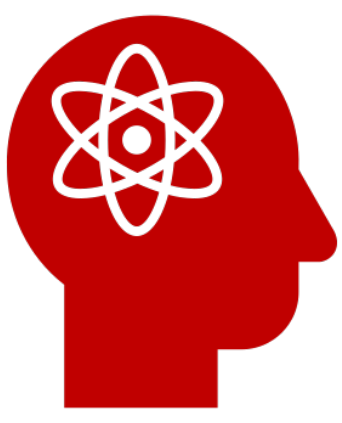
RUTGERS

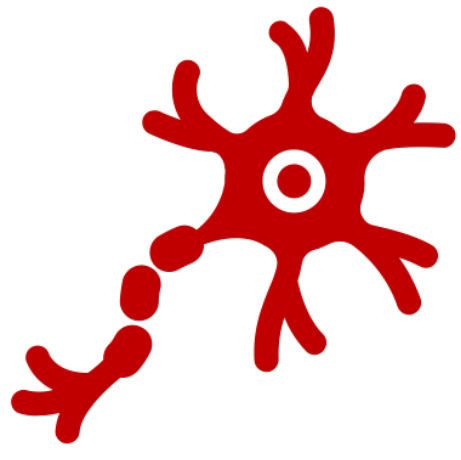
THE STATE UNIVERSITY OF NEW JERSEY

Summer Undergraduate Research Fellowship (SURF)

Final Presentations

July 27, 2023





Week	SURF Weekly Activities
1	Orientation (Lab Notebook Keeping, Reading Manuscripts, Responsible Conduct of Research)
	Advice from SURF Alumni
2	Building Your LinkedIn Profile
3	Chemicals in Our Environments with Dr. Cathleen Doherty: Overview of Lead Toxicity and Sampling
	Tie Dye Lab Coats
4	A Small Follicle Tells a Big Story with Dr. Shuo Xiao
5	Communicating Science: Crafting Your Message
6	Careers, Networking, and Painting: Meet Alumni and Trainees
7	What's in Your Personal Care Products?
8	Bristol-Myers Squibb Field Trip
	The Art of Compelling Abstracts
	Careers in Pharma with Dr. Gary Grover
9	Research Data Blitz from Pharmacy Students
10	Intro to Flow Cytometry from Dr. Ray Rancourt
	What's in Your Drinking Water? with Sean Stratton
11	Final Symposium

Session I Presentations (11:00 a.m. – 12:00 p.m.)	
Presenter	**Mentor**
Angel Beebe	Buckley
Joseph Xie	Brunetti
Kelly Nugent	Androulakis
Sadaf Gharibi	Michniak
Tom Cai	Zhou
Shuchi Merai	James
Caylee Brown	James
Armando Rios	J. Laskin

Session II Presentations (12:15 p.m. – 1:15 p.m.)	
Presenter	**Mentor**
Brendan Connor	Minko
Maria Ruszkiewicz	Suh
Nhan Huynh	Chen
Sophia Fanzini	Chen
Stephanie Mera	Guo
Kyle Yuncza	D. Laskin
Andrew Jelinsky	Gow
Brian Chan	An

Session III Presentations (1:30 p.m. – 2:15 p.m.)	
Presenter	**Mentor**
Erica Acox	Aleksunes
Mo Patel	Aleksunes
Astha Adroja	Xiao
Praytush Venkatesh	Stapleton
Nacala Gadsden	Stapleton
Adrita Dasgupta	Pilch

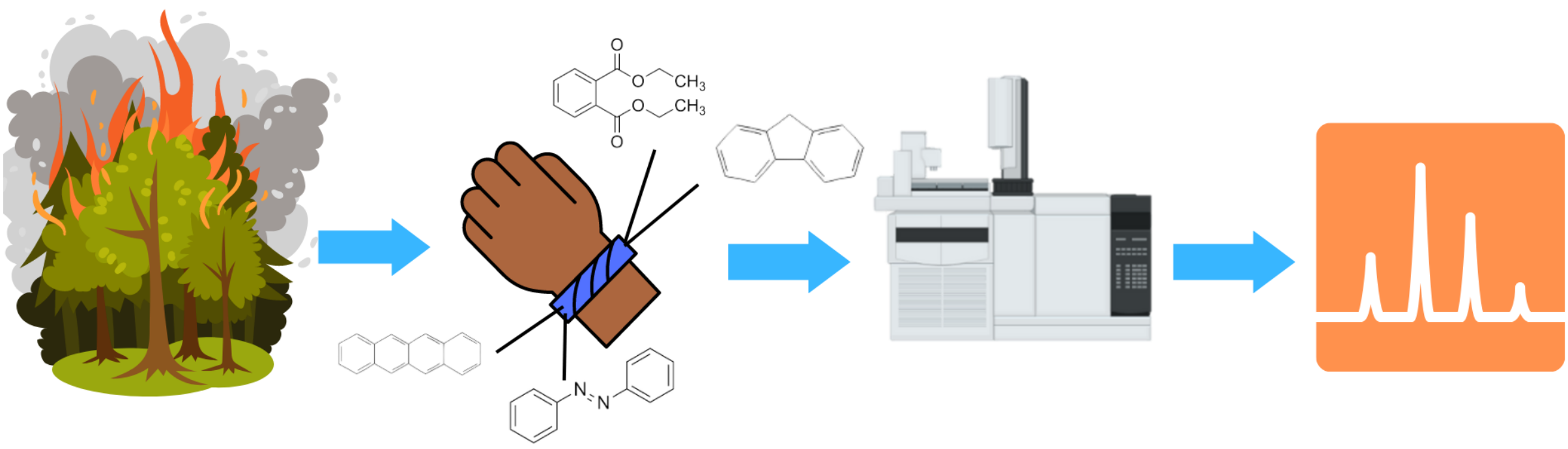

PUMA


CANCER

Tracking Environmental Toxins from the 2023 Canadian WildFire with Silicone Wristbands

Angel Beebe, Hilly Yang, Martin Geraghty, Brian Buckley
Smith College, Technological University Dublin, and Rutgers, The State University of New Jersey

Research has shown that environmental pollutants found in the air can have significant adverse health effects, some include disruption in the endocrine system, complications with reproduction, and cancer. Such classes of pollutants - polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCP), and organophosphate esters (OPEs) are ubiquitous in modern infrastructure, comprising a measurable fraction of our environmental contaminant exposures. Past research methods for estimating exposure include hand wipes, blood/urine testing, and solid-phase micro-extraction (SPME) fiber. Our aim is to use silicone wristbands to test their effectiveness in detecting exposure to different air pollutants from the 2023 Canadian forest fire. Wristbands were placed on the third-floor EOHSI patio for 4 days. Some wristbands were exposed for half the day and others for 24 hours. The exposed wristbands were extracted using methylene chloride and ethyl acetate (1:1 v:v) and evaporated under nitrogen. An Agilent Technologies 240 Ion Trap gas chromatography-mass spectrometer was used to analyze the samples. The SWBs were able to detect different classes of toxic compounds. Tentatively identified compounds (TICs), were fluorene, benzyl butyl phthalate, naphthacene, and diethyl phthalate. Knowing that the wristbands are able to effectively track one's everyday exposure, they can be used for people in different occupational settings, especially for people who are at a higher risk of being exposed such as pregnant women, children, firefighters, or a farmer. In the future human participants will wear wristbands to track their everyday exposure.



