to TCE exhibited increased body weight, fat mass, and white adipose tissue at the end of the 20 weeks. However, the males fed HFD with or without exposure to TCE showed significantly increased relative liver weights, elevated markers of liver injury—alanine aminotransferase (ALT) and aspartate aminotransferase (AST) but these remained unchanged in the females. GTT indicated glucose intolerance in both male HFD-fed groups, but in the females, only the HFD-fed group was. Histological analysis revealed increased steatosis in both HFD-fed males and females, with or without TCE exposure, with a greater effect in males, as confirmed by hematoxylin and eosin (H&E) staining.

Conclusion: The study demonstrated sex-specific differences in hepatic injury that were driven by diet, with male mice exhibiting greater susceptibility to liver injury and toxicity than females. Prior exposure to TCE did not significantly alter these sex-dependent effects.

Abstract 023 – Postdoctoral Trainee

Gestational Cigarette Smoke Exposure Alters Gut Microbial Diversity in Dams with Sex-Specific Effect in Exposed Offspring

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Background: Cigarette smoking during pregnancy poses myriad health risks to both mother and child. Even though maternal smoking rates have dropped precipitously in the United States, states such as West Virginia and Kentucky lag behind this national trend. Those born to mothers who smoke during pregnancy are more likely to exhibit low weight at birth, which predisposes them to develop metabolic disease later in life. This could be due to many possible factors, though one less explored route is through the alteration of the gut microbiome, as changes in the gut microbial community are associated with metabolic disease development.

Methods: To determine how cigarette smoking affects the gut microbiome of both the mother and her offspring, we exposed C57BL/6 mice to either filtered room air (sham exposure, n=20) or the smoke generated from one pack of Marlboro Red cigarettes (CSE, n=30) for 3 hrs/day, starting two days before mate pairing and ending the day before the birth of the litter. Fecal samples were collected from pregnant dams during the exposure period and at the time of weaning, while cecal samples were collected from offspring two days after weaning. Microbiome samples were sent to the Baylor Alkek Center for Metagenomics and Microbiome Research for 16Sv4 rRNA sequencing.

Results: We observed an increase in Operational Taxonomic Units in CSE dams during exposure (sham n=14, CSE n=15), which did not persist to the time of weaning at three weeks after last smoke exposure. Mixed-sex analyses comparing CSE vs sham weanlings (n=12-15) found differences in both alpha and beta diversity measures. We observed a sex-specific effect of CSE on beta diversity measured by unweighted UniFrac. Relative abundance analysis comparing male and female CSE offspring revealed an increased abundance of *Firmicutes* and a decreased abundance of *Bacteroidota and Cyanobacteria* in females.

Conclusions: While limited in scope, these findings suggest that gestational CSE alters gut microbial community structure in offspring, which could contribute to disease manifestation. Additionally, our findings from the dams support existing evidence that cigarette smoking alters the gut microbiome, which resolves after the cessation of smoking.

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Abstract 024 – Postdoctoral Trainee

Evaluating mitochondrial correlates of persistent alterations in sirtuin signaling pathway after developmental methylmercury exposure in hiPSC-derived cortical cultures

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Background: Methylmercury (MeHg) is a well-known environmental neurotoxicant with human exposure mainly through dietary sources such as fish and rice. Developmental exposure to MeHg can lead to long-lasting neurotoxic effects that persist in adulthood. Sirtuins (SIRT1-7), are NAD+-dependent enzymes involved in neurodevelopment and mitochondrial biogenesis. Dysregulation of SIRT signaling has been implicated in various neurodegenerative disorders, as well as in mediating the toxic effects of environmental pollutants. We hypothesized that developmental exposure to MeHg disrupts SIRT-dependent signaling, leading to persistent neurotoxic effects, and that modulation of this pathway could either mitigate or exacerbate MeHg-induced phenotypes.

Methods: Human induced pluripotent stem cells (hiPSCs) were exposed to non-cytotoxic doses of MeHg (1, 10, and 100 nM) during cortical neuronal differentiation (D7–D13). SIRT1 expression and localization were assessed by immunofluorescence at D14, D40 and D150. We assessed PGC-1α acetylation as an indirect measure of SIRT1 activity. scRNA-seq and proteomics data were analyzed using R and Ingenuity Pathway Analysis (IPA) to identify sirtuin pathway components and their connectivity to the different isoforms of SIRTs. We also modulated SIRT1 activity with a selective inhibitor (EX-527) to assess its impact on mitochondrial function, mitochondrial membrane potential, and morphology.

Results: SIRT1 protein expression and localization were not significantly altered. After EX-527 treatment, cells exposed to MeHg showed greater PGC-1α acetylation, suggesting altered SIRT1-associated deacetylation. Several differentially expressed genes detected by our scRNA-seq experiments are associated with mitochondrial electron transport chain (ETC) complex I. Proteomics showed no statistically significant changes in SIRT isoform abundance but identified sirtuin pathway proteins with mitochondrial functions. MeHg exposure reduced mitochondrial membrane potential ($\Delta\Psi m$) and basal respiration. EX-527 increased Δψm to near control levels but further impaired mitochondrial respiration in MeHg-exposed cells. These findings suggest altered downstream signaling despite unchanged SIRT1 expression and localization, with possible involvement of other sirtuins.

Conclusions: Together, these data help clarify if sirtuin signaling pathway contributes to the mitochondrial phenotypes and the extent to which it contributes to the long-term effects of MeHg-induced persistent neurotoxicity. This work was supported by NIH [R01 ES07331 (ABB) and R01 AG080917 (ABB)].

Abstract 025 – Postdoctoral Trainee

NCOA4-mediated ferritinophagy in ozone-induced asthma exacerbation

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Ground-level ozone (O_3) is a gaseous environmental hazard formed from primary air pollutants reacting with heat and sunlight. Increasing global temperatures are predicted to increase ground-level concentrations of this highly reactive gas, which causes damage to sites of respiration in plants and animals. In humans, O_3 exposure increases morbidity of cardiopulmonary diseases, including asthma. However, mechanisms of O_3-induced asthma exacerbation are understudied. It is known that O_3 inhalation induces pulmonary iron accumulation, which is independently associated with asthma severity. Iron can be released from iron-storing proteins (ferritin) through autophagy (ferritinophagy), which is also associated with respiratory disease. We hypothesized that ferritinophagy drives excess iron and mediates O_3-induced asthma exacerbation. To test this, we obtained primary human bronchial epithelial cells (HBEC) from asthmatics and non-asthmatics to assess baseline intracellular iron. We also collected bronchoalveolar lavage fluid (BALF) from asthmatic and non-asthmatic humans following exposure to filtered air (FA) or 0.200 ppm O_3. Finally, we generated a mouse model of O_3-induced exacerbation of allergic airway inflammation (AAI), whereby C57BL/6J mice (12 weeks old) were sensitized to and challenged with house dust mite (HDM) or vehicle control (PBS) and then exposed to FA or 1 ppm O_3 for 3 hours. Our preliminary data indicates that baseline iron levels are higher in HBECs isolated from individuals with asthma compared to non-asthma controls. Following an acute O_3 exposure, BALF iron levels increased in humans with asthma but not non-asthma controls. Additionally, AAI mice exposed to O_3 had a significant increase in airspace iron compared to both FA and non-AAI controls. This coincided with increased pulmonary inflammation and production of the type 2 cytokine, IL-13. Finally, O_3 increased mouse lung tissue expression of nuclear coactivator receptor 4 (*Ncoa4*) and ferritin (*Fth1*), which respectively initiates ferritinophagy and can be upregulated in response. Combined, these data suggest that the environmental toxicant, O_3, can induce NCOA4-mediated ferritinophagy, leading to excess airspace iron, and driving asthma exacerbation. Iron overload may also serve as a mediator of O_3-induced toxicity in other biological models. Future investigations will assess the efficacy of NCOA4 inhibitors in reducing O_3-induced asthma exacerbation and iron dysregulation overall.

Abstract 026 – PhD Student

Investigating the Effects of Overexpression of miR-576-5p in Human Keratinocytes

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Background: Environmental inorganic arsenic is an IARC class 1 human carcinogen affecting >200 million people that causes skin, lung, and bladder cancers. Its carcinogenic mechanism remains unclear. MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression by inhibiting translation or degrading mRNA. We identified five miRs dysregulated in both tumors from patients exposed to environmental arsenic and our in-vitro arsenic exposure skin cancer model. hsa-miR-576 (miR-576), had elevated expression in tumors and at least one timepoint in our model, suggesting a role in skin carcinogenesis.

Hypothesis: miR-576 overexpression will suppress proteins involved in biological pathways known to be dysregulated by arsenic.

Methods: Ker-CT cells were transduced with miR-576 lentiviral particles. Six clones of miR-576 transduced cells were isolated and propagated. Mature miR-576-5p and -3p expression was measured with TaqMan RT-qPCR assays using hsa-miR191-5p as a normalization control. miR-576-5p was predominantly expressed in Ker-CT cells, so miR-576-5p target mRNA predictions were produced using three miR-mRNA binding databases: TargetScan, Diana-microT and miRDip. The highest expressing miR-576 Ker-CT clone was chosen for further investigation and compared to a Ker-CT cell line transduced with a scrambled control miR (miR-SC).

Results: Compared to non-transduced Ker-CT, miR-576-5p expression increased 89–260 fold and 3p expression 9–29 fold in the transduced cell lines. Two mRNA targets (Cul3 and ZMAT3) that participate in pathways that could contribute to cellular transformation were selected from 73 predicted miR-576-5p target mRNAs common to all three databases. Cul3 and ZMAT3 are involved in ubiquitination and mitochondrial respiration pathways respectively. Steady state levels of CUL3 and ZMAT3 mRNAs were determined and CUL3 expression was lower in miR-576 overexpressing cells. CUL3 transcripts contain 6 miR-576-5p seed binding sites in the 3'UTR with two sites in proximity. CUL3 and ZMAT3 mRNA half-lives were determined by RT-qPCR in miR-576 and miR-SC cells to determine if miR-576 decreased CUL3 was caused by increased degradation rate.

Conclusions: One of the two predicted gene targets of miR-576-5p showed decreased expression by RT-qPCR. Results indicate that baseline expression of CUL3 was lower in miR-576 Ker-CT cells when compared to mir-SC Ker-CTs with no change in mRNA half-life indicating miR-576 does not suppress CUL3 mRNA by increasing degradation rate. Further studies must validate these findings for CUL3 and other targets.

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Abstract 027 – PhD Student

A Toxicology-Guided Chemically Induced Demyelination Model in Zebrafish for Multiple Sclerosis Drug Discovery

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Multiple sclerosis (MS) is a neurological disease characterized by myelin damage and impaired communication within the central nervous system. Despite advances in MS treatment, preclinical models are still needed to rapidly identify compounds with the potential to promote remyelination following demyelinating injury. This study aims to develop a dose- dependent, chemically induced larval zebrafish model as a pre-screening platform for evaluating candidate therapeutics after demyelination. Using toxicology-based dose selection, this approach is designed to identify tolerated concentrations and prioritize compounds with remyelination-associated activity.

Zebrafish provide several advantages for modeling neurological disease. Central nervous system development occurs rapidly during the first three days after fertilization, allowing neurological phenotypes to be studied early. Their rapid development and suitability for aqueous drug exposure enable efficient evaluation across multiple concentrations.

Preliminary observations from our laboratory indicate that cuprizone exposure produces demyelination-associated changes in larval zebrafish, assessed by RT-qPCR analysis of the myelin-associated genes *mbp, plp*, and *mag* and Luxol Fast Blue staining. Cuprizone altered myelin-associated gene expression and reduced myelin staining, while the MS-approved drug teriflunomide increased gene expression and staining, supporting continued dose- dependent characterization of the model. Wild-type larvae will undergo dose-dependent evaluation of candidate compounds to establish tolerability and identify biologically active concentrations below overt toxicity thresholds. Cuprizone will induce a demyelination- associated phenotype, followed by treatment with approved MS therapies or experimental compounds. Therapeutic response will be assessed using RT-qPCR and myelin staining of sectioned larvae and integrated with concentration-response data to distinguish remyelination-associated effects from toxicity. Experiments are underway, and progress and preliminary findings will be shared at the meeting.

Continued dose-response studies will define relationships among cuprizone exposure, myelin-associated gene expression, and myelin integrity. Effective candidates are expected to shift these endpoints toward control conditions while remaining within tolerated concentration ranges. By integrating dose-response relationships, toxicity thresholds, gene expression, and myelin measures, this model aims to provide a rapid in vivo strategy for prioritizing therapeutic candidates for further MS investigation.

Abstract 028 – PhD Student

PAFR–aSMase–Microvesicle signaling links thermal burn injury to systemic inflammation, amplified by aging.

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Thermal burn injury (TBI) begins in the skin, and severe TBI can rapidly trigger systemic inflammation throughout the body. However, how local injury produces distant organ effects and why the same burn may be more harmful in an older host remains unclear. We investigated the impact of burn size and aging on the Platelet-activating factor receptor (PAFR)–acid sphingomyelinase (ASM)–microvesicle particle (MVP) response and whether this pathway can be pharmacologically modified. Our hypothesis is that TBI generates PAF-laden MVPs from skin that act on gut to cause bacteria release and multiple organ dysfunction (MOD).

Young wild-type (WT), PAFR-deficient, and SMPD1/ASM-deficient mice underwent graded thermal burns. Blood plasma MVPs, mesenteric lymph node colony-forming units (MLN CFU) to assess gut permeability, and organ inflammation to assess MOD were assessed 2 hours after injury. We also evaluated young (~2 months) and aged (>18 months) cohorts after the same 12.5% total body surface area (TBSA) burn. Intestinal alkaline sphingomyelinase/ ENPP7 expression was measured by RT-qPCR and Western blotting. In HaCaT keratinocytes, functional inhibitors of acid sphingomyelinase (FIASMA) like imipramine, amitriptyline, and fluoxetine were tested for effects on burn-induced MVP release and cell viability. Exploratory pre- and post-burn treatment was also evaluated in human skin explants.

In WT mice, larger burns produced greater circulating MVP responses. MLN CFU increased about 5-fold after 25% TBSA burns and 20-fold after 37.5% burns versus non-thermal controls. Responses were reduced in PAFR- and aSMase-deficient mice. Post-burn treatment with topical imipramine was associated with lower circulating MVPs and MLN CFU in the 37.5% burn cohort. Aged mice exposed to the same 12.5% burn showed rapid early bacterial translocation, pulmonary and renal inflammation without a greater MVP response. The mechanism for the increased toxicity of burns in aged mice was attributed to decreased intestinal PAF-degrading capacity (lower gut alkaline sphingomyelinase mRNA levels and protein expression) than in young mice. FIASMAs reduced burn-induced MVP release in HaCaT cells, and exploratory human skin studies showed reduced MVP release when amitriptyline was applied immediately or 5 minutes after burn.

These findings suggest that burn severity influences the magnitude of the systemic injury signal, while aging may increase host vulnerability to that signal. The results link local skin injury to systemic inflammation and support further study of the PAFR–aSMase–MVP pathway as a potential therapeutic target to treat acute severe TBI.

Abstract 029 – PhD Student

Development and Characterization of a CRISPR/Cas9-Generated *oprm1*-Deficient Zebrafish Model for Investigating Fentanyl Toxicity

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Opioid overdose remains a critical public health challenge, with fentanyl and related synthetic opioids contributing substantially to overdose mortality through central nervous system suppression and respiratory depression. Although opioid antagonists such as naloxone are effective countermeasures, the molecular mechanisms underlying opioid-induced toxicity remain incompletely understood and additional administration is required in most cases. The μ-opioid receptor (MOR), encoded by *oprm1*, is the primary molecular target of fentanyl and mediates many of its pharmacological effects. However, the contribution of MOR-dependent and MOR-independent mechanisms to opioid-induced toxicity remains incompletely characterized. A genetically tractable vertebrate model lacking functional *oprm1* expression would provide an important tool for investigating these mechanisms.

CRISPR/Cas9 genome editing was used to target the *oprm1* gene in zebrafish. Following genome editing, candidate mutants were identified through molecular genotyping and evaluated for changes in *oprm1* expression using qPCR melt curve. *Oprm1* protein expression was assessed by Western blot analysis to further characterize the molecular phenotype. The functional response of the model to fentanyl was evaluated using a high-throughput photo-motor response (PMR) assay in 7 days post-fertilization (dpf) larvae. Locomotor activity was quantified following light-dark transitions in wild-type and *oprm1*-deficient larvae exposed to fentanyl.