2023 Canadian forest fire smoke traveled to New Jersey

Using silicone wristbands to track toxic vapor phase environmental contaminants

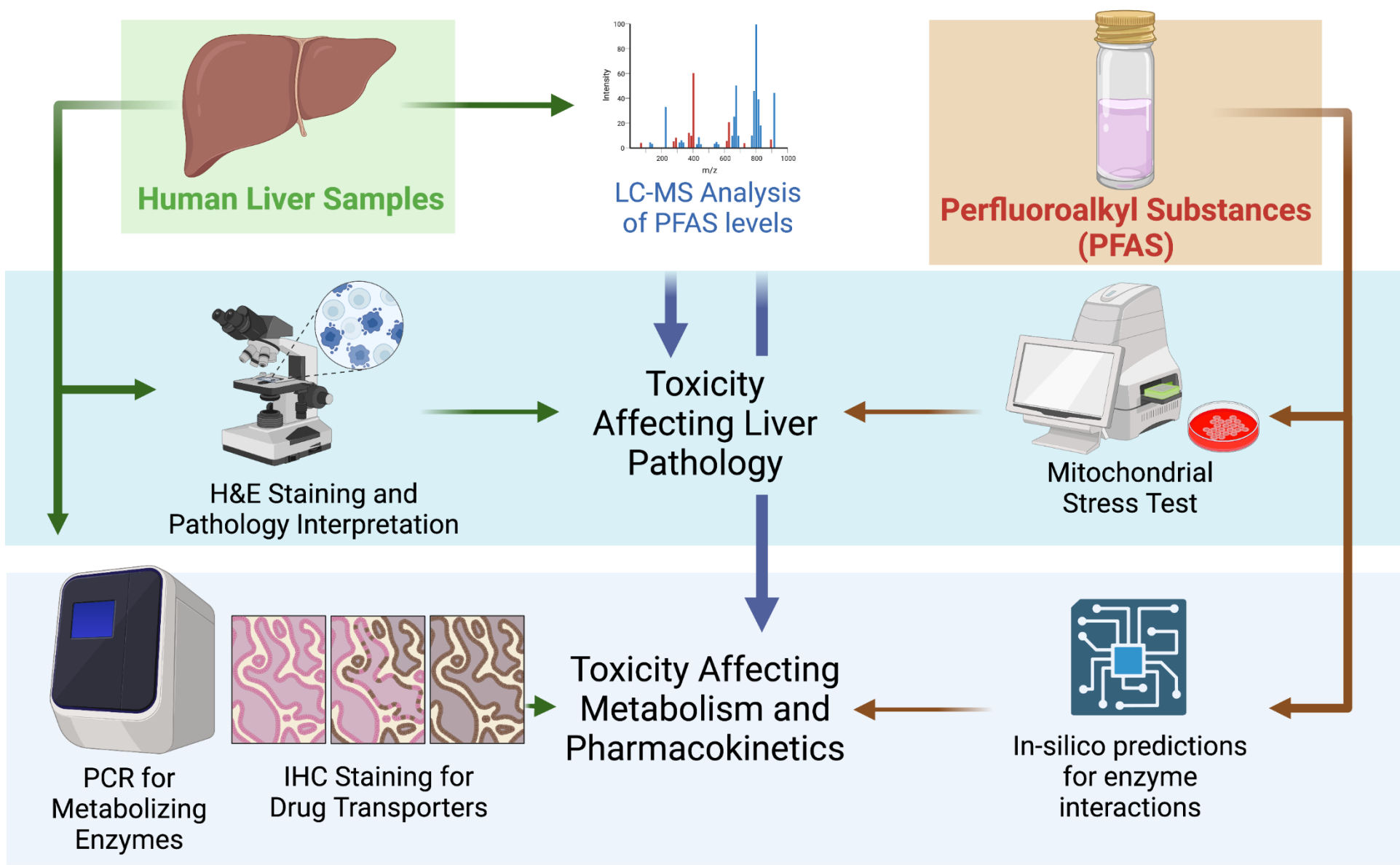
Agilent Technologies 240 Ion Trap gas chromatography used to analyze the samples from extracted wristbands

Chromatography analyzed and compounds were identified

Perfluoroalkylated Substances' Effect on Hepatocyte Pathology and Pharmacokinetically Relevant Drug Enzymes and Transporters

Joseph Xie, Moriah Anthony, Luigi Brunetti
Rutgers, The State University of New Jersey

Per/polyfluoroalkylated substances (PFAS) is a large class of chemicals associated with chronic toxicity used in products such as kitchenware, food packaging, and cosmetics. We hypothesize that the effect of PFAS on human hepatocytes can not only lead to the development of diseases such as fatty liver through mitochondrial stress, but also modify the liver's pharmacokinetics and impact drug therapeutic response. A literature search was conducted to search for the PFAS with the highest accumulation in the body, then potential candidates were input into Gastroplus for physiological based pharmacokinetic modeling analysis of liver specific accumulation and predicted enzyme inhibition. Human liver samples were also stained with hematoxylin and eosin (H&E) for pathological analysis and with immunohistochemistry (IHC) for the drug transporters OATP1B1 and OATP1B3. The liver samples will then be analyzed for PFAS levels with liquid chromatography-mass spectrometry (LC-MS) and for metabolizing enzyme expression using PCR. The effect of PFAS on mitochondrial respiration will be evaluated in-vitro using Agilent Seahorse on cultured cells. The H&E staining has been performed and several of the liver samples have been identified with hepatic lipidosis. The IHC slides have had their staining intensity quantified and the PCR has been performed, indicating a wide variety of metabolic capacity. The LC-MS and Seahorse is still being conducted and the results will be assessed in conjunction with the other data. If there is a correlation between the pathology of the liver samples, the level of drug metabolizing enzymes, and the levels of PFAS, this could indicate that PFAS can both increase the risk of liver diseases and also affect response to pharmacologic treatment. More research will then need to be performed to see if the effect is clinically impactful.



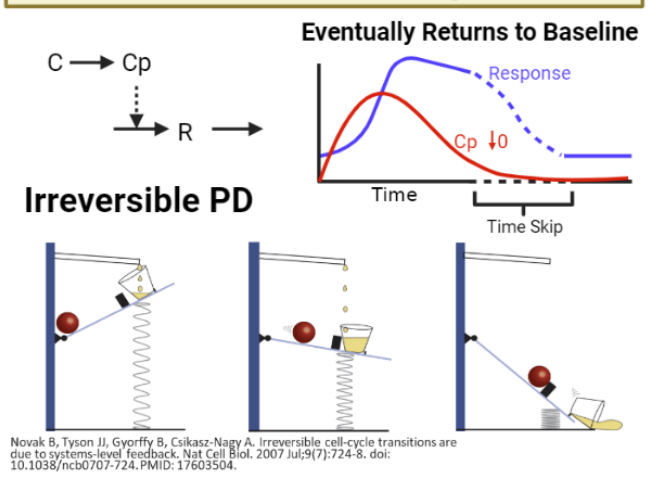
Mechanism-Based Irreversible Pharmacodynamic Models

Kelly Nugent, Ioannis Androulakis
Rutgers, The State University of New Jersey

Pharmacodynamic (PD) models describe a host's pharmacologic response resulting from drug-target interactions and are key in drug development. The pharmacologic effects may be reversible or irreversible; the difference lies in the system's response once the drug has cleared. Once the drug is eliminated from the system, a reversible response will recede concurrently, whereas an irreversible response will persist well beyond drug elimination. Irreversible effects are less straightforward in their mechanisms, and current models mimic this with slow-resolving kinetics instead of with signaling mechanisms that can induce irreversibility. In the present work, we propose mechanisms that describe drug modes of action able to generate irreversible PD effects. We hypothesize a core signaling cascade incorporating an ultrasensitive response, thus generating irreversible hysteresis and bistability. Combined with a basic pharmacokinetic model, we propose PD models utilizing this core cascade able to induce irreversible effects via a) acute (single dose) exposure; b) multiple, lower strength exposures; and c) chronic, lowest strength exposure. The PD models outlined here can emulate these conditions and provide plausible mechanisms for their inductions of irreversible effects. Additionally, the models can be applied outside the field of drug development and to the long-term effects of stress, such as the physiological damage of chronic stress-induced allostatic load. Supported by NIH R25ES020721 and the Rutgers University SURF Program.

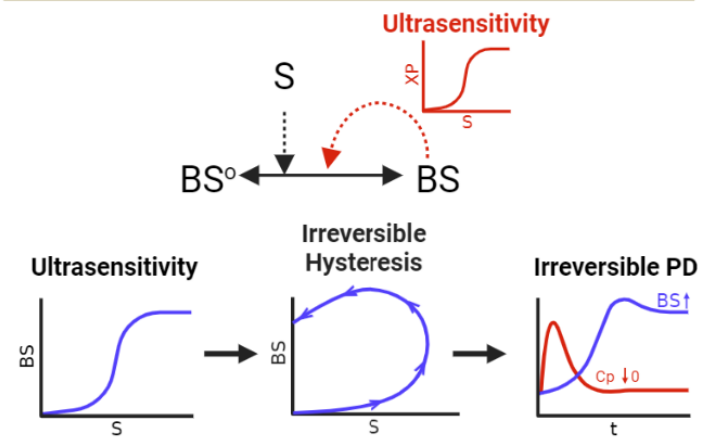
Mechanism-based Irreversible Pharmacodynamic Models
Kelly Nugent[1] and Ioannis P. Androulakis[2]
[1]Pharmaceutical Sciences, U of Michigan [2]Biomedical Engineering, Rutgers University

Irreversible Pharmacodynamics



Novak B, Tyson JJ, Gyorffy B, Csikasz-Nagy A. Irreversible cell-cycle transitions are due to systems-level feedback. Nat Cell Biol. 2007 Jul;9(7):724-8. doi: 10.1038/ncb0707-724. PMID: 17603504.

Hypothesis



Basic PKPD Model Structure

Pharmacokinetics

$$\frac{dC}{dt} = -ka \times C$$

$$\frac{dCp}{dt} = C - Cp$$

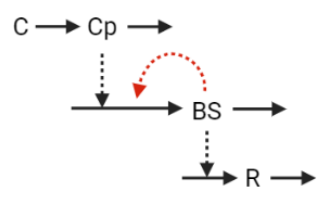
Pharmacodynamics

$$\frac{dBS}{dt} = \left(k_1 \times Cp + k_2 \times \frac{BS^n}{K^n + BS^n}\right)(BS_T - BS) - km_1 \times BS$$

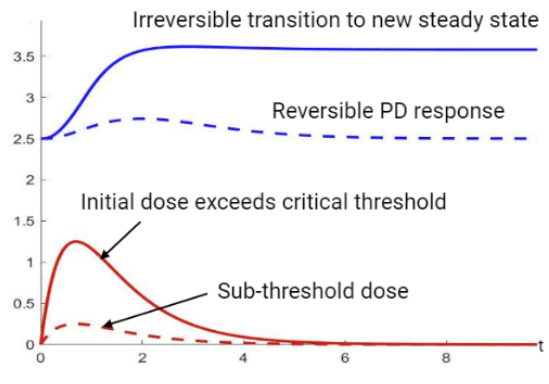
$$\frac{dR}{dt} = k_{in} \times \left(1 + \frac{BS}{1+BS}\right) - k_{deg} \times R$$

Results

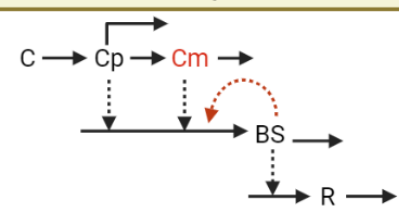
Single dose



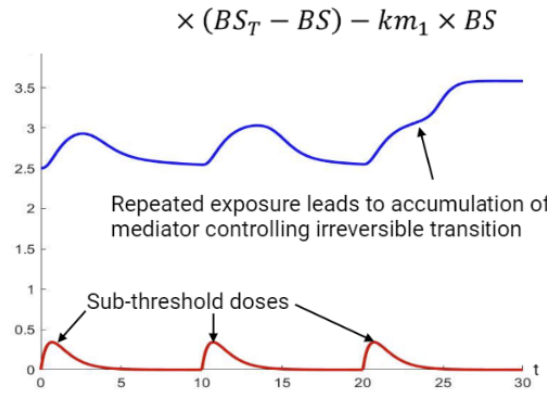
Drug concentration, Cp
Pharmacodynamic effect, R



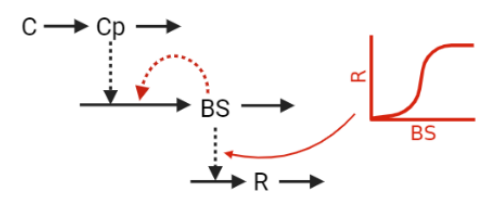
Multiple doses



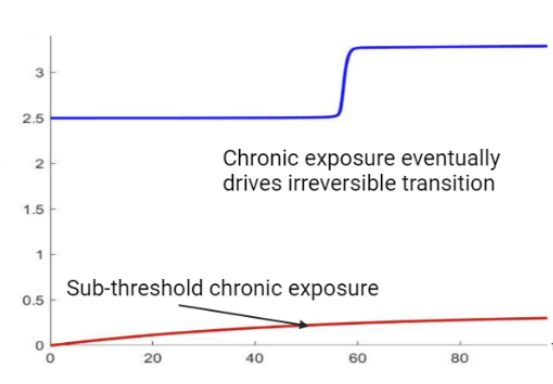
$$\frac{dBS}{dt} = \left(k_1 \times Cp + k_{cm} \times Cm + k_2 \times \frac{BS^n}{K^n + BS^n}\right) \times (BS_T - BS) - km_1 \times BS$$



Chronic dosing

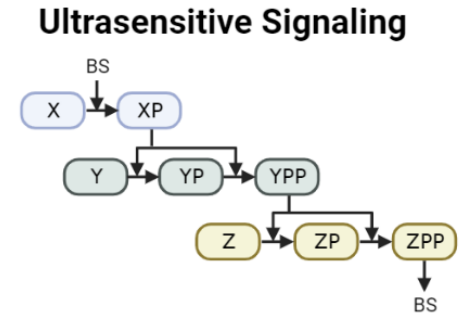


$$\frac{dR}{dt} = k_{in} \times \left(1 + \frac{BS^n}{1+BS^n}\right) - k_{deg} \times R$$



Next Steps

Ultrasensitive Signaling



Biologically relevant signaling cascades (MAPK) lead to ultrasensitivity, modeled using simplified HIll model

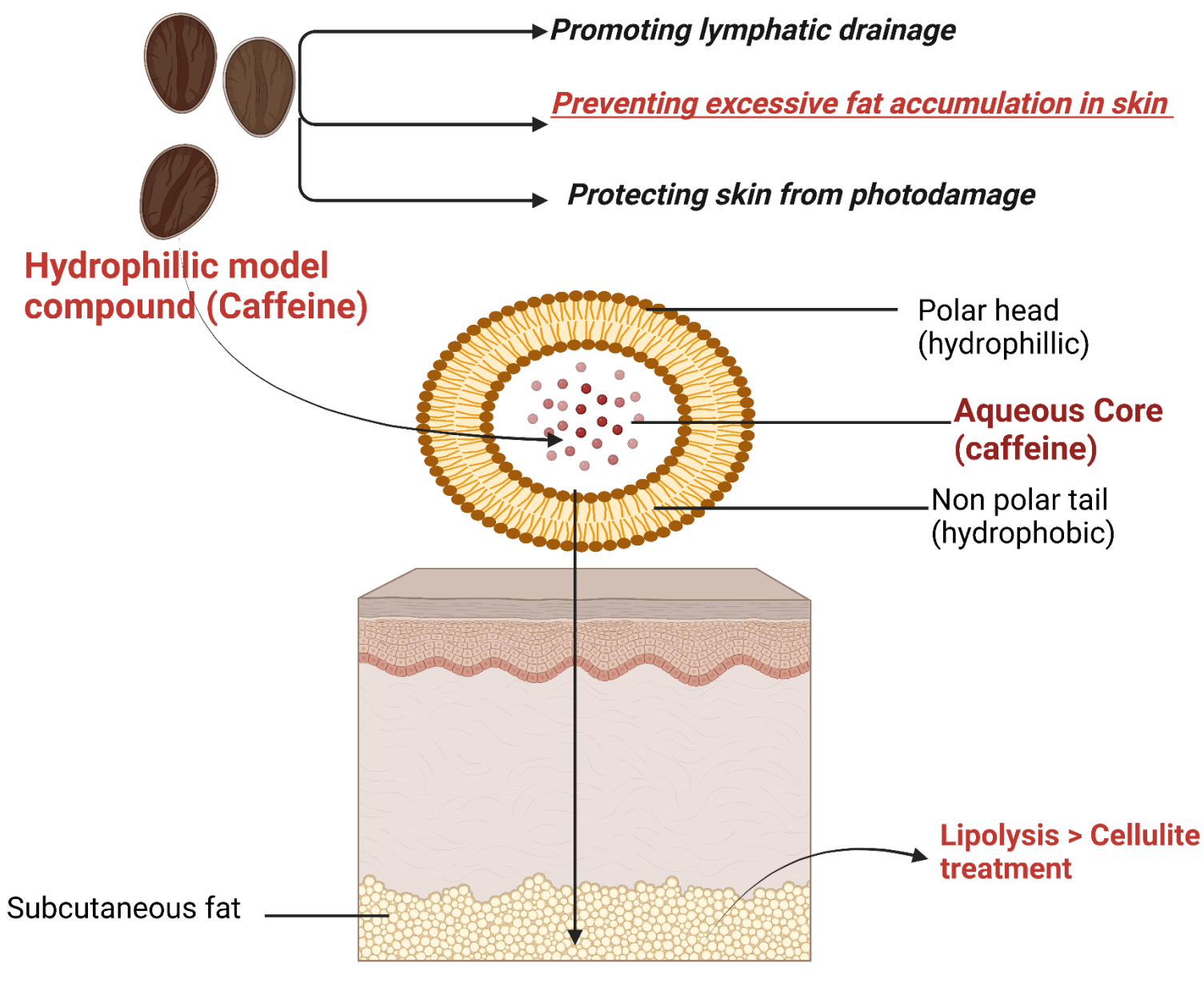
Long Term Stressors

The model can be applied to acute and chronic exposure as well as for the study of long-term effects of stress.

Development and Characterization of Caffeine Loaded Nanoparticles in Treatment of Gynoid Lipodystrophy

Sadaf Gharibi, Nubul Albayati, Bozena Michniak-Kohn
Rutgers, The State University of New Jersey