CRISPR/Cas9-mediated targeting of *oprm1* produced zebrafish with reduced *oprm1* transcript expression relative to wild-type controls. Molecular characterization by qPCR supported disruption of *oprm1* expression, while Western blot analysis demonstrated residual *oprm1* protein expression, indicating an incomplete molecular loss-of-function phenotype due to maternal transcripts. To begin functional characterization of the model, fentanyl-induced behavioral responses were assessed using the PMR assay. Fentanyl produced concentration-dependent reductions in larval locomotor activity. The behavioral phenotype observed in *oprm1*-deficient larvae did not demonstrate the anticipated complete attenuation of fentanyl-induced activity suppression, suggesting that additional mechanisms may contribute to fentanyl-induced behavioral toxicity.

We are developing and molecularly characterizing a CRISPR/Cas9-generated *oprm1*-deficient zebrafish model for investigating opioid toxicity. Reduced *oprm1* transcript expression combined with residual *oprm1* protein provides evidence of a partial molecular loss-of-function phenotype and highlights the importance of multi-level characterization when establishing genetically modified models. Continued characterization and establishment of stable mutant lines will provide a foundation for dissecting MOR-dependent and MOR-independent mechanisms of fentanyl toxicity and for evaluating potential therapeutic strategies to mitigate opioid-induced adverse effects.

Abstract 030 – PhD Student

Repetitive Traumatic Brain Injury: Exposure, Cofilin Dysregulation, and Neuroinflammation

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Traumatic brain injury (TBI) is traditionally viewed as an acute neurological event; however, repeated injuries can produce persistent cellular and molecular abnormalities. Repetitive TBI presents a toxicological challenge in which repeated mechanical insults may disrupt cellular homeostasis and increase vulnerability to long-term neurological dysfunction. The influence of exposure frequency and recovery intervals on these outcomes remains poorly understood.

Our research investigates cofilin, an actin-regulating protein that becomes dysregulated under cellular stress and can form abnormal aggregates known as cofilin rods. We hypothesize that repetitive TBI induces cofilin dysregulation, contributing to cytoskeletal abnormalities, neuroinflammation, and neurological dysfunction. Preliminary findings demonstrate increased cofilin following moderate TBI and cofilin–actin aggregate formation within microglia, suggesting a potential link between actin dysregulation and persistent neuroinflammation.

To investigate the effects of injury frequency and recovery time, we compared two mouse models of repetitive closed-head TBI: injuries administered every 48 hours versus weekly injuries. Cognitive function was assessed using T-maze alternation and Morris water maze tasks, while open field testing evaluated locomotor activity and anxiety-related behavior. Motor function was assessed using rotarod and grip strength tests. Postmortem immunohistochemistry and serum ELISA characterized cofilin distribution and associated molecular changes.

Both models exhibited expected differences relative to their respective sham controls, but no significant differences in cognitive or motor outcomes were detected between injury schedules. However, immunohistochemistry and ELISA revealed differences in cofilin localization and systemic inflammation respectively, associated with recovery time. These findings suggest that the interval between traumatic exposures may influence molecular responses even when behavioral outcomes remain comparable.

This work highlights the importance of exposure frequency and recovery intervals in determining biological responses to repeated injury. Understanding how cofilin dysregulation contributes to these responses may reveal therapeutic targets for mitigating persistent neuroinflammation and long-term neurological consequences following repetitive TBI.

Abstract 031 – PhD Student

TCDD-Induced AhR Signaling and Genetic Variation in the IGH 3' Regulatory Region: Implications for Antibody Production

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Antibodies are important components of adaptive immunity and contribute to protection against extracellular pathogens. Antibodies neutralize viruses and toxins, promote pathogen recognition and elimination by other immune cells, and contribute to vaccine-induced protective immunity. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), an environmental toxicant, has been linked to immune suppression, including inhibiting antibody responses. The aryl hydrocarbon receptor (AhR) is a transcription factor that mediates the effects of TCDD. Previous studies using a human Burkitt lymphoma cell line (CL-01) that can be stimulated to drive antibody production and induce class switch to other antibody isotypes (e.g. IgG, IgA, IgE) demonstrated an AhR dependent inhibition of IgG secretion. The immunoglobulin heavy-chain (IGH) gene plays a central role in antibody production and contains a 3' regulatory region (3'RR) that contributes to the regulation of antibody isotype produced. The 3'RR consists of three enhancer regions (hs3, hs1.2, hs4). In humans the hs1.2 enhancer is genetically variant, which may influence sensitivity of antibody production by AhR ligands. Using CRISPR-Cas9 gene editing of the CL-01 cells, we generated clones exhibiting different hs1.2 enhancer alleles. The aim of the current study is to determine if genetic variation in the hs1.2 enhancer influences AhR-mediated inhibition of antibody production. Preliminary studies suggest that sensitivity to TCDD-induced inhibition is dependent on the hs1.2 allele. Understanding this interaction will help clarify how genetic variation within the hs1.2 enhancer shapes the effects of environmental AhR ligands on antibody production.

Abstract 032 – PhD Student

Amitriptyline and imipramine, two functional inhibitors of acid sphingomyelinase, attenuate UVB-induced erythema in photosensitive rosacea subjects

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Rosacea is a common chronic inflammatory disease that is often associated with increased inflammatory responses and sensitivity to ultraviolet (UV) light, temperature changes, and alcohol consumption. Recent studies in cells, mice, and humans suggest that exposure to stressors, such as UVB, leads to the activation of the enzyme acid sphingomyelinase (ASM) in keratinocytes, which results in the release of microvesicle particles (MVPs), causing inflammation and immune suppression. Additionally, murine studies have shown that functional inhibitors of ASM (FIASMs), including two tricyclic antidepressants, imipramine and amitriptyline, have the ability to block MVP release and its resulting inflammation in models of photosensitivity. This study assessed the ability of topical 4% and 10% formulations of FIASMs imipramine and amitriptyline to block erythema reactions in subjects with photosensitive rosacea after UVB exposure.

This double-blinded, placebo-controlled clinical trial (NCT06312813) was conducted in a single center, where subjects with clinical rosacea and controls received either 4% of topical amitriptyline or imipramine on one facial side and a vehicle on the contralateral side. Following the administration of the medication or vehicle, participants were then exposed to a low-fluence (300 J/m^2) of artificial UVB light directly where the medication and vehicle were applied. Erythema, temperature, pain, and pruritus were assessed at baseline, 30 minutes after application of the FIASM, and 10, 60, 120 minutes, and 24 hours post-UVB exposure. The 4% formulations of both FIASMs significantly reduced UVB-induced erythema compared to vehicle treatment. Based on the initial findings with 10 photosensitive rosacea subjects, the trial was escalated to evaluate 10% formulations of topical FIASMs with ongoing enrollment. Additionally, two board-certified dermatologists blinded to the treatment groups graded the erythema at 24 hours post-UVB exposure. These results show that FIASMs reduced clinical erythema at the 10% formulation, but not at the 4% formulation. No side effects of topical FIASM treatment were noted. Altogether, amitriptyline and imipramine, two topical FIASMs, significantly reduced UVB-induced erythema in rosacea. These findings provide premise for a novel therapeutic target for photosensitive rosacea and possibly other light sensitive disorders.

Abstract 033 – PhD Student

FKBP-dCas13b-ALKBH5 m6A mRNA editing system for MASLD-related transcript targeting

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that humans are exposed to in the environment. PCB exposure disrupts endocrine and metabolic systems. Consequently, susceptibility increases for the initiation and progression of metabolic diseases such as metabolic dysfunction associated steatotic liver disease (MASLD). Recent human and rodent studies reported that PCBs alter epigenetics in DNA methylation, circulating miRNAs, and global RNA modifications. Our lab demonstrated that PCB exposures *in vivo* altered N-6- methyladenosine (m6A) in mRNA and other epitranscriptomic marks in total RNA in male mouse liver and decreased lipoprotein transcript stability. m6A is the most abundant mRNA modification and regulates transcript stability, splicing, and translation.

Global knockdown or knockout of m6A methyltransferase METTL3 or m6A demethylase ALKBH5 have widespread transcriptome effects. To study the importance of individual m6A modifications within lipoprotein transcripts associated with MASLD, alternative approaches are needed. Recent advances for mRNA editing use the CRISPR/Cas system. Specifically, the dCas13b protein binds and edits mRNA without RNA cleavage. Additionally, fusing ALKBH5 to an FK506 binding protein (FKBP) variant containing F36V and L106P mutations, which is stabilized by the small molecule Shield-1, allowed for site-directed m6A editing of mRNA in transfected cells. We acquired the HA-tagged FKBP*-dCas13b-ALKBH5 plasmid from Dr. Fu-Sen Liang, Case Western Reserve University. The goal of this study is to use this inducible editing system to erase specific m6A sites in our MASLD-related gene transcripts of interest including Apob.

We first examined the impact of Shield-1 on the viability of AML12 and FL83B mouse hepatocyte cells. We observed a decrease in viability of each cell line at Shield-1 concentrations > 2 μM. We tested the effect of Shield-1 on FKBP*-dCas13b-ALKBH5 protein expression in each cell line. Transfected cells without Shield-1 treatment showed less HA tag expression compared to hepatocytes treated with Shield-1, demonstrating increased stability of FKBP*-dCas13b-ALKBH5. We are currently designing sgRNAs to target m6A sites in Apob for cloning into a pC0043-PspCas13b crRNA backbone for co-transfection experiments to edit m6A in Apob and examine the effect on Apob transcript stability and APOB protein abundance in each cell line with palmitic acid and PCB exposures.

Abstract 034 – PhD Student

Development of a high-throughput assay for analyzing damaged cell-free DNA and identifying pharmacological modulators

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Cell-free DNA (cfDNA) in biological fluids is widely used for noninvasive molecular testing, including diagnosis and treatment monitoring of various diseases, yet whether cfDNA carries damaged DNA adducts has not been well studied. DNA-damaging agents such as ultraviolet B (UVB) radiation, the anti-cancer drug cisplatin, and benzo(a)pyrene diol epoxide (BPDE), a metabolite of a cigarette-smoke carcinogen, cause the formation of cyclobutane pyrimidine dimers (CPDs), cisplatin DNA adducts, and BPDE DNA adducts respectively, in genomic DNA.

We developed a high-throughput, 96-well plate-based assay to detect bulky DNA adducts in cell-free fractions from human keratinocytes exposed to these agents. After removing dead cells and apoptotic bodies by differential centrifugation, we detected DNA adducts using lesion-specific ImmunoDot blotting and measured total cfDNA and cell viability. To study the regulation of damaged cfDNA release, we used pharmacological and targeted genetic approaches to modify DNA-repair and cell-death pathways.

We detected all three types of DNA adducts in cell-free fractions. Depletion of the DNA-repair protein xeroderma pigmentosum group A increased damaged cfDNA signals, while inhibition of caspases involved in programmed cell death reduced cfDNA release. We also screened 2,320 U.S. FDA-approved drugs to identify modulators of cfDNA levels following UVB exposure. The screen identified candidate compounds with increased cell viability and reduced cfDNA levels.

This assay provides a platform for studying damaged cfDNA release and identifying pharmacological modulators. Further validation and testing in biological fluids will help assess its use for detecting DNA adducts in research and clinical samples and monitoring responses to treatment.

Abstract 035 – PhD Student

Anti-inflammatory action of 18:2 cardiolipin treatment in an ex vivo model of alcohol-associated liver disease

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Background: Alcohol-associated liver disease (ALD) is characterized by unresolved chronic inflammation that drives chronic liver injury. Previous studies have demonstrated that unsaturated cardiolipin (CL) species can inhibit lipopolysaccharide (LPS)-mediated TLR4 activation, thereby limiting inflammatory signaling. However, this has not been evaluated in models of ALD where both ethanol (EtOH) and LPS are present. We hypothesized that native 18:2 CL reduces inflammatory responses in mouse and human ex vivo models.

Methods: Whole blood was extracted from naïve male (n = 10) and female (n = 9) C57Bl6/J mice (8-12 weeks) and stimulated for 2 hours with vehicle control, EtOH (40 mM), LPS (1 ng/mL), and native 18:2 CL (1 -1000 nM). Whole blood from male (n = 10) and female (n = 10) human donors were treated in the same experimental paradigm. Cell supernatants were collected and cytokines were measured via a multiplex ELISA (MSD). Data were statistically compared by Kruskal-Wallis test followed by Dunn's post hoc analysis, and $p<0.05$ was considered statistically significant.

Results: In mouse samples, native 18:2 CL reduced IL-2, IL-6, IL-10, and TNFα across all concentrations. IL-2 showed modest reductions (1.0-1.46 -fold). IL-6 was markedly reduced by native CL (8.76-12.63-fold). IL-10 levels were also decreased following native CL treatment (1.76-2.79-fold). TNFα showed the most pronounced reductions with native CL (49.44-139.32- fold). In human samples, the same overall pattern was observed. Native 18:2 CL reduced IL- 2, IL-6, IL-10, and TNFα across all concentrations. IL-2 showed modest reductions (0.62-3.10- fold). Surprisingly, IL-6 showed the strongest response, with great reductions induced by native CL (23.83-48.27-fold). IL-10 was decreased following native CL treatment (1.31-2.01-fold). TNFα was markedly reduced with native CL (19.57-26.30-fold).

Conclusion: Native 18:2 CL attenuates inflammatory cytokine responses under combined EtOH and LPS exposure. This effect was consistent across both mouse and human ex vivo whole blood models. These findings suggest that cardiolipin composition influences inflammatory signaling, potentially through modulation of TLR4-dependent pathways and downstream cytokine production. Collectively, our results support native 18:2 CL as a potential regulator of inflammation in ALD and highlight lipid peroxidation as a key determinant of its immunoregulatory function.

Abstract 036 – PhD Student

Protective Effects of Parsnip Extract, Falcarindiol, and Nodakenin Against Cigarette Smoke Extract-Induced Cytotoxicity and Inflammatory Responses in Bronchial Epithelial Cells

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Cigarette smoking remains a leading cause of preventable disease and death in the United States and contributes significantly to chronic lung diseases through oxidative stress, inflammation, and epithelial injury. Plant-derived bioactive compounds have gained attention for their potential to mitigate smoke-induced cellular damage. Parsnip (*Pastinaca sativa* L.) contains polyphenols, furanocoumarins, and other bioactive compounds with reported antioxidant and anti-inflammatory properties. *However, the effects of parsnip on cigarette smoke-induced airway epithelial injury remain poorly understood*. We *hypothesized* that treatment with parsnip extracts, the bioactive compound falcarindiol, and the dietary bioactive compound nodakenin would reduce cytotoxicity and attenuate inflammatory responses caused by cigarette smoke extract (CSE), with aged parsnip showing greater protective effects than fresh parsnip in bronchial epithelial cells. Human bronchial epithelial BEAS-2B cells were pretreated with fresh or aged parsnip extract, falcarindiol, or nodakenin prior to CSE exposure. Cytotoxicity and cell viability were assessed by LDH and trypan blue exclusion assays, respectively, and inflammatory responses were evaluated by measuring IL-6 and IL-8 levels 24 h after exposure. Exposure to CSE significantly increased cytotoxicity and pro-inflammatory cytokine production compared with untreated controls. Treatment with aged parsnip extract, falcarindiol, and nodakenin significantly attenuated CSE-induced cytotoxicity and decreased IL-6 and IL-8 production. Protective effects were more pronounced with aged parsnip extract compared with fresh parsnip extract, suggesting that aging may influence the bioactivity of parsnip-derived compounds. Aged parsnip extract, falcarindiol, and nodakenin attenuated cigarette smoke-induced cytotoxicity and inflammatory responses in human bronchial epithelial cells. These results warrant further investigation of parsnip-derived and related dietary bioactive compounds and their mechanisms of action in preclinical models of cigarette smoke-induced lung injury. The study was supported by the Purdue AG-HHS One Health Collaborative Seed Grant and National Institutes of Health Grant R21AT012817.

Abstract 037 – PhD Student

Evaluation of Right Ventricular Dysfunction Across Type 1 Diabetic Mouse Models

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In 2023, an estimated 40.1 million Americans had diabetes, among which 2.1 million Americans had Type 1 Diabetes (T1D) which predominantly affects children. Many studies regarding diabetic complications have focused on Type 2 Diabetes (T2D), as this is the most common type (90 – 95%) in the United States. Fewer data have reported complications of patients with T1D, many of whom must manage these complications from a young age. Additionally, many studies detail function in the left ventricle (LV) while the right ventricle (RV) is often omitted. We have previously shown that diabetic female OVE26 mice independently develop LV dysfunction followed by RV dysfunction. This model, however, exhibits blood glucose levels outside of a clinically applicable range. In another preclinical study, the Akita mouse has proven useful in evaluating diabetic cardiac dysfunction in the context of T1D while maintaining more translatable hyperglycemic levels. The present study aims to determine (1) if diabetic RV dysfunction develops independently in the diabetic Akita mouse as in the OVE26 mouse and (2) identify of sex differences in diabetic RV dysfunction, to develop a better preclinical T1D mouse model for its RV and LV cardiac dysfunction.