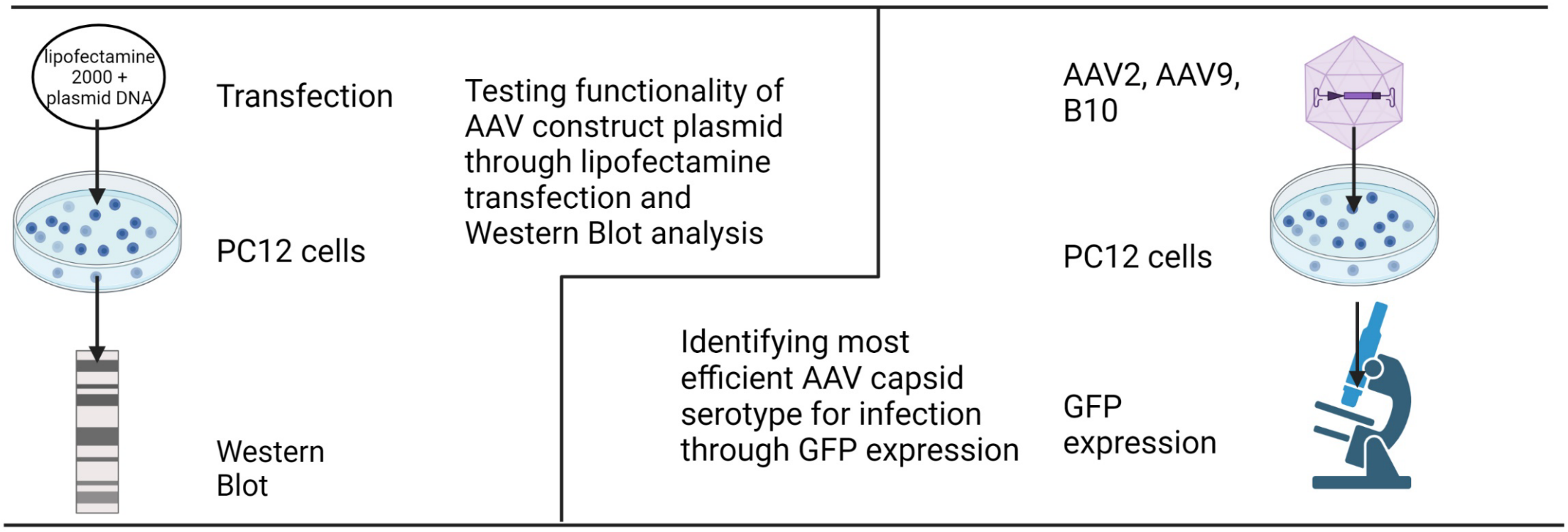
Gynoid lipodystrophy (GLD), commonly known as “cellulite” is a structural, inflammatory, and biochemical disorder of the subcutaneous tissue causing topographical skin alterations. GLD affects up to 90% of women, beginning in puberty. According to American Academy of Dermatology Association (AAD), no current treatment of cellulite is completely effective, and most improvements from currently available treatments are not long-lasting. One current treatment for cellulite is mesotherapy, which injects compounds like methylxanthines, such as caffeine, into the subcutaneous fat. Because mesotherapy is invasive, this study aimed to develop topical caffeine formulations and investigate the role of different nanocarriers and types of phospholipids in caffeine entrapment efficiency and formulation stability. Liposomes of phosphatidylcholine and cholesterol loaded with four different caffeine concentrations were prepared by thin film hydration technique and ethosomal formulations were prepared according to the ethanol injection method. Our results showed that the percentage of caffeine loaded in all liposomal formulations varied between 91% to 94%, and the percentage of caffeine loaded in all ethosomal formulations varied between 97% to 99%. Among our two tested phosphatidylcholines, Lipoid 75S showed higher zeta potential values compared to Lipoid 90G. Also, the particle size of ethosomal formulations was found to be lower than that of liposomal formulations. Our findings suggest that the high entrapment efficiency of liposomal and ethosomal formulations could enhance the penetration of hydrophilic substances such as caffeine. However, to see caffeine’s anti-cellulite effect, further in-vitro permeation assays and clinical studies need to be conducted. Supported by the Rutgers University Center for Dermal Research, NIH R25ES20721 and the SURF program.



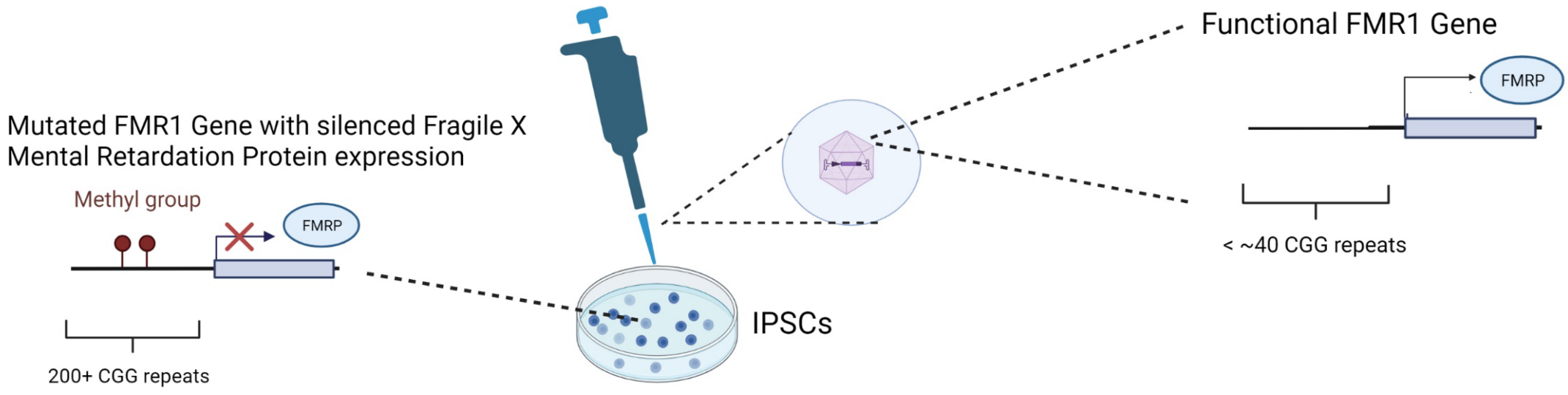
Testing AAV Vector Based Gene Therapy for Fragile X Syndrome *In Vitro*

Tom Cai, Jenny Liu, Renping Zhou
Rutgers, The State University of New Jersey

Fragile X syndrome (FXS) is an X linked dominant condition where the FMR1 gene that encodes fragile X mental retardation protein (FMRP) is silenced. The mutated FMR1 gene has an increased number of CGG repeats, leading to methylation and silencing of its promoter. Due to its monogenic nature, FXS is an excellent target for treatment using gene therapy. This study aims to evaluate the effectiveness of using Adeno Associated Viruses (AAV) as a delivery mechanism for a functional copy of the FMR1 gene in in vitro models. First, the recombinant AAV vector construct's functionality in expressing FMRP was determined through two different methods. PC12 cells were transfected with the construct plasmid DNA using lipofectamine 2000 and a Western Blot was performed to detect FMRP expression. The second method was to generate recombinant AAV virus to infect PC12 cells. They were produced through transfecting 293T cells with a helper, rep, and cap plasmid. To identify the most efficient capsid serotype for direct infection, PC12 cells were infected with AAV2, B10, and AAV9. AAV2 was determined to be most efficient. When testing the capsid serotypes on undifferentiated IPSCs, cell death occurred. Literature suggests that differentiated IPSCs tolerate infection better. Future testing will test infecting differentiated IPSCs with AAV to examine infection efficiency and FMRP expression. Supported by NIH R25ES020721.



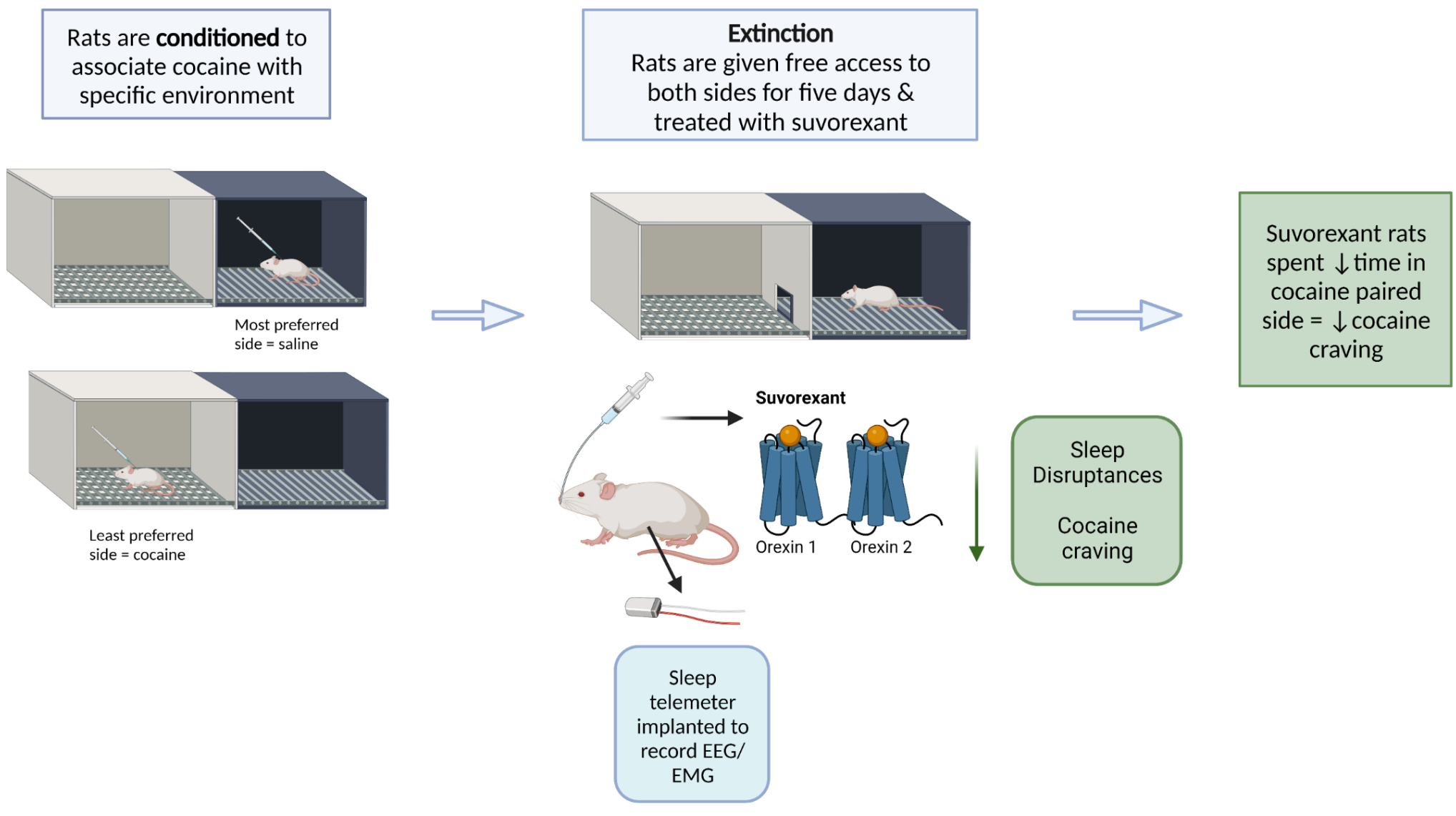
Goal: Use AAV to deliver a functional copy of the FMR1 gene to patient derived IPSCs with a mutated FMR1 gene



Suvorexant, a Dual Orexin Receptor Antagonist, Normalizes Sleep Disruptions During Cocaine Abstinence and Facilitates Extinction of Cocaine Seeking

Shuchi Merai, Utsav Gyawali, Shayna O'Conner, Charlie Olson, Nivedita Krishnakumar, Justus Williams, Morgan H. James
Rutgers, The State University of New Jersey

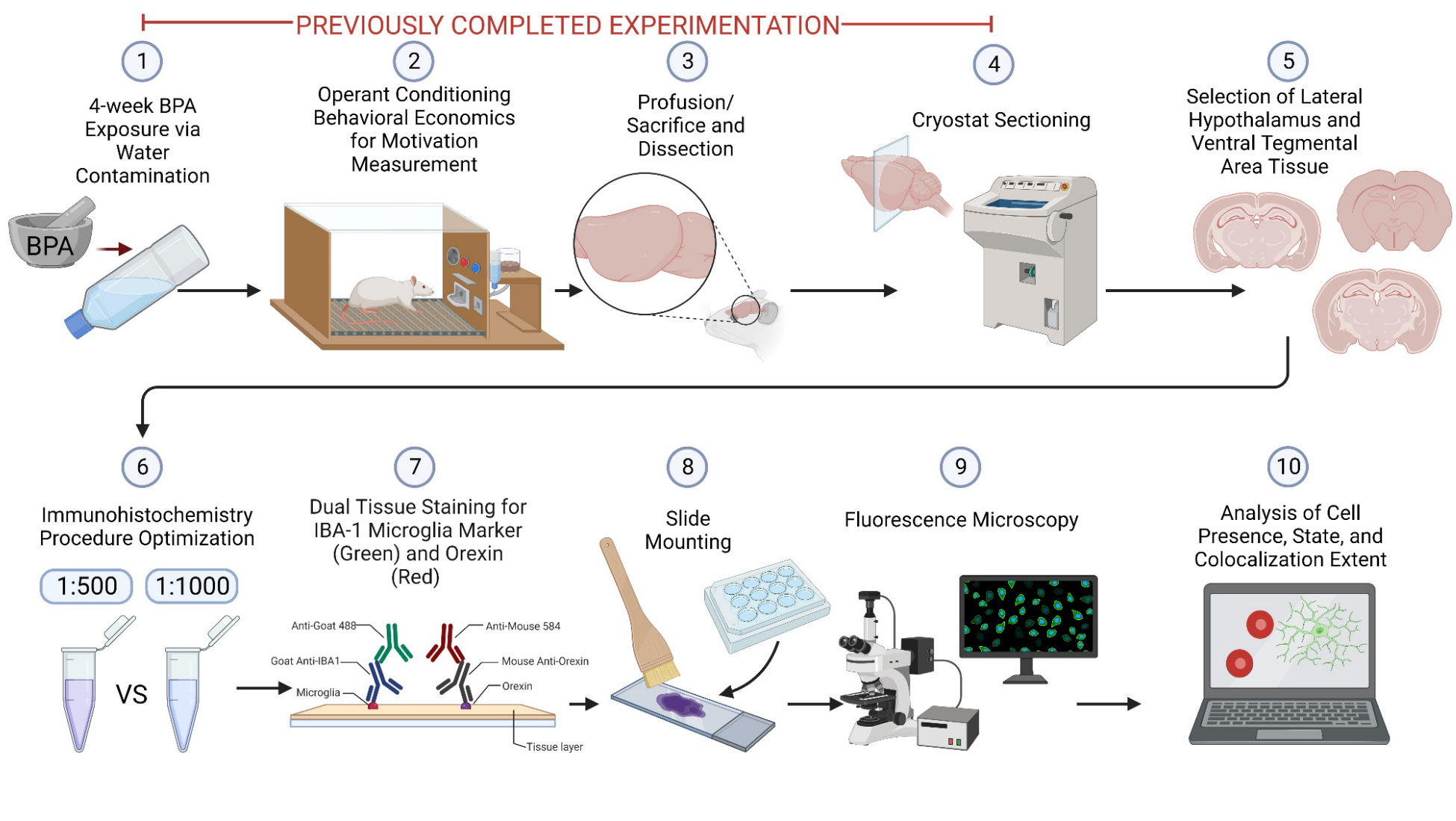
The orexin (hypocretin) system is involved in many physiological functions, including arousal, motivation, and sleep/wakefulness patterns. Cocaine seeking is associated with increased orexin system function, resulting in sleep disruptions during withdrawal. Cocaine users report sleep disturbances as a major contributor to relapse, and thus blocking orexin signaling might ameliorate sleep disruptions and reduce relapse risk. We investigated whether suvorexant, a dual orexin receptor antagonist, could normalize sleep disruptions, and decrease cocaine craving during abstinence. Rats were trained to develop a conditioned place preference for cocaine, which was subsequently extinguished. Rats were conditioned to associate distinct environments with injections of cocaine (10 mg/kg) or saline over 4 days; they then were given free access to the same environments in the absence of cocaine/saline injections over five days and the time spent in the cocaine-paired environment was measured. During extinction training, rats were treated with suvorexant (30mg/kg; p.o.) or vehicle 1h prior to their inactive period. A subset of rats were also implanted with a transmitter to record EEG/EMG activity. After conditioning, rats showed a preference for the cocaine-paired environment. During abstinence, rats treated with suvorexant showed a faster extinction in preference for the cocaine paired compartment. EEG/EMG data revealed cocaine-induced sleep disturbances were normalized by suvorexant. Thus, suvorexant decreases cocaine craving possibly by normalizing sleep, making it a strong candidate treatment for reducing relapse in cocaine use disorder. Studies supported by grants from R0DA0045765, R25ES020721 and Busch Biomedical Grant Program to MHJ.



Exploring the Role of Microglia Cells in Dysregulated Orexin System Function in Rats Exposed to Bisphenol-A During the Peripubertal Period

Caylee Brown, Michelle Bilotti, Morgan James
Rutgers, The State University of New Jersey