Male and female C57BL/6-Ins2^{Akita}/J mice were maintained with constant access to food and water. Non-fasted blood glucose levels and body weights were measured at regular intervals. At 10 or 12 months of age, mice of both sexes underwent echocardiography (Echo) to assess systolic and diastolic function (TAPSE and E/E' ratio, respectively) and cardiac morphology (RV free wall). Mice were then euthanized and tissues were collected for further analysis. Statistical analysis was performed using GraphPad Prism 10 software, with a combination of both descriptive statistics and normality tests being used to determine whether the parametric or nonparametric equivalence of the one-way ANOVA or student T-test was to be used. A p-value <0.05 was considered significant.

Diabetic male mice exhibited significantly higher blood glucose ($p<0.0001$) and lower body weight ($p<0.001$) compared to their control male counterparts. In contrast, no significant increase in blood glucose levels or decrease in body weights was seen between diabetic and control female mice in the experimental period. Echo analysis for TAPSE, PAT/PET ratio, E/E' ratio, and RV free wall did not show physiological differences between diabetes and control for both sexes of mice.

These data indicate that, unlike the female OVE26 mouse, male C57-Akita mice are better suited toward investigation of diabetic complications in the long term, which is under further investigation.

Abstract 038 – PhD Student

Integrating Acute Toxicity and Behavioral Profiling to Evaluate the Safety of Muscarinic Receptor Modulators in Larval Zebrafish

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Muscarinic acetylcholine receptor positive allosteric modulators (PAMs) are being developed as potential neurological therapeutics, yet their structural and pharmacological diversity may produce distinct toxicological outcomes.

Mortality-based toxicity screening may overlook sublethal adverse effects and functional disturbances caused by muscarinic receptor modulators. This study investigated concentration- and time-dependent toxicity and its relationship to behavioral responses during light–dark transitions in larval zebrafish.

A chemically diverse compound library was evaluated for mortality and visible abnormalities following exposure to 0.1–100 μM over 10 minutes to 24 hours. Behavioral responses were assessed at 1, 3, and 10 μM using spontaneous swimming and light–dark locomotor assays, measuring thigmotaxis, swimming distance, movement duration, and turning behavior. Compound-specific toxicity and behavioral profiles were compared alongside M1/M3 receptor pharmacology.

At 24 hours, mean compound-level mortality increased from 2.2% at 10 μM to 32.6% at 100 μM, whereas combined abnormalities or death increased from 11.7% to 66.9%. Toxicity varied substantially between compounds and over time. At 10 μM, compounds without recorded combined adverse outcomes also exhibited distinct first-dark-cycle thigmotaxis responses, including CW-6-77 (−0.72) and GD-1-34 (+0.59). These findings indicate that behavioral alterations can occur without concurrent overt morphological toxicity.

Together, these results demonstrate the complementary value of morphological, lethal, and behavioral endpoints in evaluating muscarinic modulators. Integrating light–dark responses with time-dependent toxicity profiles may improve the identification of functional disturbances and help distinguish overt toxicity from pharmacological effects.

Abstract 039 – PhD Student

Neurodevelopmental Impacts of PFAS on the Glutamatergic Pathway of Larval Zebrafish (*Danio rerio*)

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with a fluorinated carbon backbone with polar functional end groups, contributing to unique amphipathic properties responsible for widespread distribution, persistence, and bioaccumulation of PFAS, rendering the name 'Forever Chemicals'. Nearly 90% of tested human populations show an average of ~5 µg PFAS/ L blood, even pregnant women. The wide array of PFAS variants escapes the current toxicological evaluation frameworks relevant to them. Current studies indicate PFAS exposure causes neurological complications through altering the abundance of neurotransmitters, expression of synaptic proteins, and calcium signaling during key developmental stages. Vertebrate embryos are susceptible to exposure during development due to the increased body burden, lack of protective barriers, and sensitive developmental processes that leave them vulnerable to malformations. Excess glutamate, the main excitatory neurotransmitter, induces excitotoxicity that generates reactive oxygen species (ROS) leading to increased neuronal cell death and necrosis of brain regions that results in declined cognition, memory, and learning. In this study *Danio rerio* (zebrafish) embryos were exposed beginning at 1 hour post fertilization (hpf) to PFOS at concentrations ranging from 0.004-4 ppb (µg/ L) or to GenX or PFBA at concentrations ranging from 0.04-40 ppb (µg/ L) through 72 hpf and outcomes compared to a negative control. Behavioral changes were assessed using the visual motor response (VMR) test at 144 hpf and the open field test (OFT) at 168 hpf. At 120 hpf (i.e., 48 hours after exposure cessation) glutamate was quantified in whole larvae using commercial ELISA kits. Parametric data was analyzed using a one-way ANOVA and Tukey's post hoc test, while nonparametric data was analyzed using a Kruskal-Wallis and Dunn post hoc test (α=0.05). In addition, the VMR was analyzed using repeated measures ANOVA (α=0.05). In the VMR test, hyperactivity occurred in all GenX treatment groups but only in the highest PFBA treatment concentration. PFOS exposure showed a hypoactive response at distinct phases in the 0.04 and 4 ppb treatment groups. No significant changes were observed in the OFT or in glutamate concentrations across all PFAS treatments. This data warrants further investigation to address the intricate implications of PFAS exposure along the glutamatergic and additional neurotransmission pathways to assess mechanisms driving behavioral alterations as observed in the VMR test.

Abstract 040 – PhD Student

Sex-Dependent Effects of Trichloroethylene and High Fat Diet on the Development of Metabolic Syndrome in a Humanized PPARα Mouse Model

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Metabolic syndrome (MetS) is a cluster of adverse health conditions including high blood pressure, elevated blood glucose levels, increased abdominal circumference, low HDL cholesterol, and hypertriglyceridemia. A patient must display three out of these five conditions to be diagnosed with MetS. Nearly 40% of American adults have MetS. MetS is a risk factor for the development of cardiovascular disease, type 2 diabetes, stroke and hepatic steatosis. High fat diet (HFD) consumption is a major risk factor for the development of MetS. Toxicant exposures may also contribute to MetS. Trichloroethylene (TCE) is an industrial solvent that has been linked to metabolic dysfunction and hepatic steatosis. Several TCE metabolites agonize PPARα, a regulator of lipid metabolism with notable species differences between mice and humans. This research tests the hypothesis that TCE exposure exacerbates HFD-induced metabolic dysfunction in a sex-dependent manner in humanized PPARa (hPPARα) mice. Male and female hPPARα C57BL/6J mice (9-12 weeks old) were placed on either a low-fat diet (LFD; 10% kcal from fat) or a HFD (42% kcal from fat) with concurrent exposure to 0.5 mg/mL of TCE or 1% ethoxylated castor oil in drinking water for 12 weeks. HFD feeding increased body weight, fat mass, and white adipose tissue (WAT) weight, elevated total plasma cholesterol, and induced hepatic steatosis in both sexes of mice. HFD feeding also impaired glucose tolerance only in male mice. Interestingly, TCE exposure reduced the negative effects of HFD-feeding in the female mice by significantly decreasing body wight, fat mass, and WAT relative to HFD-fed females. HFD feeding caused hypertrophy of adipocytes in the WAT of both sexes of mice. Further analysis of the WAT via qPCR showed that HFD significantly reduced fatty acid synthase, adiponectin, stearoyl-CoA-1, fatty acid translocase, and increased leptin mRNA levels, in both sexes of mice. TCE significantly reduced mRNA levels of adiponectin, fatty acid synthase, peroxisome proliferator-activated receptor gamma, and stearoyl-CoA-1 in female LFD-fed mice when compared to vehicle control. This study revealed marked sexual dimorphism in diet- and TCE-induced metabolic outcomes in hPPARα mice where male mice were more susceptible to diet induced injury, whereas female mice displayed alterations in systemic fat deposition and gene expression in WAT when exposed to TCE.

Abstract 041 – PhD Student

Sex-Dependent Diet and Polychlorinated Biphenyl Exposure-Induced Metabolic-Associated Steatotic Liver Disease in Mice

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Metabolic-associated steatotic liver disease (MASLD) is prevalent in individuals with chronic nutrient overload and dysfunctional glucose and lipid metabolism. In humans and rodents consuming a high fat diet (HFD), exposure to metabolic-disrupting environmental toxicants, such as polychlorinated bisphenols (PCBs), increases the risk of MASLD and accelerates progression to metabolism-associated steatotic hepatitis (MASH). Previously, we demonstrated that PCB exposure in HFD-fed male mice promoted MASH and altered lipid metabolism. Additionally, N(6)-methyladenosine (m6A) is the most abundant chemical modification in mRNA and its levels in lipoproteins were altered during the progression to MASLD in HFD-fed male mice. Whether sex differences in liver biology impact HFD + PCB exposure associated m6A and transcriptome changes in MASLD is unknown. To investigate this, male and female mice were fed either a normal diet (ND) or a high-fat, high fructose diet (HFHFD). Mice were gavaged with corn oil (control) or with Aroclor1260+PCB126 (3.33μg/kg + 3.33mg/kg) every other week for 12 wks. At 11 wks., EchoMRI and glucose tolerance tests (GTT) were performed. Following euthanasia, blood and livers were collected and analyzed. Histological analysis was conducted with H&E and Sirius red staining to determine steatosis and fibrosis, respectively. Representative liver samples (n=5/group) were selected for downstream RNA sequencing analysis based on histology and plasma markers. HFHFD significantly increased plasma alanine transferase (ALT) in males and very low-density lipoprotein (VLDL) in females, but decreased VLDL in males. While a significant increase in steatosis was observed in all mice fed a HFHFD, PCB-exposed HFHFD male mice showed a decrease compared to the HFHFD vehicle control. There was no difference in fibrosis between sexes or treatment groups. RNA-seq, m6A-RNA immunoprecipitation followed by Nanopore direct RNA-sequencing (DRS meRIP-seq), and Iso-seq will be used to identify transcriptomic and epitranscriptomic changes associated with sex-specific HFHFD and PCB exposures-induced MASLD progression.

Abstract 042 – PhD Student

Relating Childhood Lead Surveillance to Current Risk Predictions and In-Vivo Bone Lead Measurements

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A growing number of studies are identifying lead (Pb) as a leading cause of negative health outcomes, with a major focus shifting to negating exposure in individuals below the age of 7. In this study we aim to evaluate the strength of current Indiana statistics on lead risk and surveillance. We utilized Indiana Childhood Lead Surveillance data, the Indiana Childhood Lead Risk Assessment Map, and lab collected X-ray Fluorescence data of at-risk individuals for this evaluation. Results from the Childhood Lead Surveillance matched with data from the Risk Assessment map show weak correlation between predicted risk and actual elevated cases. Lab collected bone lead date matched with blood lead surveillance data showed an increase in the rate of elevated levels of individuals from the observed counties. It can be concluded that there are risk factors that are potentially being neglected for consideration or the weight of a risk factor is being neglected. It can also be concluded that a greater effort needs to be made in the testing of youth for lead exposure.

Abstract 043 – PhD Student

Lipocalin 2 Production Mitigates Ozone Induced Lung Injury and Inflammation

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Exposure to air pollutants including ground level ozone (O_3) contributes to increases in morbidity and mortality of cardiopulmonary diseases. One mechanism by which this may occur is through O_3-induced dysregulation of iron homeostasis. Previous work from our lab has shown elevations in airspace iron and iron associated proteins such as Lipocalin 2 (LCN2) are seen following an acute O_3 exposure. Although LCN2 is shown to be essential in the pulmonary host defense, its biological role following exposure has not been explored. Therefore, we hypothesis that O_3-induced elevations in airspace iron stimulate production of LCN2, mediating O_3-induced lung injury and inflammation.

To test this hypothesis, female C57BL/6J (WT) mice or LCN2 deficient (*Lcn2*$^{-/-}$) mice were exposed to filtered air (FA) or 1ppm O_3 for 3h. Mice were euthanized 24- and 48h post-exposure and bronchoalveolar lavage fluid (BAL) and plasma were collected. BAL total protein and immune cell recruitment were measured within BAL. 24h following O_3, iron concentrations within the BAL were quantified through inductively coupled plasma-mass spectrometry. To better understand the cellular compartment responsible for LCN2 production following O_3 exposure, male WT and *Lcn2*$^{-/-}$ mice underwent a bone marrow transplant (BMT) to generate bone marrow chimeras. Once fully engrafted, BMT mice were then exposed to FA or 1ppm O_3 for 3h and 24h following exposure BAL and plasma were collected.

24h following O_3 exposure, *Lcn2*$^{-/-}$ mice had a significant increase in airspace neutrophilia and total BAL protein compared to O_3-exposed WT mice. Neutrophilia and markers of lung injury remained elevated until 48h post exposure within *Lcn2*$^{-/-}$ mice, while WT mice only sustained increased total protein within their BAL between FA and O_3 exposure. O_3 exposure increased airspace iron concentrations in WT mice when compared to FA exposed mice which was further augmented in *Lcn2*$^{-/-}$ mice. Following BMT, WT recipients given *Lcn2*$^{-/-}$ bone marrow had significantly increased airspace LCN2 when compared WT mice receiving WT bone marrow. Regardless of donor bone marrow genotype, *Lcn2*$^{-/-}$ recipients has elevated BAL total protein as a marker for lung injury, while no significant differences were seen in immune cell recruitment.

Taken together, these data suggest that LCN2 is protective against O_3-induced lung injury and inflammation perhaps through decreasing airspace iron concentrations. Furthermore, LCN2 production following O_3 exposure is structural cell dependent. Ongoing studies will identify which structural cells are responsible for LCN2 production following O_3 exposure and elucidate the mechanism by which iron is released.

Abstract 044 – PhD Student

Dietary Intervention Modulates Pulmonary Inflammation and Lipid Metabolism Following Silver Nanoparticle Exposure in Metabolic Syndrome Mice

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Chronic diseases, including metabolic syndrome (MetS), are associated with increased susceptibility to environmental exposures, contributing to greater toxicity and disease risk. MetS is a progressive disease characterized by worsening lipid dysregulation, which has been associated with altered inflammatory responses to inhaled particulates. Because lipids serve as key mediators of inflammation, persistent lipid dysregulation may contribute to altered responses to inhaled exposures throughout MetS progression; however, whether susceptibility to exposure can be reversed following dietary treatment of MetS remains unknown. We hypothesized that dietary intervention in MetS mice may restore inflammatory responses and lipid metabolism to levels similar to healthy mice. To evaluate this, 6-week-old mice were given a healthy diet or high-fat Western diet for 24 weeks, or a high-fat diet for 12 weeks followed by 12 weeks of healthy diet. Mice were then exposed to 50 µg silver nanoparticles (AgNPs) or water control via oropharyngeal aspiration. At 24 hours post-exposure, serum, bronchoalveolar lavage fluid (BALF), and lung tissue were collected to evaluate metabolic outcomes, pulmonary inflammation, gene expression, and lipid profiles. AgNP exposure increased BALF neutrophils and total protein across diet groups; however, the magnitude of these responses was significantly attenuated in MetS mice compared to their healthy and diet-switch counterparts. Similarly, gene expression analysis revealed AgNP-induced upregulation of the pro-inflammatory chemokines and cytokines *CCL2*, *IL-6*, and *IL-1β*, with induction lower in MetS mice and restored to levels similar to healthy mice following dietary intervention. In contrast, lipid metabolism remained altered following dietary intervention, with *COX-2* and *SEH* altered following AgNP exposure only in MetS and diet-switch mice. Lipidomic analysis via MRM profiling further demonstrated that lung lipid profiles and lipid mediators of inflammation in diet-switch mice were an intermediate between healthy and MetS groups under both control and AgNP conditions. Collectively, these findings suggest that chronic MetS may suppress immune responsiveness to AgNP exposure, an effect that may be reversed through dietary intervention. Although switching to a healthy diet may restore pulmonary inflammatory responses following AgNP exposure, persistent alterations in lipid metabolism remain and may reflect lasting effects of prior MetS-associated lipid dysregulation.

Abstract 045 – PhD Student

Effects of a novel glutamate transporter-1 modulator, MC-350012, compound on the liver injury markers in C57BL/6 mice exposed chronically to ethanol

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Alcohol-associated liver disease (ALD) and its complications are major health problems worldwide. Early diagnosis and treatment of ALD are difficult because of asymptomatic progression, which frequently leads to serious consequences. Chronic exposure to ethanol affects a wide range of ALD, causing inflammation, fibrosis, and steatosis. The present study investigated the effects of a novel glutamate transporter-1 modulator, MC-350012, compound in the liver of C57BL/6 mice, exposed chronically to ethanol. Control group received control Lieber−DeCarli diet ad libitum, and other groups were fed with ethanol Lieber−DeCarli diet ad libitum containing 5% (v/v) ethanol throughout the study. Ethanol feeding groups were given an oral gavage of 31.5% (v/v) ethanol while the control group was given an oral gavage of 45% (w/v) maltose dextrin solution. Mice were administered 50 mg/kg (i.p) of MC-350012 or saline for seven days. Chronic ethanol exposure increased serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), malondialdehyde (MDA), pro-inflammatory cytokines (IL-1β), (IL-6) levels as well as fat droplet content in the liver. However, MC-350012 attenuated ethanol-induced increases in fat droplet content, ALT, AST, TG, MDA, IL-1β, and IL-6 levels in the liver. These findings suggest that MC-350012 has potential in attenuating liver injury induced by chronic exposure to ethanol.

Abstract 046 – PhD Student (Poster withdrawn – Presenter could not attend)

Hexavalent Chromium Exposure and Regulation of Antibody Production in Human B-Cells

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B-cells produce antibodies and play a central role in regulating adaptive immune responses against extracellular pathogens. Antibody production is tightly regulated and influenced by environmental stressors through alterations in cellular signaling and gene regulation. The immunoglobulin heavy chain (*IGH*) locus is critical for antibody expression and is sensitive to cellular stress and transcriptional regulation. Hexavalent chromium [Cr(VI)] is a widespread environmental contaminant and human toxicant, however, its potential effects on B-cell function and antibody production remain poorly understood. While previous studies of Cr(VI) exposure have reported limited and inconsistent immune effects, these studies did not directly evaluate B-cell specific responses. Environmental toxicants like Cr(VI) induce oxidative stress and DNA damage, which may disrupt processes mediating efficient antibody production. The objective of this study was to evaluate the concentration-dependent effects of Cr(VI) on antibody production in human B-cell line models and to determine whether direct exposure of Cr(VI) alters B-cell function independent of overt cytotoxicity. Human B-cell lines, including SKW6.4, CL-01, and CL-01 gene-edited clones (4D5 and 4E10), were exposed to Cr(VI) concentrations ranging from 0.01-5 μM and 0.2-200 μM in the presence or absence of CD40L and IL-4 stimulation. Cell viability was assessed by trypan blue, and antibody production was quantified by ELISA. Preliminary analysis identified 0.01-0.1 μM Cr(VI) as non-cytotoxic concentrations suitable for evaluating antibody production. In CL-01 WT cells stimulated with CD40L+IL-4, Cr(VI) had minimal effects on IgM secretion and no apparent effects on IgG or IgA secretion. In contrast, CD40L+IL-4+CD28 stimulation suggested an inhibitory effect on IgA secretion. These findings indicate that low, non-cytotoxic Cr(VI) concentrations may differentially affect antibody production depending on activation conditions. Future studies will evaluate subchronic 28-day exposure on B-cell function.

Abstract 047 – PhD Student

Perfluorooctane sulfonate (PFOS) induced oxidative stress and altered lipid profiling using zebrafish

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Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic chemicals that are made of a chain of carbon (C) atoms with multiple fluorine (F) atoms. Among all the PFAS, long-chain PFAS (C>6) are more bioaccumulative, persistent, and toxic. Perfluorooctane sulfonate (PFOS) belongs to the long chain PFAS group and has eight carbons. It is of major concern because it persists in the environment and bioaccumulate in fish tissues, which are consumed by humans and pose a serious threat to public health. PFAS exposure is associated with neurotoxicity, developmental neurotoxicity, carcinogenicity, endocrine disorder, and immunotoxicity. However, oxidative stress and lipid-level mechanism underlying its neurotoxic effects remain poorly understood. This study used zebrafish (Danio rerio) embryos to investigate the developmental neurotoxicity of this compound. Zebrafish embryos were exposed to PFOS at concentrations of 0, 0.004, 0.04, 0.4, 4, or 40 ppb (µg/L) from 1 to 72 hours post-fertilization (hpf). At 72 hpf, larvae were rinsed three times with embryo water to remove PFOS solution. Oxidative stress markers such as reactive oxygen species (ROS), antioxidant enzyme activity, thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation, and lipidomic profiling were assessed at 120 hpf. ROS were measured using florescence intensity, while antioxidant enzyme activity, and TBARS levels were assessed using spectophotmetrically. Lipid profiling were measured using multiple reaction monitoring (MRM) profiling. To check the differences among treatment groups, a one-way ANOVA followed by a Tukey's post hoc test were used (α=0.05). PFOS showed increased levels of ROS in all concentrations compared to the negative control group (0 ppb). Catalase significantly decreased in 0.4, 4, and 40 ppb whereas, glutathione reductase did not change. TBARS levels increased in almost all the concentrations compared to the negative control group. Lipidomics profiles revealed upregulation of phosphatidylcholine (PC) and fatty acid (FA), accompanied by downregulation of sphingomyelin (SM) and phosphatidylinositol (PI) at 120 hpf. These lipid classes contribute to synaptic plasticity, cell membrane integrity, cellular signaling (e.g., cell growth), and apoptosis. These findings suggest PFAS exposure increases oxidative stress through the induction of ROS generation leading to lipid peroxidation, disruption of the oxidative balance, and altered lipid profile. However, future studies are needed to further investigate the neurotoxic mechanisms underlying PFOS-induced oxidative stress and altered lipid profiling to determine if these alterations may affect neuronal function and neurobehavioral.

Abstract 048 – PhD Student

Physicochemical and Metal Influences on Biocorona Formation and Toxicity in Exposure to Real-World Micro- and Nanoplastics

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Micro- and nanoplastics (MNPs) are environmentally abundant as plastic production has increased. Degradation of plastic waste generates MNPs, which are commonly found in air, water and soil, where interactions occur with environmental pollutants. Due to their environmental abundance and potential to interact with other pollutants, MNPs may pose a significant risk to human health. In the body, MNPs are rapidly coated with biomolecules, forming a biocorona. The biocorona can modify MNP characteristics and cellular interactions. Variations in MNP physicochemical properties and biological matrix composition dictate biocorona formation. MNP exposure can initiate systemic inflammation, oxidative stress and cytotoxicity, promoting disease, which could be affected by the biocorona. Traditional studies use engineered polystyrene nanomaterials (E-PS) with uniform shape and size, however, environmental MNPs have large size distributions and irregular shapes, which may impact biocorona formation. We hypothesize real-world plastic waste MNPs will differentially form biocoronas, influencing cellular responses. Representative E-PS, bare MNPs generated from salt milling (PET, PS), MNPs with Pb adsorbed, or MNPs with metal mixture adsorbed (Mn, Co, Zn, Cd, Pb) were incubated in pooled male human serum at 50 µg/ml for 24-hours. The biocoronas were characterized using lipidomic and proteomic approaches. Unique biocoronas formed according to composition, indicative of physicochemical-dependent differences. MRM profiling identified Pb adhered MNPs to form the most compositionally diverse lipid coronas, followed by MNPs alone and metal mix adhered MNPs. Proteomic profiling identified the same for PET MNPs; however, PS MNPs with the metal mix had the most diverse protein components followed by Pb adhered PS and then bare PS. Across all groups, biocoronas had significantly different lipid and protein abundances compared with E-PS. Enrichment and pathway analysis using KEGG databases determined biomolecules involved in inflammation. Overall, MNPs generated distinct biocoronas, indicating differences in their physicochemical properties result in unique biocorona formation potentially affecting toxicity.

Abstract 049 – PhD Student

Macrophage response to LPS challenge is altered in offspring after *in utero* exposure to E-cigarette aerosol

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Rationale: Use of E-cigarettes (ECs) by pregnant women can lead to deleterious effects on offspring exposed *in utero* to EC components. Up to 7% of women use ECs during pregnancy, but the consequences of EC aerosol exposure *in utero* have yet to be fully elucidated. The effects of *in utero* exposure to EC aerosol on immune function in offspring are unknown.

Methods: Pregnant mice were exposed to filtered air (FA), EC aerosol alone (50:50 propylene glycol;vegetable glycerin, PV), or EC aerosol with 2% nicotine (PVN), 4 hrs/day, 5 days/week for the length of gestation. We isolated bone marrow from adult male and female F1 mice and cultured the cells in DMEM with M-CSF for 7 days to differentiate the cells into bone-marrow derived macrophages (BMDMs). F1 BMDMs were exposed to a single dose of LPS at concentrations ranging from 1ng/mL to 100ng/mL for 24 hours, then harvested. Cell culture supernatant was collected to determine cytokine concentration via ELISA. RNA was extracted from cell lysates, and cDNA was generated to perform qPCR to determine gene expression levels of cytokines associated with macrophage-mediated pro- and anti-inflammatory immune response, as well as angiogenic factors. ELISA was performed on cell culture supernatant to determine cytokine expression levels. Data were analyzed via ANOVA.

Results: At low doses (10ng/mL LPS), BMDMs from male PV and PVN offspring secreted more TNFa in culture supernatant as compared to FA offspring ($p = 0.01$ and $p = 0.08$, respectively). At higher concentrations (100ng/mL LPS), this effect was not seen. However, following 100ng/mL LPS exposure, *Tnfa* gene expression in male BMDMs was increased in FA and PV offspring ($p < 0.03$), but not PVN offspring, while *Tnfa* expression in female BMDMs was increased in all groups ($p < 0.0004$). *Il-10* expression was increased in female BMDMs from FA and PV offspring ($p < 0.009$), but not PVN offspring. Conversely, no effect was seen in male BMDMs. *Il1b* expression was increased in female BMDMs from PV and PVN offspring ($p < 0.002$), but not FA offspring, but this effect was not seen in male BMDMs. *Vegfa* expression decreased in male BMDMs from FA offspring in a dose-dependent manner following LPS exposure ($p < 0.008$), but this effect was abrogated in BMDMs from PV and PVN offspring, and no change in *Vegfa* expression was seen in female BMDMs from any group. *Hif1a* expression was increased in female BMDMs from FA and PVN offspring ($p <= 0.0001$) but not PV offspring, while this effect was not seen in male BMDMs.

Conclusion: *In utero* exposure to EC aerosol resulted in altered expression of pro- and anti-inflammatory factors at gene and protein levels following LPS exposure in BMDMs. The altered expression of genes associated with macrophage immune response to LPS may have implications for the effects of EC aerosol exposure on immune health of offspring.

Abstract 050 – PhD Student

Investigating the role of ZFP36L1 and ZFP36L2 on host immune response and antiviral activity against RNA viruses

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CCCH-type zinc finger 36 family proteins (ZFP36) are RNA-binding proteins that regulate mRNA turnover by poly-A tail deadenylation. ZFP36 proteins bind AU-rich elements at the 3'UTR of mRNA, subsequently recruiting exosomes and exonucleases to facilitate decay. In humans, the ZFP36 family contains three members, ZFP36, ZFP36L1, and ZFP36L2. As key regulators of mRNA degradation, ZFP36 proteins also bind to viral RNA, facilitating its degradation and controlling virus replication. ZFP36L1 inhibits viral replication by several other pathways. ZFP36L1 inhibits influenza A viral protein translation and coronavirus replication by directly binding to the nucleocapsid open reading frame. ZFP36L2 has demonstrated antiviral activity in flavivirus infection via 5'-3' XRN1-mediated decay. Since viral infection triggers inflammation, it is also hypothesized that ZFP36 proteins could break down mRNA of pro-inflammatory cytokines, moderating virus-induced inflammation. We have found that the stable overexpression of ZFP36L1 through lentivirus transduction significantly reduces rotavirus and norovirus titer as well as reduction in expression of pro-inflammatory cytokines like IFN-α and TNF-α while knockdown of ZFP36L1 enhances viral titers and immune responses. Knockdown of ZFP36L1 in RAW 264.7 cells showed more migration when stimulated by bacterial LPS when compared to wildtype and overexpressed cells, suggesting an influence in other immune responses. Preliminary data in our lab shows overexpression of ZFP36L2 inhibits coronavirus replication while knockdown of ZFP36L2 enhances viral replication. RNA viruses have a high mutation rate, allowing them to evade pre-existing host immunity and the potential for inter-species transmission. RNA viruses are the primary etiological agents of past emerging diseases and are a candidate for future pandemics. Studies from our lab and others suggest ZFP36L1 and ZFP36L2 may have broad-spectrum antiviral efficacy, making them a potential therapeutic target. The aims of this study are to investigate the antiviral efficacy ZFP36L1 and ZFP36L2, and their role in mitigating virus-induced inflammation and damage.

Abstract 051 – PhD Student

Transcriptomic Analysis Reveals Heterocyclic Amines Commonly Found in Cooked Meats Affect Pathways Associated With Metabolic Syndrome in Human Hepatocytes

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Affecting over 1 in 3 adults worldwide, metabolic syndrome is a cluster of conditions that increases one's risk for developing type 2 diabetes and cardiovascular disease. The hepatic manifestation of metabolic syndrome is metabolic dysfunction-associated steatotic liver disease (MASLD), an increasingly prevalent chronic liver disease driven by insulin resistance. Exposure to heterocyclic amines (HCAs), mutagenic and carcinogenic toxicants formed from high temperature or prolonged cooking of meats, has been linked to insulin resistance, type 2 diabetes, and MASLD, but the underlying mechanism is largely unknown. The study objective was to use transcriptomics to investigate the effect of common dietary HCAs, including PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) and MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline), on gene expression in human hepatocytes, particularly genes involved in metabolic syndrome. Cryopreserved human hepatocytes were exposed to a vehicle control (DMSO) or 25 μM PhIP or MeIQx for 24 h (four replicates per condition). RNA was isolated for RNA-sequencing and bioinformatic evaluation by Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetaCore pathway enrichment analyses. The threshold cutoff was p and $q < 0.05$, and MetaCore pathway maps were filtered by "Nutritional and Metabolic Diseases" and a |fold change (FC)| ≥ 1.5. KEGG analysis revealed global disruption and molecular shifts associated with metabolic syndrome, including mitochondrial dysfunction, insulin resistance, steatosis, and lipotoxic inflammation. MetaCore analysis revealed multiple affected metabolic pathways, including "GLP-1 in beta cell apoptosis in Diabetes Mellitus, Type 2", "Glucagon-induced glucose upregulation in type 2 diabetes in liver", "TNF-alpha, IL-1 beta induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes", "GLP-1 in the regulation of beta cell proliferation and function in Diabetes Mellitus, Type 2", and "Dysregulation of Adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome". Notably, within the map "Glucagon-induced glucose upregulation in type 2 diabetes in liver", a key gluconeogenic gene, PCK1, was upregulated upon HCA exposure despite suppression of upstream glucagon signaling. These findings suggest that HCAs regulate pathways associated with metabolic syndrome in human hepatocytes and drive hepatic glucose output via PCK1 upregulation in a hormone-independent stress pathway.

Abstract 052 – PhD Student

Dev-Vape Exposure Impairs Hepatic Response to Acetaminophen Challenge in C57BL/6NCrl mice but not in C57BL/6J mice

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Introduction / Background: Electronic cigarette (e-cigs) use during pregnancy is increasing, yet the long-term impacts remains poorly understood. E-cigarette aerosols contain nicotine, metals and other constituents that may induce oxidative stress and alter cellular signaling. We have developed a new model of inhalation exposure to a commercially available product to examine impacts on fertility, birth outcomes and gene expression. The liver regulates metabolism and detoxification, and developmental disruption of hepatic pathways may increase susceptibility to injury during later exposure. We used an acute sublethal acetaminophen (APAP) challenge to assess hepatic susceptibility in juvenile offspring.

Hypothesis / Objective: Gestational vaping exposure impairs juvenile offspring hepatic response to acute sublethal acetaminophen challenge.

Methods: Female 8-12 weeks old C57BL/6 mice were exposed to Vuse™ Alto Golden Tobacco aerosol (5% nicotine; 1 h/day) or filtered room air beginning 4 days prior to mating, with 2 mating cycles, and continuing throughout gestation until day before birth. At postnatal day 25–28, fasted offspring were challenge with APAP. Blood ALT/AST was measured via hepatic panel kit with a Piccolo Gen 2. Statistical significance was defined as $p < 0.05$.