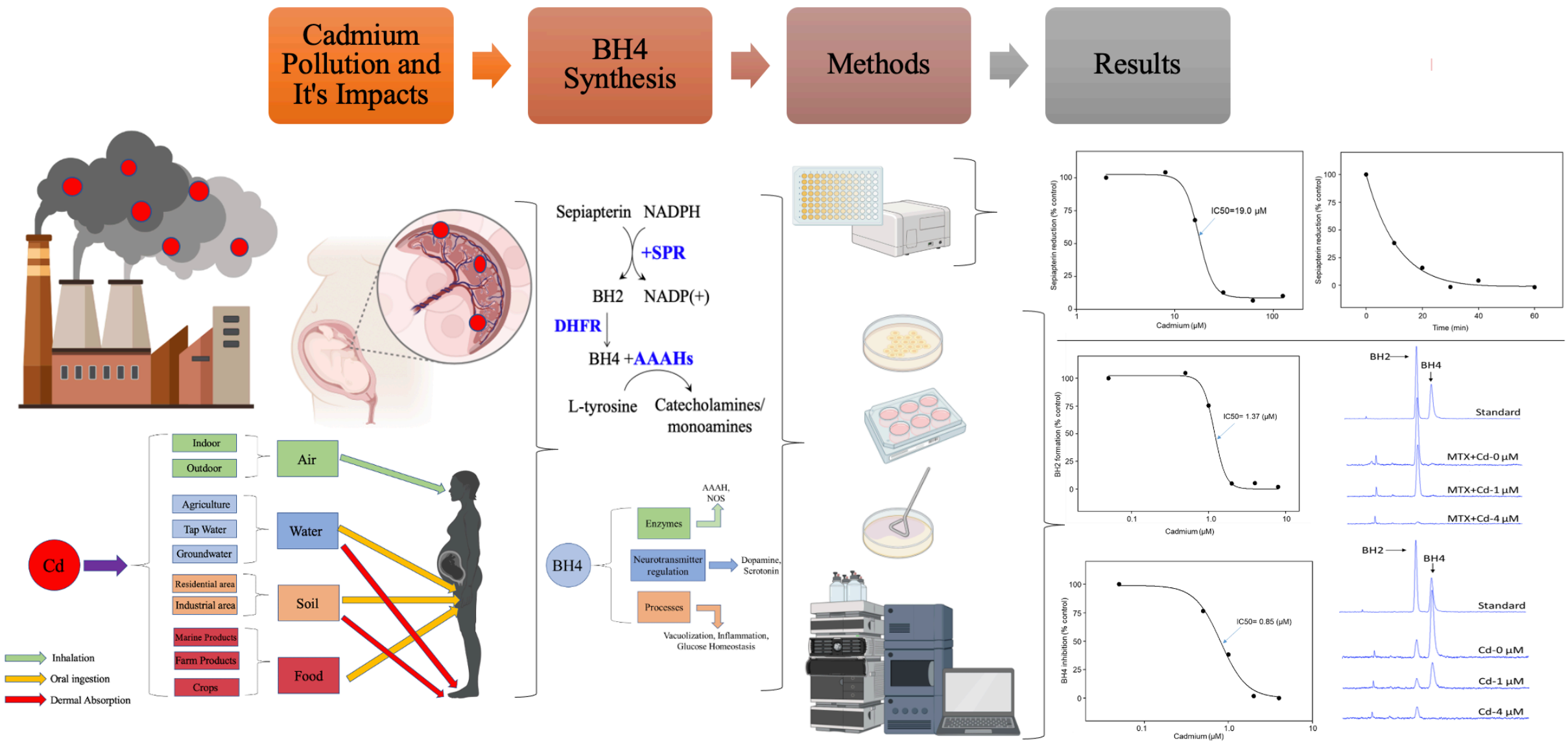
Early life exposure to endocrine-disrupting compounds (EDCs), such as bisphenol-A (BPA), have been associated with increased risk of depression and anxiety, particularly in young adult females. BPA is known to cause hormone dysregulation following neonatal exposure, yet there is a lack of research on the impact of BPA exposure during puberty, a developmental period characterized by heightened hormonal reorganization. Our lab has previously shown that female rats exposed to BPA during postnatal days 28-56 exhibit decreased reward motivation, which is accompanied by a reduction in the number and activity of neurons that produce orexin, a hypothalamic neuropeptide involved in motivated behavior. The mechanisms underlying these changes remain unclear. Microglia are the resident immune cells of the brain. They survey their environment in a ramified morphological state before being activated by cellular distress signals. This project aims to investigate whether peripubescent BPA exposure is associated with increased neuroinflammation which may, in turn, affect orexin system functionality. To test this, we processed brain tissue from BPA-exposed (0, 25, 250ug/kg/d) rats for immunohistochemical detection of orexin-containing neurons and microglia. Based on the decrease in motivation and orexin expression indicated by previous studies, we hypothesize that there will be lower numbers of orexin-containing neurons, a higher presence of activated-state microglia, and higher extent of colocalization between these cell populations in BPA-treated rats than untreated controls. This study will contribute to our understanding of how BPA exposure during puberty impacts motivational behavior in adolescents and young adults, predisposing them to motivation-linked disorders. This work is financially supported by the NIH R25ES020721 Grant, a NIDA R00 award (DA045765), a New Jersey Health Foundation award, a NIEHS P50 Pilot Grant Award, and a NIH T32 Training Grant.



Impacts of Cadmium on Placental BH4 Cofactors

Armando J. Ríos Padín, Shaojun Yang, Jeffrey D. Laskin
Rutgers, The State University of New Jersey and
University of Puerto Rico Río Piedras Campus

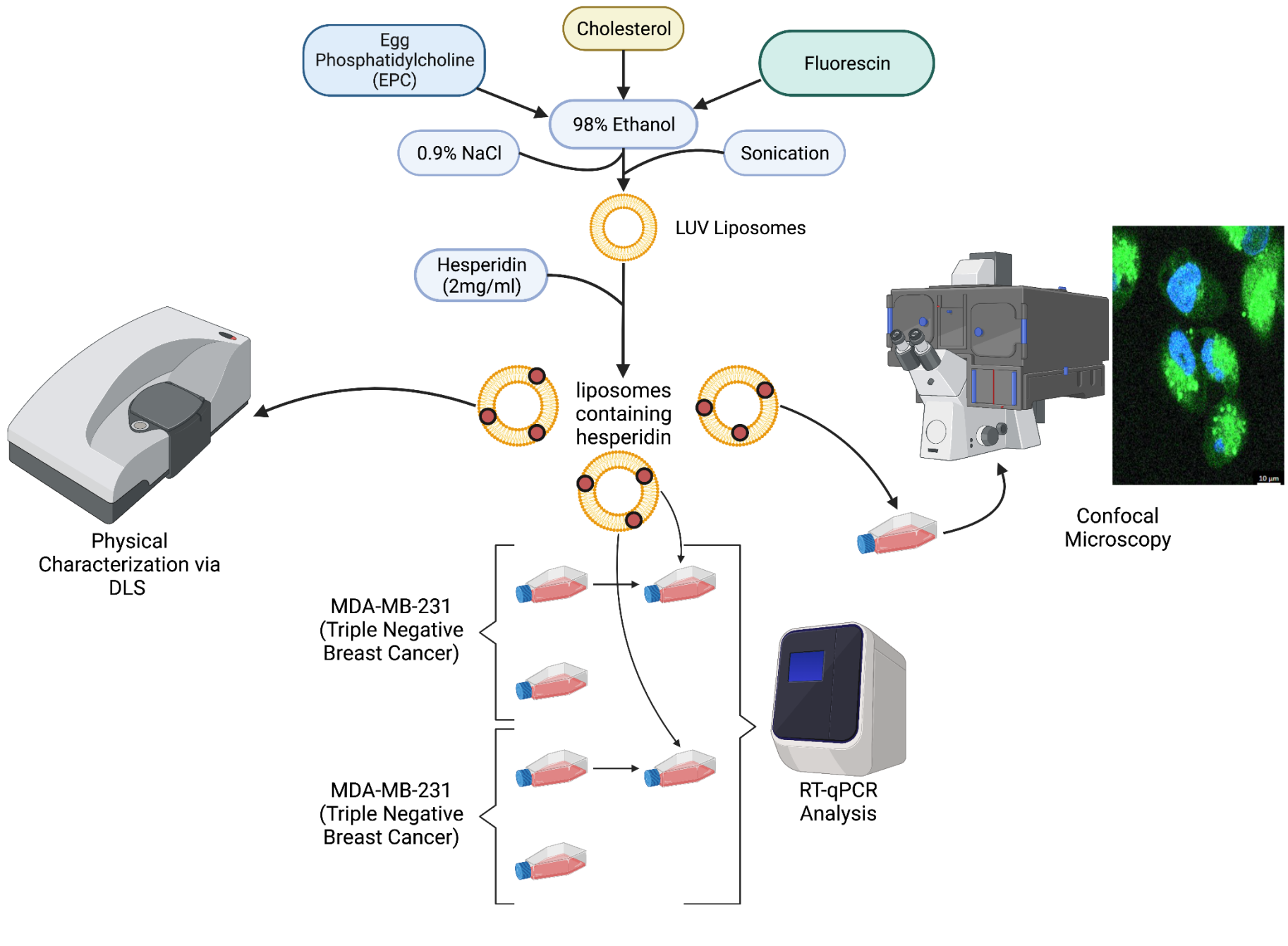
Rapid industrial development has led to extensive environmental pollution, particularly from the toxic heavy metal cadmium (Cd). This pollution has negative effects on human health and fetal development through contamination of the atmosphere, water, soil, and food. Cadmium exposure disrupts placental development, gene expression, and nutrient exchange, leading to abnormal fetal development and impaired placental function. It also interferes with the synthesis of the essential cofactor tetrahydrobiopterin (BH4), causing metabolic disorders. Therefore, it is crucial to investigate the impact of cadmium on placental BH4 cofactors and evaluate the potential protective effects of BH4 supplementation. Our hypothesis is that cadmium inhibits the placental BH4 pathway, disrupting essential metabolic processes, and BH4 supplementation can mitigate the detrimental effects of cadmium exposure. Our study confirmed that cadmium was found to inhibit recombinant sepiapterin (SPR) (IC50 = 19.0 µM), it also confirmed direct concentration and time-dependent inhibition of BH4 synthesis and observed its inhibition of BH2 and BH4 production from SPR in human placental cell lines. Furthermore, cadmium specifically inhibited SPR activity (IC50=1.37 µM) and BH2-mediated BH4 formation (IC50=0.85 µM) in cells after a 24-h incubation. These findings support BH4 supplementation as a potential treatment to counteract cadmium-induced inhibition of the BH4 pathway, enhance placental function, and promote normal fetal development. This research project is supported by the NIH R25ES020721 Grant, Society of Toxicology Intern Program, and RISE/SURF program at Rutgers University.