Results: C57BL/6NCrl mice offspring exposed during fetal development to vaping demonstrated a 1-2 pup reduction in litter size ($p = 0.03$) and a trend of higher birth weights ($p = 0.09$). C57BL/6J mice exposed in utero to vaping showed no differences in litter size and birth weight. At weaning, offspring from both strains were phenotypically normal. In juvenile C57BL/6NCrl offspring, the acetaminophen challenge did not elicit greater increases in ALT. For juvenile C57BL/6NCrl offspring exposed during development to vaping, demonstrated a greater proportion of high responders with a plasma ALT level greater than 2000 U/L ($p = 0.041$) at 4- and 8- hour post challenge. C57BL/6NCrl vape offspring exhibited a correlation between necrosis and ALT. C57BL/6J offspring showed no difference in ALT based on prenatal exposure group.

Conclusion: These studies report the development of an inhalation model for maternal vaping in two mice strains using a commercially available vaping product. The greater impact on offspring liver response to drug challenge noted in the C57BL/NCrl strain, as compared to C57BL/6J strain, mirrors the known lesser susceptibility to APAP in the C57BL/6J strain. This indicates that strain specific resistance to DILI is not modulated by developmental vaping exposure.

Abstract 053 – PhD Student

Development and Characterization of a Murine Model of Dev-CSE: Sex-Dependent Hepatic Responses in Young Adult Offspring

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Smoking tobacco during the periconceptional and prenatal periods remains a major health concern despite public health awareness. In 2023, smoking during pregnancy was reported in 8.7% of Kentucky births compared with 3.0% nationally. Prenatal CSE is associated with fetal growth restriction, low birth weight, childhood obesity, and increased risk of chronic diseases later in life. These outcomes align with the Developmental Origins of Health and Disease (DOHaD) framework. Previous studies indicate that maternal CSE alters offspring hepatic metabolism and oxidative balance, increasing hepatic vulnerability later in life. However, the sex-dependent hepatic molecular alterations of Dev-CSE in the absence of low birth weight remain incompletely understood. This study examined whether Dev-CSE induces sex-dependent changes in hepatic gene and protein expression in young adult offspring.

C57BL/6 dams received whole-body cigarette smoke or filtered-air exposure for 3 hr/day beginning 2 days before mating through parturition. Liver was collected from postnatal week 10 offspring for RNA-seq [N=33, MC=10, MS=7, FC=9, FS=7] and proteomics [N=24, n=6/group]. Analyses were stratified by sex. Differential gene expression was analyzed using DESeq2 with adjusted $p< 0.05$) were analyzed using MetaboAnalyst 6.0 with PLS-DA; features with VIP≥1.25 were selected for pathway analysis. Upregulated and downregulated protein sets were submitted separately to ShinyGO for KEGG enrichment (FDR ≤ 0.05).

RNA-seq PCA identified sex as the dominant source of hepatic transcriptomic variation, with PC1 explaining 28.37% of total variance. Dev-CSE produced a stronger transcriptomic response in males than in females. At adjusted $p<0.05$, 89 genes were altered in MC vs MScompared with one gene in FC vs FS. Among the top downregulated genes in MC vs MS were *Cyp2a4, Cyp2c40, Cox7a2, and Ndufb6*. KEGG pathway analysis identified downregulation of oxidative phosphorylation and chemical carcinogenesis-reactive oxygen species pathways in the male transcriptomics analysis. Proteomic KEGG analysis identified reduced glutathione metabolism and xenobiotic and drug metabolism pathways only in male offspring. FC vs FS proteomic responses favored cellular stress, protein homeostasis, and inflammation.

Our findings reveal prenatal Dev-CSE induces sex-dependent hepatic alterations months after cessation of exposure. Males show greater suppression of glutathione defense, xenobiotic, and mitochondrial function genes and proteins. These alterations may increase hepatic vulnerability to a second-hit challenge.

Abstract 054 – PhD Student

Identifying Conserved Molecular Signatures of Persistent Neurotoxicity Across Lead (Pb) Exposure Concentrations in Human iPSC-Derived Cortical Neurons

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Even today, humans are continually exposed to varying amounts of lead through consumer products, diet, dust, and water. Exposures occurring during sensitive windows of neurodevelopment are of particular concern because even transient exposures can induce long-lasting biological alterations that persist after exposure ceases. Although the implications of developmental lead (Pb) exposure are well documented, we have yet to identify the exact mechanisms by which persistent toxicity from lead exposure occurs. Furthermore, how dose impacts alterations in cellular pathways remain unclear. In this study, we attempt to define the relationship between dose-dependent persistent Pb toxicity. Specifically, by leveraging a hIPSC-derived cortical neuronal model (through small molecule-based dual SMAD inhibition), we classified the persistent effects of developmental Pb exposure 70 days after exposure in two hiPSC cell lines using single-cell transcriptomics. Four non-cytotoxic lead concentrations, determined by Alamar Blue and carboxyfluorescein diacetate cytotoxicity assays, were chosen to gain an encompassing view of alteration differences and biological relevance identified in epidemiological data, including 0.3, 3, 30, and 300 nM concentrations. Analysis of glutamatergic neuronal populations identified 27 persistently altered pathways that were conserved across all lead exposure conditions, including oxidative phosphorylation, Parkinson's disease signaling, and EIF2 signaling. It is important to note, however, that across shared altered pathways, the dose response to developmental lead exposure was nonlinear, implying a more complex mechanism of persistent neurotoxicity. Overall, we have classified altered pathways specifically affecting glutamatergic neurons long after developmental lead exposure and evaluated the dose-dependent relationships.

Abstract 055 – PhD Student

Maternal Cadmium Exposure and Hepatocellular Carcinoma Outcomes in Offspring Under Metabolic and Carcinogenic Stress

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Hepatocellular carcinoma (HCC) is associated with metabolic dysfunction and environmental exposures. Cadmium (Cd) may influence hepatic disease and cancer development, but effects of maternal Cd exposure on offspring HCC outcomes under metabolic and carcinogenic stress remain unclear. We evaluated HCC-related outcomes and hepatic metal profiles following maternal Cd exposure.

C57BL/6J female mice received drinking water with or without 5 ppm Cd for 1 week before breeding through weaning. Offspring received no Cd after weaning and were assigned to control, diethyl nitrosamine/high-fat choline-deficient diet (DEN/HFCD), Cd, or Cd+DEN/HFCD groups. 0After weaning, offspring received one dose of DEN (25 mg/kg), followed by HFCD feeding. Body weight was monitored longitudinally. Males were euthanized at weeks 12, 18, and 24 and females at weeks 18, 24, and 30. The incidence of mice with liver cancer was counted, while liver and kidney weights were measured and the number of liver tumor nodules per liver was analyzed. Hepatic Cd, Zn, Fe, Cu, and Mg concentrations were assessed by inductively coupled plasma mass spectrometry (ICP-MS). Data was analyzed by two-way analysis of variance with appropriate multiple comparisons.

Body weight differed across groups and time ($p<0.0001$). Liver and kidney weights normalized to tibia length were greater in DEN/HFCD and Cd+DEN/HFCD groups than controls in both sexes ($p<0.0001$). Liver tumor incidence and tumor burden were greater in Cd+DEN/HFCD than DEN/HFCD in male ($p=0.009$) and females ($p=0.011$) offspring. Hepatic Cd was highest in Cd-only mice and decreased over time. In males, hepatic Zn, Fe, Cu, and Mg were decreased in DEN/HFCD and Cd+DEN/HFCD groups ($p=0.029$, $p=0.0051$, $p=0.0030$, and $p=0.0134$, respectively). These findings associate maternal Cd exposure with altered hepatic metal homeostasis and HCC-related outcomes. The underlying mechanisms remain unclear and continue further investigation.

Abstract 056 – PhD Student

Analysis of RNA modifications in fibroblasts and myofibroblasts after arsenite exposure

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It is thought that exposure to the environmental toxicant, arsenic, can lead to stress-induced cellular reprograming and downstream changes in protein translation influenced by tRNA modifications. Modifications made to transfer RNA (tRNA) influences its stability, protein translation, and codon biases. Interestingly, nucleases, such as angiogenin or Dicer, can cleave tRNA at specific ribonucleotides to produce tRNA-derived small RNAs (tsRNA). Research has shown tsRNA can control a variety of biochemical functions including transcription regulation. Notably, production of tsRNA increases following exposure to heavy metal toxicants such as arsenic. Our lab developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the separation and quantification of over 60 modified ribonucleosides. With this method we previously found that tRNA modification levels varied between different mammalian cell lines and across tissues. Furthermore, we found that tRNA modifications levels changed in response to metabolic stress in mice fed a high fat diet. Metabolic stress such as from diabetes, UV radiation, and aging can lead to myofibroblast generation. Arsenic exposure also activates fibroblasts to the myofibroblast state; however, the impact of arsenic on pre-existing myofibroblasts and on tRNA and subsequent formation of tsRNA remains understudied. In this study, analysis was conducted using both naïve fibroblasts and cytokine-activated myofibroblasts exposed to a physiologically relevant range of arsenic. We treated cells with arsenic at sublethal dosages despite morphological changes indicative of stress. We observed alterations in the modification levels of both tRNA and tsRNA following activation of fibroblasts into myofibroblasts and at all exposure concentrations of toxic arsenic. Treated cells were evaluated for fibrotic gene expression, cell viability, and phenotypic behavior. Overall, this study aimed to further understand how tRNA and tsRNA modifications change in response to different stressors.

Abstract 057 – Master's Student

The impact of psychological support on treatment outcomes in cancer patients

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Living with a chronic illness such as cancer involves not only physical challenges but also substantial emotional and psychological stress. Extended hospital stays, complex treatment regimens, and uncertainty regarding disease outcomes can place considerable strain on patients and their families. These challenges often result in significant psychological distress, including anxiety, depression, and emotional fatigue, which markedly impact the quality of life. Despite increasing recognition of the mind-body connection, mental health care remains only partially integrated into standard medical practice and is often considered complementary rather than an essential component of treatment. This review aims to explore the impact of mental health interventions on cancer patients, with a particular emphasis on outcomes such as anxiety, treatment adherence, and daily functioning. Evidence from randomized trials, observational studies, and real-world clinical experiences indicates that cancer patients and their caregivers experience substantial psychological and emotional challenges. Importantly, mental health support has been shown to provide emotional relief, improve physical well-being, enhance treatment compliance, and strengthen social connections. Therefore, early identification of patients' and caregivers' psychological and emotional needs, together with timely and sustained counseling and psychosocial support is important for comprehensive cancer care. To that end, integrating psychological support as a standard routine cancer care may contribute to more holistic, patient-centered treatment and improved overall well-being.

Abstract 058 – Master's Student

Defining the role of the platelet-activating factor receptor pathway in the cytotoxic response to epidermal growth factor receptor inhibitor and its combination with antihistaminic drugs in non- small cell lung cancer

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Introduction: Non-small cell lung cancer (NSCLC) is a leading cause of lung cancer-related mortality worldwide. Frequent mutations in oncogenic drivers contribute to resistance to standard therapies, making lung cancer treatment challenging. Cancer cell survival pathways, including epidermal growth factor receptor (EGFR) and platelet-activating factor receptor (PAFR) signaling, contribute to tumor progression and metastasis.

Objective/Aims: Notably, EGFR mutations are among the most prevalent oncogenic alterations in lung adenocarcinoma. Evidence suggests crosstalk between EGFR and PAFR pathways through shared downstream signaling pathways, including MAPK and ERK. Therefore, this study aims to investigate dual targeting of EGFR and PAFR signaling using the third-generation EGFR tyrosine kinase inhibitor (TKI) osimertinib and a PAFR antagonist. Specifically, we evaluated the role and underlying mechanisms of PAFR signaling in osimertinib-mediated effects and assessed the effects of osimertinib alone and in combination with a PAFR agonist or antagonist.

Methods: Experiments were conducted using EGFR-mutant (T790M/L858R) H1975 and EGFR-wild-type/KRAS-mutant A549 lung adenocarcinoma cell lines. Cell viability, migration, and colony formation assays were performed, and quantitative analyses were conducted using GraphPad Prism and ImageJ.

Results: Osimertinib decreased cell survival in a dose- and time-dependent manner in both cell lines, with greater effects in H1975 cells than in A549 cells. Using optimal doses and time points, we demonstrated that PAFR activation by CPAF attenuated, whereas PAFR antagonism by WEB2086 enhanced, the anti-migratory and anti-clonogenic effects of osimertinib compared with osimertinib alone. Given the emerging potential of antihistaminic medications in cancer therapy, ongoing studies are evaluating their effects in combination with osimertinib and PAFR antagonism.

Conclusion: Collectively, these findings support a role for PAFR signaling in modulating the response to osimertinib and provide a rationale for investigating PAFR-targeted combination strategies to enhance therapeutic responses in NSCLC.

Abstract 059 – Master's Student

Platelet-activating factor-receptor signaling inhibits the efficacy of targeted Signal transducer and activator of transcription 3 pathway inhibitors in non-small cell lung cancer models

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Background/Objectives: Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality, with a 5-year survival rate of less than 30%. Therapy resistance remains one of the major challenges to effective treatment, highlighting the need to identify signaling pathways that contribute to therapeutic resistance. Studies, including ours, have shown that Platelet activating factor-receptor (PAFR) signaling plays a critical role in promoting several hallmarks of lung cancer, including tumorigenesis and therapy resistance. PAFR is also known to interact with several downstream mediators to drive oncogenesis, including the transcription factor called Signal transducer and activator of transcription 3 (STAT3). In this regard, several STAT3 inhibitors have demonstrated promising anticancer properties; however, the impact of PAFR signaling on the efficacy of STAT3 inhibitors has not been investigated. We hypothesized that PAFR activation would attenuate the anticancer effects of STAT3 inhibitors, whereas PAFR antagonism would enhance their anticancer effects in NSCLC cells.

Methods: We assessed the effects of the PAFR agonist (cPAF) and antagonist Apafant (WEB2086) on the activity of the STAT3 small-molecule inhibitors, C188-9 and Stattic in NSCLC cell lines A549 and H1299 through cell viability, cell migration, and soft-agar anchorage-independent colony formation assays.

Results: C188-9 and Stattic inhibited the survival and migration of A549 and H1299 cells in dose- and time-dependent manner. PAFR activation by cPAF attenuated the anti-migratory and anti-clonogenic effects of these STAT3 inhibitors. In contrast, PAFR antagonism with WEB2086 synergized with C188-9 to enhance its anti-migratory effect.

Conclusions: Although studies investigating the underlying mechanisms and potential combination strategies are ongoing, our findings demonstrate that PAFR activation attenuates the efficacy of STAT3 inhibitors in NSCLC, highlighting PAFR-STAT3 signaling axis as a potential therapeutic target.

Abstract 060 – Master's Student

Relevance of platelet-activating factor receptor (PAFR) signaling in the efficacy of Salinomycin and its combination approaches

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Introduction and Background: Non-small cell lung cancer (NSCLC) remains a clinical burden due to therapeutic resistance, disease recurrence, and metastasis, highlighting the need for novel therapeutic interventions. Salinomycin, a polyether ionophore, has been reported to target cancer stem cells and other tumor-promoting pathways. As aggressive tumor behavior is largely driven by signaling pathways that promote metastasis and tumor survival, G protein-coupled platelet-activating factor receptor (PAFR) signaling has been implicated in promoting these processes in NSCLC. However, whether PAFR signaling modulates the response to salinomycin in NSCLC remains unknown.

Hypothesis: We hypothesized that activation of PAFR signaling would attenuate the inhibitory effects of salinomycin, whereas PAFR antagonism would enhance the effects of salinomycin in NSCLC.

Aim: This study aimed to investigate (1) the role of PAFR signaling in salinomycin-mediated responses in NSCLC and (2) rational combination approaches using standard chemotherapeutic agents with salinomycin.

Methods: A549 and H1299 NSCLC cell lines were treated with salinomycin alone or in combination with the PAFR agonist CPAF or antagonist WEB2086. Cell migration and clonogenic ability were assessed using migration and colony formation assays.

Results: Salinomycin decreased cell survival in a dose- and time-dependent manner in both cell lines compared with controls. Using optimal doses and time points, we demonstrated that PAFR activation by CPAF attenuated, whereas PAFR antagonism by WEB2086 enhanced, the anti-migratory effects of salinomycin compared with salinomycin alone. Furthermore, salinomycin significantly decreased colony formation, whereas CPAF partially restored the colony-forming ability of NSCLC cells.

Conclusion: While ongoing studies are evaluating the effects of salinomycin along with the standard chemotherapeutic agents, gemcitabine and docetaxel, with and without a PAFR antagonist, these findings indicate that PAFR signaling modulates the response of NSCLC cells to salinomycin and support further investigation of salinomycin in combination with PAFR antagonism as a potential therapeutic approach for NSCLC.

Abstract 061 – Master's Student

Mechanism of microvesicle particle release following Photodynamic therapy and Thermal burn injury

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Photodynamic therapy (PDT) and Thermal burn injury (TBI) induce cellular stress which can be followed by subcellular vesicle release involved in extracellular signaling. Microvesicle particles (MVPs), which are generated through remodeling of plasma membrane, have been shown to be released from keratinocytes following PDT and TBI. Although the exact mechanism is unclear, we hypothesize translocation of acid sphingomyelinase (ASM) from lysosome to cell membrane and conversion of sphingomyelin into ceramide dependent ceramide release which leads to outward budding and release of MVPs is involved. The hypothesis states that ASM contributes to ceramide-dependent ceramide signaling. This study investigates the mechanisms regulating stress induced MVP release, with more emphasis on ASM-dependent ceramide release. HaCaT keratinocytes WT and CRISPR-generated ASM-knockout (ASM-KO) were evaluated following PDT and TBI. ASM expression was assessed using RT-qPCR and Western blotting. C2-ceramide and dihydroceramide were used as positive and negative controls for MVP release. MVP release was evaluated using Nanosight tracking analysis. Phorbol ester TPA and Platelet-activating factor agonist CPAF were used as positive controls. PDT and TBI induced expected MVP response in HaCaT WT cells, with appropriate response to the positive controls. However, HaCaT cells deficient in ASM released less MVP, suggesting involvement of this enzyme. These findings suggest ASM is required for PDT and TBI to cause MVP shedding, and could result in new treatments for TBI injuries.

Abstract 062 – Master's Student

Understanding ribonucleotide reductase in DNA replication checkpoint regulation

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Precise regulation of deoxyribonucleotide (dNTP) availability during the cell cycle is critical for DNA replication and repair, the two cellular processes essential for genome integrity. Ribonucleotide reductase (RNR) is the key enzyme that maintains balanced cellular dNTP pools by converting ribonucleotides into deoxyribonucleotides. The importance of RNR-mediated nucleotide regulation is underscored by human disease: mutations in RRM2B, a small subunit of human RNR, are associated with mitochondrial DNA depletion syndrome, developmental delays and hypotonia. Like human RNR, the RNR in the fission yeast Schizosaccharomyces pombe, consists of larger cytoplasmic subunit Cdc22 and smaller nuclear subunit Suc22. In our laboratory, we identified a suc22-S239F mutation that increases cellular sensitivity to chronic replication stress induced by hydroxyurea (HU), an RNR inhibitor and a cancer chemotherapeutic agent. To better understand the RNR regulation, we investigated whether a secondary genetic mutation can compensate for the suc22-S239F defect through an alternative mechanism of RNR regulation or replication-stress adaptation. We performed a suppressor screen for mutations that convert the HU-sensitive suc22-S239F to HU-resistant cells. Following high-throughput genomic sequencing and genetic analysis, we identified both intragenic suppressor mutations within the suc22 gene and intergenic suppressor mutations affecting a list of candidate genes of diverse cellular functions, such as cdc22, mug178, but2, rad51, cdc17, atp6, med15, pdi4, wtf18, wtf4, pam17, erg8 and pan3. These findings indicate cells can overcome impaired Suc22 function through multiple genetic routes, including changes within the RNR complex and pathways outside of its canonical components. Characterization of these suppressor mutants using genetic and biochemical approaches will define how these mutations restore cellular fitness under replication stress and determine how they influence RNR function and nucleotide homeostasis. Because dysregulated nucleotide metabolism and replication stress are prominent features of cancer cells, these mechanisms may provide insights into cellular vulnerabilities relevant to disease prevention and therapeutic development.

Abstract 063 – Master's Student

Efficacy and Safety of Chemoradiotherapy in Small Cell Lung Cancer

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Background/Introduction: Limited-stage small cell lung cancer (SCLC) is a rapidly growing and aggressive disease, which responds well to the combination of chemotherapy and radiotherapy. This underscores the importance of refining radiation schedules and considering patient-specific factors, such as overall health, to achieve optimal outcomes. Several platinum-based chemotherapy regimens combined with radiotherapy are commonly used because of their proven effectiveness in improving tumor response and survival. Across major studies, this combined approach has demonstrated high response rates, with side effects such as esophagitis generally being manageable. Current research is exploring whether modifications in radiation dose or delivery methods can further improve outcomes. The rapid progression of SCLC has also prompted investigations into reducing the overall length of treatment schedules. Advanced technologies such as intensity-modulated radiation therapy (IMRT) and positron emission tomography-computed tomography (PET-CT) may help spare healthy tissue and minimize toxicity. Additionally, emerging biomarkers may guide patient selection to identify those most likely to benefit from specific treatment strategies. The goal of this study is to highlight the effectiveness of chemoradiotherapy in limited-stage SCLC and discuss current and future approaches to optimize therapy.

Aim: To evaluate the effectiveness of concurrent chemoradiotherapy in the treatment of limited-stage small cell lung cancer and its impact on survival and tumor control.

Methods: Across major studies, we examine combined chemotherapy and radiotherapy regimens, including platinum-based combinations and advanced technologies such as IMRT and PET-CT.

Results: High response rates have been demonstrated, with manageable side effects like esophagitis. Emerging radiation strategies and advanced imaging and delivery technologies may further improve treatment outcomes while reducing toxicity.

Conclusion: Concurrent chemoradiotherapy remains an effective treatment approach for limited-stage SCLC. Ongoing efforts to optimize radiation schedules, treatment duration, radiation delivery, and patient selection may further improve survival and tumor control while minimizing treatment-related toxicity.

Abstract 064 – Master's Student

Acid Sphingomyelinase Regulates Microvesicle Release from Keratinocytes Following Environmental Stress

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Environmental stress induces complex responses in keratinocytes, including extracellular vesicle release. Acid sphingomyelinase (ASM) is a key regulator of membrane-associated signalling and cellular stress responses through its generation of ceramides. However, its contribution to keratinocyte responses to environmental injury remains incompletely understood. This study aimed to characterize extracellular vesicle responses in HaCaT ASM knockout (ASM KO) keratinocytes and determine whether loss of ASM alters microvesicle particle (MVP) release following environmental stressors. Two cell lines, HaCaT wild type (WT) and HaCaT ASM knockout (ASM KO) cells, were exposed to thermal burn injury (TBI) or cold injury at −20°C and −80°C, and MVP release was assessed relative to non-treated (NT) controls. MVP release was also evaluated following ultraviolet B (UVB) exposure.

ASM KO cells showed altered MVP responses to environmental stressors compared with WT cells. Following −20°C cold injury and TBI, ASM KO cells released fewer MVPs than WT cells, while no differences between WT and ASM KO cells were observed following −80°C cold injury MVP release. In additional experiments examining UVB exposure, UVB treatment of HaCaT ASM KO cells did not significantly increase MVP release compared to the large amounts released following UVB irradiation of HaCaT cells. Together, these results demonstrate that ASM deficiency alters MVP release in HaCaT keratinocytes following selected environmental stress conditions. The different responses observed following −20°C, −80°C, TBI, and UVB exposure indicate that regulation of MVP release varies according to the environmental stressor. These preliminary findings support a role for ASM in keratinocyte responses to environmental injury and provide a foundation for further characterization of ASM-dependent changes in extracellular vesicle release and cellular stress responses. These studies have therapeutic relevance as pharmacologic ASM inhibitors could have use in treating acute environmental injuries.

Abstract 065 – Master's Student

The Toll of Cortisol: How Genetic Variation in hs1.2 Governs Antibody Isotype Secretion in Human B Cells

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The human immunoglobulin heavy chain (*IGH*) locus is regulated by the *3'IGH* regulatory region (*3'IGH*RR), which contains enhancer elements (i.e. hs3, hs1.2, hs4) that control antibody isotype expression. The polymorphic hs1.2 enhancer has a ~53 bp invariant sequence (IS) varying naturally in the human population. This enhancer consists of transcription factor binding sites whose activity may be altered by hormonal stressors and xenobiotics which may further influence immunoglobulin expression and susceptibility to immune-related disorders.

This study investigated whether the hormonal stressor cortisol alters antibody production and whether the hs1.2 enhancer polymorphisms modify these responses in human B cells. Human CL-01 B cells and CRISPR-Cas9–edited hs1.2 enhancer variant clones were treated with hydrocortisone under stimulated and unstimulated conditions. Immunoglobulin isotype secretion (IgM, IgG, IgA) was measured via ELISA to assess genotype-dependent responses to hydrocortisone.

Hydrocortisone significantly increased basal IgG and IgA secretion but had no effect on stimulation-induced antibody secretion. In addition, hs1.2 enhancer variant clones did not exhibit increased IgG or IgA following hydrocortisone treatment but instead showed increased IgM production, demonstrating a genotype-dependent shift in isotype expression. These findings provide direct evidence that variation in the hs1.2 enhancer alters human B-cell responsiveness to hormonal stressors. Ongoing studies are examining the effects of hydrocortisone on immunoglobulin transcription. This work identifies a novel gene–environment interaction that may contribute to individual differences in immune responses and susceptibility to immune-related diseases.

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Abstract 066 – Master's Student

Exploring the efficacy of chlorpromazine as a repurposed drug in glioblastoma

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Background/Introduction: Glioblastoma is an aggressive and malignant primary brain tumor characterized by rapid progression and a poor prognosis, with a median survival of approximately 14–20 months. Epigenetic alterations play a critical role in glioblastoma progression and contribute to therapeutic resistance. The current standard of care includes surgical resection followed by chemotherapy and radiotherapy. However, patient outcomes remain poor, particularly when standard treatment is ineffective. Drug repurposing offers a promising strategy to reduce development time and cost while improving therapeutic outcomes. Chlorpromazine, a typical antipsychotic drug, has emerged as a potential candidate for repurposing in glioblastoma treatment, particularly to overcome resistance to temozolomide associated with an unmethylated O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter. The goal of this study is to evaluate the efficacy and safety of chlorpromazine as a repurposed therapeutic agent in the treatment of glioblastoma.

Methods: Published preclinical and clinical studies of chlorpromazine in glioblastoma were reviewed, focusing on its effects on tumor cell growth, treatment resistance, and outcomes when combined with temozolomide.

Results: Preclinical studies suggest that chlorpromazine inhibits glioblastoma cell growth through multiple mechanisms, including downregulation of connexin-43, a protein implicated in chemotherapeutic resistance through enhanced DNA repair mechanisms. Furthermore, a Phase II clinical trial demonstrated that combining chlorpromazine with temozolomide during the adjuvant phase significantly prolonged progression-free survival in patients with an unmethylated MGMT promoter. These findings suggest that repurposing chlorpromazine may represent a promising therapeutic strategy with manageable side effects for overcoming temozolomide resistance in glioblastoma.

Conclusion: Current evidence suggests that chlorpromazine represents a promising repurposed therapy for overcoming temozolomide resistance in glioblastoma. Further clinical studies are needed to confirm its efficacy, safety, and therapeutic role.

Abstract 067 – Master's Student

Differential Effects of Perfluorooctanoic Acid (PFOA) on a Human B-cell line (CL-01) and its hs1.2 Variants

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Perfluorooctanoic acid (PFOA) is a man-made environmental contaminant resistant to biodegradation and associated with potential health effects. Previous in vitro studies using human peripheral blood mononuclear cells (PBMCs) have shown that PFOA can suppress antibody response, which may increase susceptibility to infection and reduce vaccine efficacy. However, the direct effects of PFOA on antibody production by human B cells, particularly in relation to genetic variants, remain unclear. Antibody secretion depends on transcription of the immunoglobulin heavy chain (*IGH*), which is partially regulated by the duplicated 3'*IGH* regulatory region containing three enhancer regions (hs3, hs1.2, hs4). The hs1.2 enhancer is polymorphic due to variation in the tandem repeats of an approximately 53-bp sequence, resulting in four alleles (*1, *2, *3, and *4) containing one, two, three, and four repeats, respectively. These allelic differences may contribute to variation in antibody responses. Using the human CL-01 B-cell line [Wild type (WT), *2/*4] and its hs1.2 allelic variants [4D5 (*1/*3) and 4E10 (*1/*4)], we evaluated the concentration-dependent effects of PFOA (0.0125-0.1 µg/mL) on IgM, IgG, and IgA secretion under stimulated and unstimulated conditions. Under stimulated conditions, PFOA did not significantly affect IgM in any of the three cell lines. In a nonmonotonic concentration response, 0.025 µg/mL of PFOA increased IgA secretion and trended towards an increase in IgG secretion in CL-01 WT (*2/*4). Whereas 4D5 (*1/*3) and 4E10 (*1/*4) cells demonstrated a nonsignificant upward trend for IgG across PFOA concentrations and no effect on IgA secretion. Under unstimulated conditions, PFOA did not significantly impact IgM, IgG or IgA secretion in any of the three cell lines. Altogether, these findings suggest that PFOA exposure may affect antibody production in an isotype- and hs1.2 variant-dependent manner.

Abstract 068 – Master's Student

Evaluating the synergistic antitumor activity of immune checkpoint inhibitors and nab-paclitaxel combination in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is an aggressive and metastatic subtype of breast cancer characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). This limits the effectiveness of hormone-based and HER2-targeted therapies, leaving chemotherapy as a primary treatment option despite its nonspecific toxicity, limited efficacy, and high rates of tumor recurrence. The high metastatic potential, tumor heterogeneity, and immune evasion associated with TNBC contribute to treatment resistance and disease recurrence. Therefore, combining nab-paclitaxel with immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1), has emerged as a potential strategy to enhance antitumor immunity and improve clinical outcomes. This study examines the clinical rationale and emerging evidence supporting the combination of immune checkpoint inhibitors with albumin-bound paclitaxel (nab-paclitaxel) to overcome the limitations of conventional chemotherapy.

Abstract 069 – Master's Student

Influence of the Aryl Hydrocarbon Receptor on Human Antibody Production

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The aryl hydrocarbon receptor (AhR) responds to environmental chemicals and can influence B-cell antibody production. In mice, AhR activation reduced antibody production by inhibiting a transcriptional regulatory region within the immunoglobulin heavy-chain (IgH) gene, called the 3′IgH regulatory region (3′IgHRR). This region regulates IgH expression and class switching from IgM to other antibody isotypes. The human B-cell line CL-01 can produce multiple isotypes, including IgM, IgG, IgA, and IgE; IgG includes subclasses IgG1–IgG4, and IgA includes subclasses IgA1–IgA2. However, the role of AhR in human B cells remains unclear because of differences between human and mouse IgH genes. In prior work, shRNA-mediated AhR knockdown in CL-01 cells markedly decreased IgG production while leaving IgM production largely unchanged. In the current study, we are using CRISPR-Cas9 gene editing to disrupt AhR in CL-01 cells and assess isotype-specific Ig expression (IgG1–IgG4, IgA1–IgA2, and IgE) at the mRNA and protein levels. The prior findings suggest that AhR may differentially regulate antibody isotypes, motivating further investigation of its role in human B-cell antibody production.

Abstract 070 – Undergraduate Student

Comparing Rates of Airborne Particulate Matter 2.5 Along Major Roadways in Northern Kentucky and Greater Cincinnati

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Particulate Matter 2.5 (PM 2.5) consists of microscopic particles that can include solids or liquid acids created during combustion processes. These microparticles, when inhaled, can enter the lungs and bloodstream which worsens pre-existing respiratory issues and is linked to inflammatory processes in the heart and brain. Groups especially at risk of these harmful effects include those with heart or lung disease, children, and the elderly. For this project, PM 2.5 rates were collected by various preinstalled Purple Air Monitors across Northern Kentucky and Greater Cincinnati. This data was downloaded and analyzed to determine if any trends could be found in PM 2.5 rates based on proximity to different levels of traffic-related air pollution (TRAP). Time of day, time of year, and location were main factors taken into account. Thirteen monitors in the Purple Air network were selected and eight additional Purple Air monitors were installed for data collection including three control sites. The control sites were in two suburban and one rural away from heavy traffic and other sites (e.g. power plants) known to be sources of PM 2.5 pollution. Our preliminary findings demonstrated a clear relationship between proximity to major highways and significant increases in PM 2.5; however, a surprising finding was an overnight peak in Northern Kentucky which might be linked to heavy truck traffic from the Greater Cincinnati airport which has multiple warehouse hubs for delivery services such as FedEx, DHL and Amazon.