Inhibition of Programmed Death Ligand 1 (PD-L1) in Triple-Negative Breast Cancer Cells by Liposomal Hesperidin

Brendan Connor, Natalia Pogrebniak, Olga Garbuzenko, David Lee,
Andrew Shen, Tamara Minko
Rutgers, The State University of New Jersey

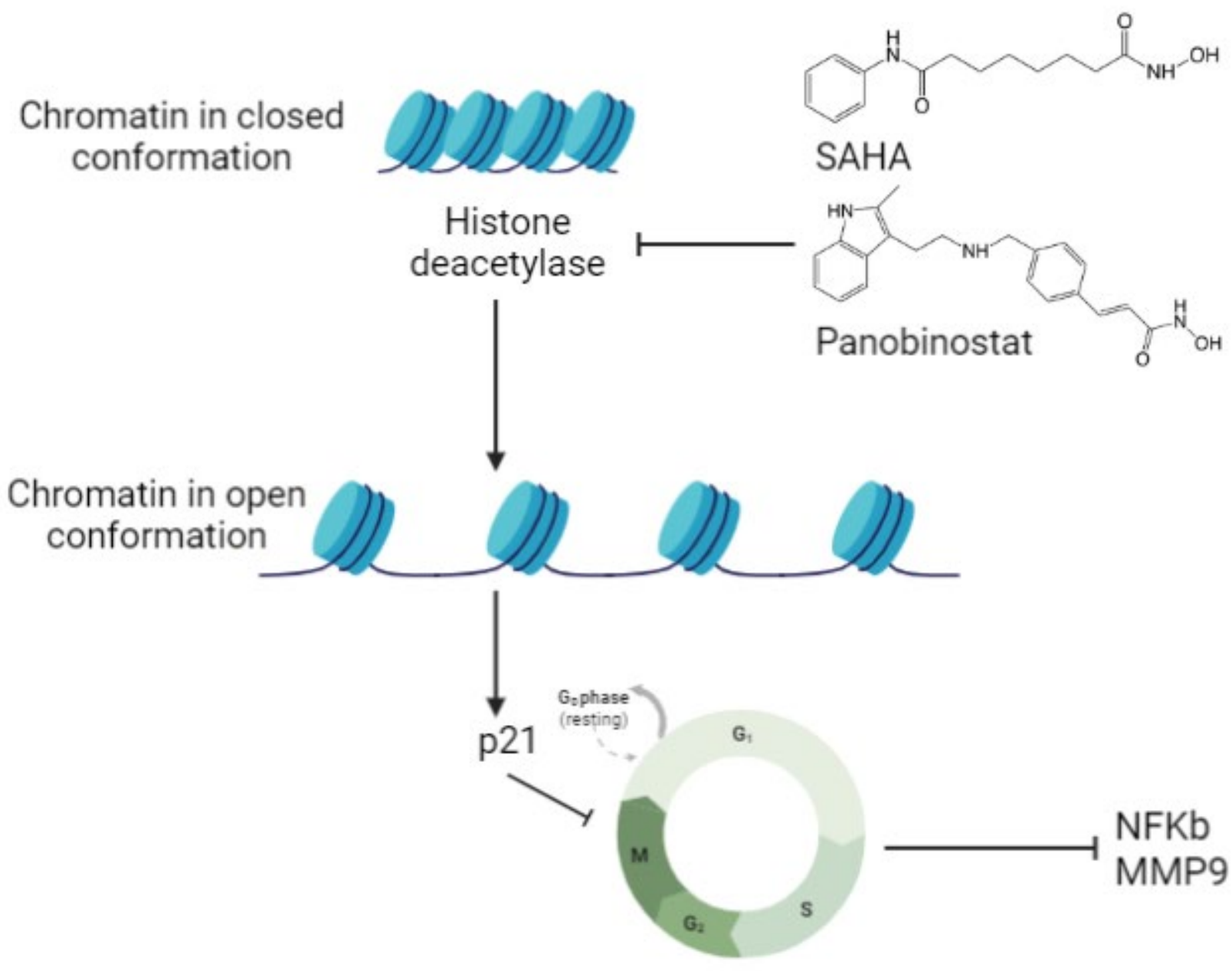
264,000 people are diagnosed with breast cancer annually in the US, with 10% to 20% of diagnoses attributed to triple-negative breast cancer (TNBC). TNBC is characterized by the lack of progesterone, estrogen, and human epidermal growth factor receptor 2 (HER2). Additionally, programmed death ligand 1 (PD-L1) is overexpressed in TNBC, aiding tumor growth by inducing immune escape. Hesperidin, a natural bioflavonoid, has recently been shown to inhibit the expression of PD-L1 in breast cancer cells. However, Hesperidin's anticancer activity is low due to its limited solubility. We hypothesized that delivering hesperidin through liposomes could overcome its low solubility and enhance treatment efficacy. The present study aims to verify this hypothesis and demonstrate that liposomal hesperidin (Lip-H) can suppress PD-L1 expression in TNBC cells. MDA-MB-231 (TNBC) and MCF-7 cells were incubated with Lip-H for 48 h. RNA was extracted from the harvested cells, and expression of the PD-L1 gene was measured by real-time quantitative polymerase chain reaction (RT-qPCR). Confocal microscopy revealed that the liposomes were internalized by cancer cells and localized in the cytoplasm and nuclei. PD-L1 gene expression in the untreated TNBC cells was 40x higher than the untreated MCF-7 cells, and Lip-H significantly inhibited PD-L1 expression in TNBC cells. Consequently, we verified the hypothesis and showed that the inclusion of hesperidin in liposomes strongly decreased the expression of PD-L1. This work supports the viability of this approach and shows the high therapeutic potential of liposomal hesperidin in the treatment of triple-negative breast cancer. This research was supported by R01CA251438 and R25ES020721 grants.



Histone Deacetylase Inhibitors Reprogram Triple Negative Breast Cancer to be Less Aggressive by Targeting Cell Proliferation, M etastasis, and Cancer Stemness

Maria Ruszkiewicz, Ge Yang, Ah-Ng Tony Kong, Cassandra Winz,
Philip Furmanski, Nanjoo Suh
Rutgers, the State University of New Jersey

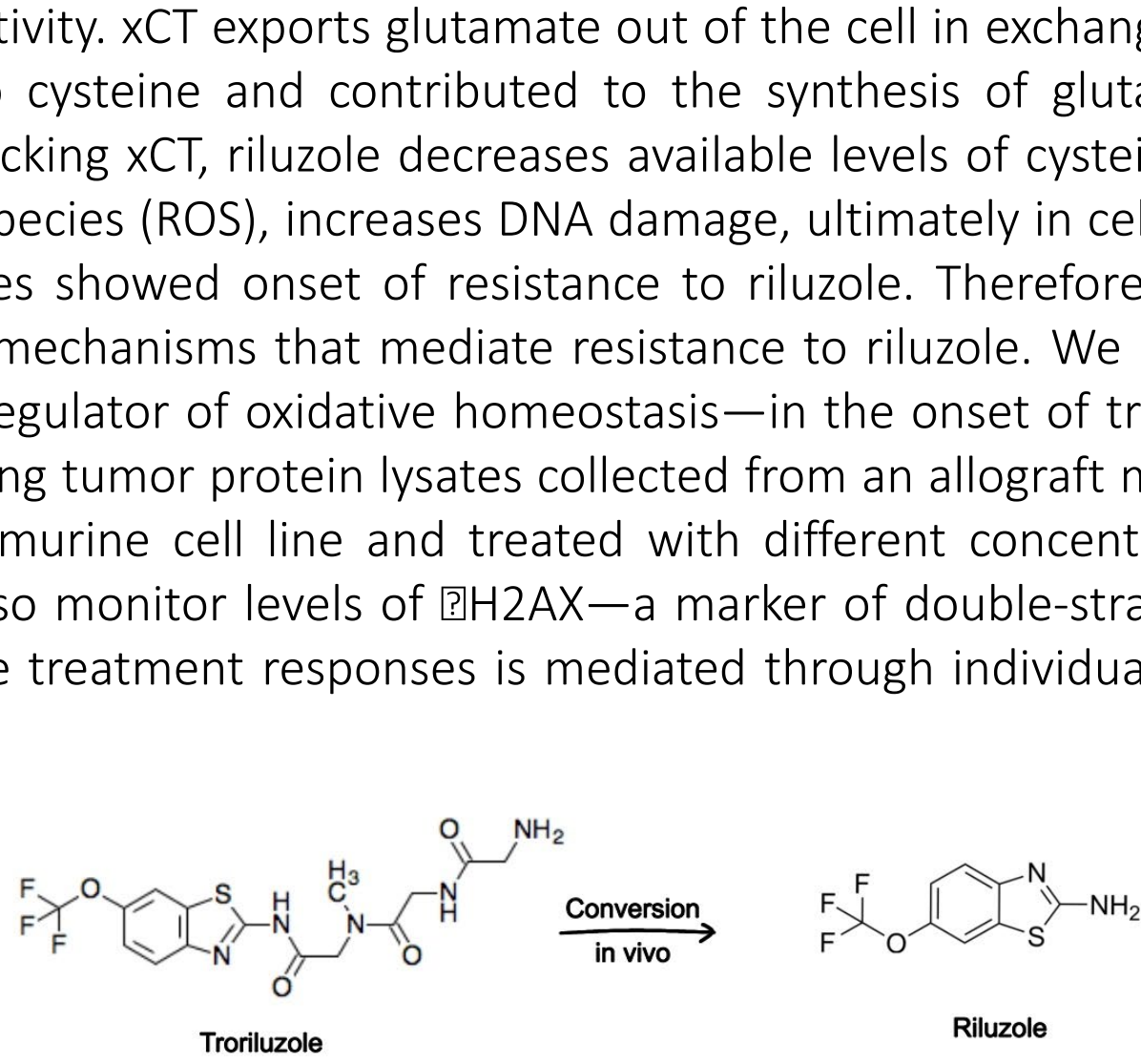
Triple negative breast cancer (TNBC) is highly aggressive and the most difficult type of breast cancer to treat due to its lack of receptors to target. Novel studies have explored the epigenetic mechanisms to i nhibit or reverse aggressive characteristics of this subtype of breast cancer. Histone deacetylases (HDA Cs) control gene expression by changing the chromatin conformation to a closed state, which leads to t ranscriptional repression of certain genes. Recent studies have suggested that specific inhibitors targeti ng HDAC may cause tumor suppressor genes to be re-expressed in cancer cells, reversing cancer progr ession. Epigenetic mechanisms may also play a role in reprogramming cancer stem cells (CSCs) in TNBC . CSCs are primarily drivers of recurrence and metastasis, and therefore associated with poorer progno ses. In this study, we have examined the effects of HDAC inhibitors on cell proliferation, metastasis, and cancer stemness in TNBC cells. Two TNBC cell lines, 4T1 and SUM-159, were treated with the HDAC inh ibitors, Vorinostat (SAHA) and Panobinostat, and their effects on cell proliferation, tumor suppressor ge nes and metastatic markers were determined by MTT assay and qPCR analysis. HDAC inhibitors on the formation of mammospheres and the stemness properties were further determined. We found that H DAC inhibitors decrease cell proliferation and downregulate cancer genes such as MYC, NFKb, and MM P9 while upregulating tumor suppressor genes such as CDKN1a. HDAC inhibitors have a modest effect on mammosphere forming efficiency and the CD24low/CD44high subpopulation. In conclusion, HDAC i nhibitors target the cell proliferation pathway and to a lesser extent cancer stemness in TNBC. Supporte d by NIH R25ES020721.