Abstract 071 – Undergraduate Student

Developmental Neurotoxicity of Trifluoroacetic Acid (TFA) in Zebrafish: Cellular, Mitochondrial, and Neurotransmitter Responses

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Trifluoroacetic acid (TFA) is an ultrashort-chain per- and polyfluoroalkyl substance (PFAS) formed from the degradation of certain fluorinated chemicals. Despite increasing environmental TFA contamination, little is known about its effects during early development. Therefore, this study used the zebrafish (*Danio rerio*) model to investigate the effects of TFA on cell viability, mitochondrial function, and neurotransmitter responses. Zebrafish embryos were exposed to 0, 0.04, 0.4, 4, 40, or 400 ppb (µg/L) TFA beginning within 0–2 hours post-fertilization (hpf) and maintained in the exposure solutions until 72 hpf (i.e., throughout embryogenesis). At 72 hpf, larvae were washed three times with fresh embryo water and maintained until 120 hpf. Cell death was assessed using SYTOX Blue staining. Mitochondrial superoxide, mitochondrial membrane potential, MitoTracker fluorescence, and ATP were assessed using fluorescence-based assays. Dopamine and glutamate levels were measured using ELISA-based assays. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test in SAS 9.4 (α=0.05). TFA exposure increased cell death at 40 and 400 ppb. No significant changes were observed in mitochondrial superoxide, MitoTracker fluorescence, dopamine, or glutamate. These findings suggest that TFA exposure increases cell death at higher concentrations, while the mitochondrial and neurotransmitter endpoints measured in this study were not significantly altered. Further studies are needed to understand the mechanisms underlying the increased cell death following developmental TFA exposure.

Abstract 072 – Undergraduate Student

6PPD and 4HDPA Photochemistry and Photophysis

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6PPD is a tire antidegradant that can be found in aquatic environments, where it undergoes transformation through oxidation, hydrolysis, or through exposure to UV light. Comparing the influence of different solvent properties on its UV photodegradation is important for predicting the behavior of these molecules in real-world environments. This research investigated the effect of solvent on the photodegradation of 6PPD and its hydrolysis product, 4HDPA. Solutions of each compound were prepared in multiple solvents at different initial concentrations as determined by UV-Vis spectroscopy. Samples were exposed to UV light for 30 s to over 20 min, and changes were monitored by UV-Vis spectroscopy and liquid chromatography–mass spectrometry. Photodegradation rates differed among solvents, with photodegradation in water being much slower for 4HDPA than in alcohols, demonstrating that solvent strongly influences photodegradation kinetics.

Abstract 073 – Undergraduate Student

Size Dependent Cytotoxicity, Genotoxicity, and Metal Ion Uptake of Glutathione-Protected Silver Nanoclusters in Human lung Fibroblast Cells

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Silver nanoclusters (AgNCs) are small, precisely structured silver assemblies of fewer than 100 atoms that are increasingly being explored for use in inhaled therapies targeting chronic lung infections. AgNCs are attractive antimicrobials because they act as tunable reservoirs of Ag^+, with larger clusters decomposing faster and delivering larger doses. Unlike larger silver nanoparticles (10-100 nm in diameter) that most toxicology studies have focused on, AgNCs have an atomically defined core size (~1-2 nm in diameter) and glutathione surface coating that could lead to very different biological effects – but we do not yet have data to describe what those effects actually are, especially in lung tissue.

Lung fibroblasts are an ideal cell type to study in this context because they are imperative for lung structure and health – maintaining tissue structure, regulating inflammation, and helping with repair after injury. If silver exposure disrupts fibroblast function, it could contribute to things like fibrosis or impaired healing, which could be a real concern for any inhaled therapeutic. We know from existing literature that silver can cause oxidative stress, DNA damage, and cell death – but how cluster size and surface chemistry change that picture in human lung fibroblasts specifically has not been assessed. Prior work showed that larger clusters (Ag45 and Ag66) kill planktonic *Staphylococcus* and *Pseudomonas aeruginosa* at minimum concentrations of 4.5 uM and 1.5 uM, respectively, whereas smaller clusters (Ag15(SG)12 and Ag32(SG)19) required approximately 150 uM and 5 uM, respectively.

We are running three endpoints in WTHBF-6 human lung fibroblast cells after a 24 hour exposure: clonogenic survival for cytotoxicity, chromosomal aberration analysis for genotoxicity, and silver uptake. We tested two AgNC formulations (mixtures of larger clusters with either cysteine or glutathione ligands) at 1 uM and 10 uM alongside ionic silver nitrate ($AgNO_3$) as a baseline, at concentrations of 1 uM to 10 uM and an untreated control. So far, clonogenic survival data from three biological replicates show that $AgNO_3$ causes a clear, concentration dependent decrease in survival in WTHBF-6 cells, giving us a solid ionic silver baseline to compare against the clusters. Genotoxicity and metal ion uptake work for the cluster formulations is still ongoing. Once complete this dataset should give us a clearer picture of how core size and surface ligand chemistry drive toxicity differences across the AgNC panel in a human lung fibroblast model. These data will be valuable for determining safety threshold of AgNCs for therapeutic use.

Abstract 074 – Undergraduate Student

Miniaturizing X-Ray Fluorescence Spectroscopy Through Simulation-Driven Vacuum Chamber Design

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X-ray fluorescence (XRF) spectroscopy has become an increasingly prevalent technology due to its atomic detection capabilities. This technique involves firing x-rays at a substance within a vacuum to trigger a shift in the atoms' higher orbital electrons. The subsequent release of energy can be analyzed to identify the atomic composition of the sample. Current downscaling efforts aim to decrease the size of such machines and the time to yield results while maintaining accuracy.

This project aims to create a downscaled XRF setup specialized in detecting Per- and polyfluoroalkyl substances (otherwise known as PFAS). PFAS is a forever chemical linked to hormonal disruptions and other complications and is commonly found in plastic consumer products. PFAS is composed of two components: a fluorinated carbon chain and a functional group. Because of the high presence of fluorine, the detection of fluorine via XRF in consumer product samples can serve as a proxy for determining if the sample contains PFAS.

In order to construct this miniaturized setup, our team utilized Geant4, an open-source Monte Carlo simulation toolkit for particle-matter interactions, to perform parametric simulations of a mock XRF system, allowing us to identify the optimal geometric configuration to maximize elemental detection while minimizing background radiation. The optimized parameters, together with cost constraints and other considerations, guided the design of the setup in SIEMENS NX (CAD software). Finite element analysis in Ansys Mechanical and Ansys Fluent was then conducted to test the model's mechanical properties against inherent forces in the setup. These include the weight of the XRF components and the pressure created inside the internal vacuum chamber.

Currently, our team has utilized the data from the simulation to construct and manufacture the first prototype for the XRF setup. We have found two main weak points: the first is that the prototype's large flat face needed for sample loading is susceptible to deformation from the force of the vacuum. Additionally, the prototype currently lacks adequate gaskets/sealing which is required to sustain the vacuum for the duration of the irradiation process. The simulation is currently undergoing design changes as well to better incorporate parameters like detector/source angle, source window material, and sources of air leakage. These parameters will help bridge the gap between an ideal simulation world and reality.

Abstract 075 – Undergraduate Student

Oxidative Stress and Antioxidant Responses to Trifluoroacetic Acid Exposure in Developing Zebrafish

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Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals that do not readily degrade in the environment. Long-chain PFAS such as PFOA and PFOS have been extensively studied and are associated with several toxic effects. Short-chain PFAS have been introduced as alternatives, but recent studies have also raised concerns about their toxicity. Ultra-short-chain PFAS, particularly trifluoroacetic acid (TFA), are increasingly detected in the environment. However, far less is known about their developmental toxicity. This study examined whether TFA exposure induces oxidative stress and alters antioxidant responses during early zebrafish (Danio rerio) development. Embryos were exposed to 0, 0.04, 0.4, 4, 40, or 400 ppb (µg/L) TFA from 0–2 to 72 hours post-fertilization (hpf), throughout embryogenesis. At 72 hpf, larvae were washed three times with filtered embryo water and allowed to develop until 120 hpf. Lipid peroxidation (LPO) was assessed using a TBARS assay. Antioxidant responses were examined by measuring catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test in SAS 9.4 ($\alpha = 0.05$). LPO increased at 40 and 400 ppb. CAT activity also increased at 40 and 400 ppb, while GPx activity decreased at 4, 40, and 400 ppb. TFA exposure did not significantly alter GR activity at any concentration. These findings suggest that developmental TFA exposure can disrupt oxidative balance and antioxidant defense in zebrafish. Further studies are needed to better understand the mechanisms underlying these responses.

Abstract 076 – Undergraduate Student

Hormonal Impact on Immunoglobulin Production

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Variations in immunoglobulin production are responsible for numerous physiological responses. Increases in immunoglobulin production are responsible for enhancing immune response as well as enhancing vaccine efficacy. Negative repercussions can include increased rate of autoimmune disease and allergic responses. With a decrease in immunoglobulin production these effects are displayed inversely. Autoimmune diseases tend to occur more frequently in women as compared to men with estrogen being considered inflammatory and testosterone being considered immunosuppressive. Testosterone has displayed significant suppression of immunoglobulin production from human peripheral blood mononuclear cells (PBMCs). In contrast, previous data from our lab has shown that estradiol does not directly alter antibody production in human B cells. However, the study on testosterone used mixed PBMCs so the direct impact of testosterone on human B cells is unresolved as well as how it compares with the impact of estrogen on human B cells. Previous data from our lab on hydrocortisone has shown hydrocortisone increased production of some immunoglobulin isotypes. The effect of these hormones compared to their effect alongside hydrocortisone on human B cells also remains an avenue for further investigation.

The overall objective is to determine if testosterone directly impacts antibody production using a human B-cell line model (CL-01). A physiologically relevant concentration range will be utilized for testosterone and hydrocortisone. We will utilize CL-01 cells, which can be stimulated by T-cell-derived factors such as CD40 ligand and IL-4 to induce antibody production and initiate class-switch recombination. Antibody isotype production will be quantified in culture supernatants by sandwich ELISA after 96 hours of incubation. Based on prior findings in PBMC cultures and our previous data evaluating hydrocortisone, we anticipate that testosterone will suppress stimulation-induced and hydrocortisone-induced antibody production in human CL-01 B cells.

Abstract 077 – Undergraduate Student

Developmental Lead (Pb) Exposure Triggers Brain Oxidative Stress in the Brain of Male Adult F1 Zebrafish in a Multigenerational Assessment

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Lead (Pb) contributes to increased reactive oxygen species (ROS) in different biological systems. In turn, ROS generates oxidative stress: an imbalance between free radicals and antioxidants in cells triggering alterations in the cell cycle, lipid degradation, and cellular apoptosis. Our recent studies have shown early-life multigenerational changes generated from developmental Pb exposure. However, the multigenerational late-in-life neurological effects from this developmental Pb exposure remain unstudied. Therefore, this study investigates how low-level Pb exposure affects the F1 generation (the first generation of fish born from exposed parents) aged 1 year. We aim to understand if developmental Pb exposure in parents changes oxidative stress markers in the F1 adult brain. It is hypothesized that Pb affects the antioxidant system multi-generationally. To address these questions, zebrafish (Danio rerio) were exposed to low concentrations of Pb (0, 0.01, 0.1, or 1 ppb; parts per billion, µg/L) from 1 to 72 hours post-fertilization and then Pb exposure ceased. These fish were raised to adulthood and bred to produce an F1 generation. When the F1 reached 1 year of age, markers of oxidative stress were evaluated on whole-brain tissue including mitochondrial and cytoplasmic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) as indicators of the enzymatic antioxidant system. In addition, reduced glutathione (GSH) and oxidized glutathione (GSSG) were assessed as indicators of the non-enzymatic antioxidant system. Outcomes were analyzed using a one-way ANOVA followed by Tukey's post hoc test ($\alpha = .05$) and assessed by sex ($n=6$) to understand sex-specificity. Data showed significant decreases in GPx, GSH, and CAT antioxidant defense activity and an increase in TBARS displaying increased lipid peroxidation in the two highest concentrations (0.1 and 1 ppb) in males, but no changes were measured in the females. With that said, it is now evident that parental Pb exposure has lasting effects on biomolecular health in the next generation and significantly impairs antioxidant defenses in males compared to females.

Abstract 078 – Undergraduate Student

Can exercise mitigate the effects of developmental benzo[a]pyrene exposure on visual recognition memory and spatial learning and memory?

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Benzo[a]pyrene (BaP) is a neurotoxic polycyclic aromatic hydrocarbon that can be found in grilled foods, cigarette smoke, vehicle exhaust and other sources of fossil fuel combustion. Previous studies found that children exposed to BaP and other polycyclic aromatic hydrocarbons during early brain development had lowered IQ and behavioral issues that persisted into adolescence. Our studies using a mouse model to mimic human variation in genes associated with BaP metabolism found that *Cyp1a2(-/-)* knockout mice were more susceptible to developmental BaP exposure compared with *Cyp1a2(+/+)* wild type mice. In our follow-up studies, we used daily exercise as an intervention to determine if that could mitigate the adverse effects of BaP. Pregnant mice were treated with corn oil or 10mg/kg/day BaP dissolved in corn oil from gestational day 10 (G10) to postnatal day 25 (P25). Half of the offspring had daily access to running wheels for 1hr a day from P30 to P60 when neurobehavioral testing began. We used Novel Object Recognition to assess visual recognition memory and Morris Water Maze to assess spatial learning and memory. We found no sex differences. There was a significant gene x treatment interaction in Novel Object Recognition ($p = 0.029$) and a trend toward significance for a treatment x exercise interaction ($p = 0.065$). The Cued phase demonstrated that all mice could swim and could visually locate the escape platform. Offspring that exercised out-performed control mice on Days 1 ($p < 0.001$), 2 ($p < 0.05$) and 4 ($p < 0.05$). We also found a benefit of offspring exercise for BaP-exposed mice in the Reverse phase, which is more challenging. There was a significant treatment x exercise interaction on Days 4 and 6 ($p < 0.05$) and overall better performance on 5 of 6 days of testing. Together, these results suggest that offspring exercise had a modest benefit on spatial learning and memory.

Abstract 079 – Undergraduate Student

Exercise effects on anxiety-like behaviors in two genotypes of mice after developmental exposure to benzo[a]pyrene

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Exposure to benzo[a]pyrene (BaP) happens daily through vehicle exhaust, cigarette smoke, power plant emissions and grilled foods. Those especially vulnerable to its harmful effects are the offspring of pregnant women. Prior studies have found persistent neurological deficits in children exposed to high levels of polycyclic aromatic hydrocarbons (PAH) during early brain development. These deficits can include impaired learning and memory, behavioral issues and an increase in anxiety. We use BaP as a prototypical PAH with well-characterized mechanisms of toxicity and mice with variations in CYP1 enzymes known to metabolize BaP, and our previous studies found that *Cyp1a2(-/-)* knockout mice were more susceptible to developmental BaP exposure compared with *Cyp1a2(+/+)* wild type mice. I In these studies, we hypothesized that voluntary offspring exercise would mitigate anxiety-like behavior. We focused on exercise as a cost-effective way to intervene with the long-term goal of translating our findings to the most susceptible human populations. Pregnant mice from the two genotypes (were fed either the corn oil vehicle or 10mg/kg/day BaP dissolved in corn oil from gestational day 10 to weaning at postnatal day 25 (P25). The offspring had access to running wheels 1h/day from P30 to P60 when neurobehavioral testing began. We used complimentary tests of anxiety-like behavior, the elevated zero maze and marble burying to compare males and females in each treatment group and genotype. We found significant benefits of exercise for all groups. Exercise reduced anxiety-like behavior for three of the four outcomes measured in the elevated zero maze. Mice that exercised had significantly shorter latencies to leave the closed quadrant ($p = 0.037$), more head dips ($p = 0.025$), and more zone crossings ($p < 0.001$) compared with mice that did not exercise. Interestingly, mice that exercised spent less total time in the open quadrants ($p < 0.001$). Exercised mice also buried significantly fewer marbles with wild type mice showing the greatest difference from non-exercised controls ($p < 0.001$). There were similar reductions when comparing BaP-exposed mice with corn oil treated control ($p < 001$). Together, these results suggest that regular exercise is beneficial at mitigating the deleterious effects of developmental BaP exposure on anxiety-like behaviors.

Abstract 080 – Undergraduate Student

Effect of Perfluorodecanoic Acid (PFDA) on Glutathione Related Antioxidant Defense and Mitochondrial Membrane Potential in Developing Zebrafish

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Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals. PFAS are commonly used in industrial and consumer products due to their resistance to heat, water, and oil. However, their strong carbon-fluorine bonds make them highly persistent in the environment, giving them the reputation as “forever chemicals”. Long-chain PFAS such as perfluorodecanoic acid (PFDA, C-10), are particularly concerning because of their high detection and persistence in water, soil, food sources, and biological tissues. Exposure to PFAS has been associated with many adverse health outcomes, including developmental toxicity, endocrine disruption, and neurotoxicity. Mitochondria produce cellular energy through oxidative phosphorylation and is critical for neuronal function. Mitochondria disruption can lead to decreased ATP production and impaired cellular processes. PFDA has been found to trigger mitochondrial dysfunction; however, its neurodevelopmental toxicity and underlying mitochondrial mechanism remain largely unknown. This study used the zebrafish (*Danio rerio*) model to assess the developmental neurotoxicity of PFDA focusing on glutathione-dependent antioxidant defense and mitochondrial membrane potential. Zebrafish embryos were exposed to PFDA at concentrations of 0, 0.004, 0.04, 0.4, 4, or 40 parts per billion (ppb, µg/L). Exposure occurred from early developmental stages (initiated at 1-2 hours post fertilization, hpf) through 72 hpf encompassing embryogenesis. After exposure, larvae were rinsed three times and collected 48 hours after exposure cessation at 120 hpf for further analysis. Antioxidant enzyme and mitochondrial membrane potential were measured spectrophotometrically and using JC-1 dye, respectively. JC-1 dye accumulates in mitochondria and emits different fluorescent signals depending on the membrane potential. A higher ratio of red/green fluorescence indicates polarized mitochondria, whereas lower red/green fluorescence indicates depolarized mitochondria. A one-way ANOVA followed by a Tukey's post hoc test ($\alpha = 0.05$) was used to check the differences among treatment groups. Antioxidant enzyme levels were increased compared to the negative control group. The red/green fluorescence ratio decreased in the highest PFDA treatment group (40 ppb), suggesting PFDA exposure may disrupt mitochondrial membrane potential. These results indicate PFDA can impair antioxidant defense and mitochondrial function. Future work will focus on mitochondrial reactive oxygen outcomes and ATP measurements to further assess their role in mitochondrial dysfunction.

Abstract 081 – Undergraduate Student

Reducing UVB induced microvesicle particle release using functional inhibitors of acid sphingomyelinase

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Patients with photosensitive disorders like xeroderma pigmentosum type A (XPA) show increased sensitivity to sunlight, specifically UVB irradiation. Photosensitive reactions cause sunburns with severe reactions causing hospitalization due to multiple organ dysfunction (MOD) including the lungs, gut, and kidney. Even though a significant portion of the population is photosensitive, the underlying mechanism is not fully understood. Our group has shown that lipid mediator Platelet-activating factor (PAF) binds to its receptor PAFR which activates the enzyme acid sphingomyelinase (ASM) when exposed to UVB. ASM causes the release of extracellular vesicles called microvesicle particles (MVPs) by converting sphingomyelin into ceramide. We hypothesize that MVPs transport PAF from the skin and bind to PAFR in the gut causing bacterial translocation (BT) which results in MOD. Additionally, we hypothesize that inhibition of ASM will cause a reduction in MVP release and therefore reduce systemic toxicity.

Using HaCaT keratinocytes, we investigated the release of MVPs induced by UVB exposure in wild type (WT), ASM-deficient, and photosensitive XPA-deficient cells. MVP release was significantly increased in XPA-deficient cells treated with UVB in comparison to HaCaT cells. Additionally, cells were treated with functional inhibitors of ASM (FIASMs) amitriptyline and fluoxetine, to determine if FIASMs could lower MVP release in XPA-deficient and WT cells. Treatment with FIASMs before UVB exposure reduced MVP release in XPA-deficient cells. ASM-deficient cells showed no significant change in MVP release when treated with UVB alone or in combination with FIASMs but do release MVPs when treated with ceramide.

Additionally, UVB induced MVP release, BT, and MOD were investigated in WT C57Bl/6, XPA-deficient, ASM-deficient, and PAFR-deficient mice. MOD was measured using qPCR and histology. XPA-deficient mice showed significant BT and MOD. Furthermore, WT and XPA-deficient mice were treated with FIASMs amitriptyline and fluoxetine before UVB exposure. FIASMs showed a significant reduction in both BT and MOD in XPA-deficient mice. In conclusion, this data indicates that FIASMs amitriptyline and fluoxetine could be novel therapeutics for systemic toxicity associated with photosensitivity.

Abstract 082 – Undergraduate Student

microRNAs and Their Role in Arsenic-induced Skin Cancer: Validating Predicted Gene Targets of hsa-miR-452 Through in-vitro Study

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Inorganic arsenic (iAs) is a natural, toxic metalloid and group I human carcinogen. Over 2 million people in America are exposed to iAs through contaminated well water. iAs causes cancer through the disruption of biological processes such as DNA repair, chromosomal stability, alternative mRNA splicing, and microRNA (miRNA) expression. miRNAs are short, non-coding RNAs that regulate gene expression largely through inhibiting mRNA translation. Our lab has shown the miRNA hsa-miR-452 is dysregulated in skin tumors in iAs-exposed patients and in our *in-vitro* model of iAs-induced skin cancer. We hypothesized that hsa-miR-452 overexpression will suppress proteins linked to biological pathways altered by iAs exposure in human keratinocytes (Ker-CT). Three miRNA/mRNA binding databases (TargetScan, mirDIP, and MicroT) were used to predict mRNA targets of miR-452-5p (mature miR-452). Over a thousand genes were identified as potential targets: 1,054 from MicroT, 192 from mirDIP, and 182 from TargetScan. 54 genes were predicted to miR- 452-5p in all three databases, then filtered for genes involved in pathways known to be disrupted by iAs: DNA repair, chromosomal stability, alternative splicing, alternative nucleotide methylation, cell cycle progression, cellular proliferation, as well as association with cancer. From this list *RPA1*, *SF3B1*, and *HP1BP3* were selected as validation targets of miR-452-5p binding. These targets are involved in DNA damage repair, alternative splicing, and chromosomal stability, respectively. String networks were created in STRING database to predict protein-protein interactions of miR-452-5p targets. miRNA expression was measured in Ker-CT overexpressing miR-452 or a scrambled miRNA control (SC-miR). The protein and mRNA expression of the three validation targets were measured in both groups in triplicate. miRNA expression was significantly higher in the miR-452 overexpressing cell line compared to SC-miR. RPA1 expression was found to be significantly reduced in miR-452 overexpressing cells. mRNA expression remained unchanged in both lines. These findings support the prediction of at least one gene target of hsa-miR-452 through experimental validation. Further study will seek to validate other targets, investigate the functional consequences of RPA1 dysregulation, and determine if miR-452 overexpression accelerates malignant transformation in our *in-vitro* chronic iAs exposure model.

Abstract 083 – Undergraduate Student

Effects of PFOA and Ethanol Co-Exposure on Gut Integrity and Metabolic Signaling in Mice

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Background: Alcohol-associated liver disease (ALD) remains a major public health challenge, yet only a subset of individuals with heavy alcohol consumption develop severe disease, suggesting that modifiable risk factors such as environmental exposures may influence susceptibility. Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants linked to hepatotoxicity and metabolic dysfunction. Our ongoing studies of perfluorooctanoic acid (PFOA) and ethanol co-exposure have revealed hepatic lipid accumulation and molecular evidence of metabolic stress. However, whether intestinal alterations accompany these effects and potentially contribute to liver injury remains unclear.

Hypothesis/Goal of Study: We hypothesized that PFOA modifies ethanol-associated ileal homeostasis by altering mucosal and antimicrobial defenses, tight junction-associated signaling, inflammation, and ethanol metabolism.

Methods: Male C57BL/6J mice received pair-fed or ethanol-fed Lieber-DeCarli diets and were concurrently administered vehicle or PFOA (2 mg/kg/day) via oral gavage for 10 days. Ethanol-fed mice then received an ethanol binge (5 g/kg) and were euthanized 5–6 h later. Ileal tissues were harvested for qPCR analysis of mucosal defense, tight junction, inflammatory, ethanol metabolism, and aldehyde detoxification genes. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparisons.

Results: Compared with ethanol alone, PFOA-ethanol co-exposure significantly downregulated ileal *Defa1* (P=0.0030) and *Defa4* (P=0.0363) mRNA expression and upregulated *Adh1* (P=0.0160). Relative to PFOA alone, co-exposure significantly decreased *Muc2* (P=0.0106) and the tight junction-associated transcript *Cldn3* (P=0.0352), indicating altered mucosal and epithelial junction-associated gene expression. No significant differences in *Il1b, Il6*, or *Tnfa* expression were detected between ethanol-alone and co-exposure groups. Ethanol increased *Aldh2* mRNA expression in both vehicle-treated (P=0.0064) and PFOA-treated (P=0.0175) mice.

Conclusions: Collectively, these findings suggest that PFOA-ethanol co-exposure alters ileal transcript profiles, with reduced expression of antimicrobial defensin expression, modified mucosal and tight junction-associated responses, and changes in ethanol metabolism. These alterations may be relevant to gut-liver axis dysfunction and warrant further investigation of intestinal barrier function and potential contributions to hepatic injury.

Abstract 084 – Undergraduate Student

Measurement and Mapping of Metal Leaching Inside Common Vapes

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Vaping has become a widely used alternative to conventional smoking, with more than twenty million Americans using electronic cigarettes. One potential health risk is the inhalation of metals released into e-cigarette aerosols from device components. Heating elements and wicks of the devices are particularly relevant due to their repeated elevated temperatures and direct contact with the e-liquid. This study evaluated twelve vapes with varying models and brands to investigate potential metal transfer from heating elements to organic cotton wicks.

Portable X-ray fluorescence (XRF) measurements were used to characterize the elemental composition of heating elements and compare the measured compositions with manufacturer information. Iron (Fe), nickel (Ni), and chromium (Cr) were among the most common elements identified.

Micro-XRF provided spatially resolved measurements of metal distribution across the cotton wick, with particular attention to the elements of toxicological concern, including chromium and nickel, and their distribution within samples. Elemental maps were analyzed statistically to identify regions with characteristic X-ray counts exceeding three standard deviations above the mean, indicating potential metal deposition hotspots.

From this analysis, we found that every wick contained Fe, Ni, and Cr with variation in the amount of contamination. With 50% of samples containing a highly elevated Ni hotspot, and eight samples containing highly elevated Fe hotspots. These findings provide evidence of spatial variation in metal distribution and suggest potential transfer of metals from heating elements to wicks. Further investigation is needed to determine the extent to which deposited metals contribute to metal concentrations in e-cigarette aerosols and the associated potential health risks.

Abstract 085 – Undergraduate Student

Spatial Characterization of Zinc in Human Hair and Nail Specimens Using Tabletop Micro-XRay Fluorescence

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Zinc (Zn) is an essential trace element, but commonly used biomarkers may not capture spatial variation within keratinized tissues. This study evaluated whether tabletop micro-X-ray fluorescence (μXRF) could characterize Zn distribution in intact human hair and nail specimens collected from individuals with no known change in Zn intake. Nineteen specimens were analyzed, including five fingernails, eight toenails, and six hair samples. Elemental maps were generated by μXRF, regions of interest were defined, and each specimen was divided into 10 sequential sections representing estimated distance across the measured region. Zn signal at each position was normalized to the mean Zn signal of the individual specimen before averaging within tissue type. Within-specimen heterogeneity was also described using the 99th-percentile-to-mean signal ratio and coefficient of variation (CV). Fingernail and toenail profiles showed lower normalized Zn signals near the ends of the measured region and higher signals toward the middle, with peak values of approximately 1.26 and 1.31–1.32, respectively. Hair profiles were comparatively stable, ranging from approximately 0.91 to 1.09 across positions. The p99-to-mean ratio ranged from 5.23–8.33 in fingernails, 6.04–9.92 in toenails, and 6.01–10.07 in hair, with mean CV values of 1.54, 1.80, and 1.87, respectively. These findings show that Zn signal is spatially heterogeneous within individual hair and nail specimens and that tabletop μXRF can preserve and visualize this variation without chemical digestion. Spatial profiling may complement bulk elemental measurements and support future studies examining changes in Zn exposure over time.

Abstract 086 – High School Student

Cadmium induces pro-fibrotic signaling in human renal proximal tubules by down-regulating NF-E2 expression

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Cadmium (Cd) is a non-essential heavy metal, non-biodegradable environmental toxin, and accumulates in the body with an estimated half-life of 10-30 years. Cd exposure occurs from a wide range of sources, including drinking water, food, ambient air, smoking, contaminated dust and soil and is known to cause chronic damage to the kidneys. The major mechanism of kidney damage is attributed to Cd-induced oxidative stress. Therefore, identifying mechanism underlying cadmium induced ROS production may lead to the identification of more therapeutic targets.

Human renal proximal tubule cells (HK-11) were cultured and treated with varying doses of Cd for 24 to 72 hours. Cell lysates were prepared and subjected to immunoblot analysis with various antibodies. Cd treatment induced expression of profibrotic protein Fibronectin (FN) in a dose dependent manner and decreased expression of nuclear factor- erythroid derived protein 2 (NF-E2). To determine the role of NF-E2 in modulating Cd-induced pro-fibrotic signaling in HK-11 cells, we transfected HK-11 cells with pUSE cDNA or NF-E2 wild type, NF-E2 S170A or NF-E2 S170D cDNA. Twenty-four hours after transfection, cells were treated with/without 50 μM $CdCl_2$ and cells were lysed after 24 hours and subjected to immunoblot analysis.

Cd-induced FN, PAI-1, and KLF6 expressions in HK-11 cells which were significantly inhibited by overexpressing NF-E2 WT cDNA. We are currently assessing effects of NF-E2 phosphorylation mutants on modulating Cd-induced pro-fibrotic signaling in the renal cells. Our studies have identified NF-E2 as a possible cellular target that will alleviate Cd-induced oxidative damage and fibrosis in HK-11 cells by possibly inducing its cellular target glutathione.

Abstract 087 – Early-Stage Investigator

hsa-miR-362 Overexpression in Human Keratinocytes Recapitulates Phenotypes of Arsenic Exposure

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Background: Inorganic iAs (iAs) is an environmental carcinogen that causes skin, lung, and bladder cancers. Over 200 million people are exposed to iAs contaminated groundwater. iAs dysregulates DNA repair, chromosomal stability, mRNA expression, alternative splicing, and miRNA (miR) expression. hsa-miR-362 (miR-362) overexpression (OE) was observed in iAs-induced skin tumors and has been associated with several types of cancer, suggesting a role in carcinogenesis.

Hypothesis: miR-362 OE will dysregulate pathways known to be affected by iAs.

Methods: Ker-CT cells were transduced with lentivirus containing a miR-362 or scrambled miR control (SC-miR) OE construct. Monoclonal cultures were produced and assayed for mature miR-362-5p expression. Three miR-mRNA binding databases were cross-referenced and predicted mRNA targets in pathways known to be affected by iAs were identified and ranked by combined score. Translational repression of three targets (RMI1, CENPK, and LUC7L3) was confirmed with immunoblot and RT-qPCR, followed by functional assays. T-tests were used to determine statistical significance; $\alpha = 0.05$.

Results: RMI inhibition causes Bloom-like syndrome which can be diagnosed by increased sister chromatid exchange (SCE) and elevated SCE is observed in patients exposed to environmental iAs. SCE analysis confirmed miR-362 Ker-CT cells and had more crossing over events than SC-miR cells. LUC7L3 inhibition leads to genomic instability including the formation of multipolar spindles, a known effect of iAs exposure. γ-tubulin immunofluorescence revealed multipolar spindles in miR-362 Ker-CT cells. Inhibition of CENPK results in cells stalling at the G1/S checkpoint, and iAs causes cell cycle disruptions. Dysregulated cell cycle was confirmed with flow cytometry; results indicated prolonged S phase and decreased G2/M phase compared to SC-miR. OE of miR-362 suppressed the expression of proteins involved in DNA repair, maintenance of chromosomal stability, and alternative splicing, and resulted in phenotypic changes consistent with iAs exposure.

Conclusions: These results indicate a mechanistic rationale for miR-362's contribution to iAs carcinogenesis.