Involvement of DNA Damage and Oxidative Stress in Differential Sensitivities to Glutamatergic Signaling Inhibitor

Nhan Huynh, Anna Fateeva, Suzie Chen
Rutgers, The State University of New Jersey

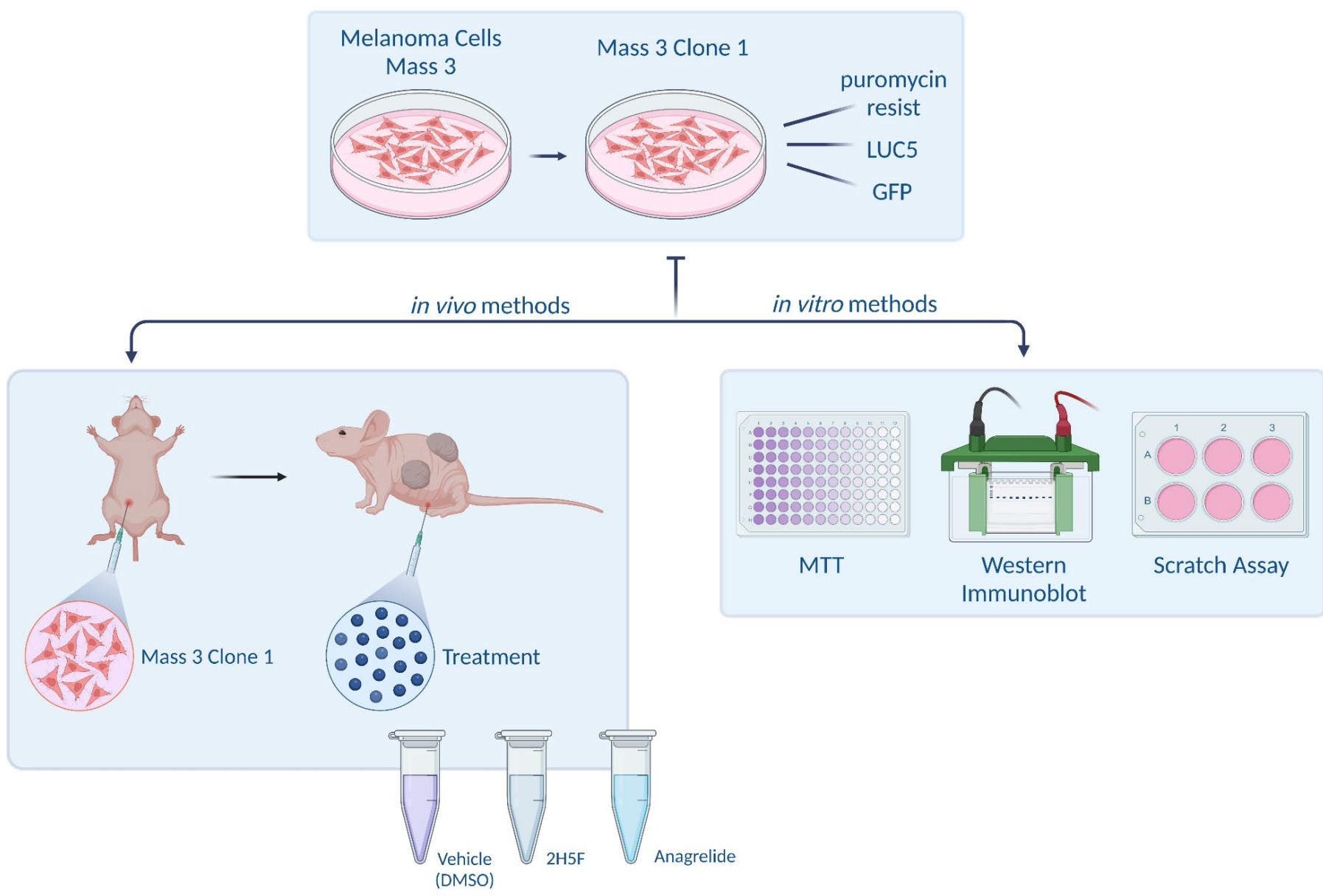
Melanoma is the most threatening type of skin cancer, as it has high potential for developing metastasis resulting in poor patient outcomes. In our lab, metabotropic glutamate receptor 1 (mGluR1) has been demonstrated as a driver of melanocytic transformation in vitro and melanoma tumorigenesis in vivo. mGluR1 expression in melanocytes leads to hyperactivation of MAPK and PI3K/AKT pathways, resulting in anti-apoptotic signals and increased proliferation rates. Riluzole, an FDA approved drug for amyotrophic lateral sclerosis (ALS) and an inhibitor of glutamatergic signaling, was shown to decrease extracellular levels of glutamate, melanoma cell proliferation in vitro and tumor growth in vivo. Based on these results, several clinical trials have demonstrated that mGluR1 is a potential target for further development in melanoma therapeutic. We propose a potential mechanism of action for riluzole is by blocking xCT antiporter activity. xCT exports glutamate out of the cell in exchange for import of cystine, which is then reduced to cysteine and contributed to the synthesis of glutathione (GSH), a major antioxidant in cells. By blocking xCT, riluzole decreases available levels of cysteine, reduces GSH levels, elevates reactive oxygen species (ROS), increases DNA damage, ultimately in cell transformation and/or cell death. Previous studies showed onset of resistance to riluzole. Therefore, the current project is aiming at elucidating the mechanisms that mediate resistance to riluzole. We begin to investigate the involvement of NRF2—a regulator of oxidative homeostasis—in the onset of treatment resistance. We perform Western blots using tumor protein lysates collected from an allograft mouse model inoculated with an mGluR1-positive murine cell line and treated with different concentrations of troriluzole, a prodrug of riluzole. We also monitor levels of □H2AX—a marker of double-stranded DNA damage—to confirm if onset of riluzole treatment responses is mediated through individual's reaction to oxidative stress and DNA damage.



Minimizing Metastatic Spread of Melanoma using Antagonists to L1, a Cell Adhesion Molecule

Sophia Fanzini, Stefano Boccadamo, Suzie Chen
Rutgers, The State University of New Jersey

Metastatic melanoma is a highly aggressive skin cancer. When melanoma is metastasized, the host has a 5-year 15-20% survival rate according to the American Cancer Society. The Chen lab was the first to identify the correlation between melanoma growth and the abnormal expression of GRM1, a normal neuronal receptor not normally expressed in melanocytes. The ectopic expression of this receptor led to spontaneous development of metastatic melanoma. L1, a cell adhesion molecule, was found to upregulate its expression in tumor cells including melanoma. It was postulated that L1, through its motility, can generate an invasive phenotype to promote metastasis. This study determines if two L1 antagonists, 2-hydroxy-5-fluoropyrimidine (2H5F) and anagrelide, may affect cell viability and/or cell migration. We assessed the protein levels of L1 in vivo and in vitro. The in vitro Mass 3 Clone 1 cell line and the in vivo isolated Mass 3 Clone 1 2H5F-resistant cell line were tested using MTT cell viability/cell proliferation assays. The assay spanned 96 hours and consisted of treatments of growth media containing vehicle (DMSO) and 100uM of anagrelide and 2H5F. We also tested both cell lines for cell motility using scratch assays and measured the changes in distances of the space made when we scratched the surface of the culture plates. These plates contained different concentrations of anagrelide and 2H5F at 1uM , 10uM, or 100uM. Protein expression was verified with the same sets of cells in the scratch assays. Cell proliferation appeared to be unaffected by the two L1 antagonists, whereas cell motility appeared to be reduced in the presence of either inhibitor. Western immunoblots demonstrated the presence of L1 for each sample. Future directions for L1 studies include additional examination in in vivo settings. Supported by NIH R25ES020721

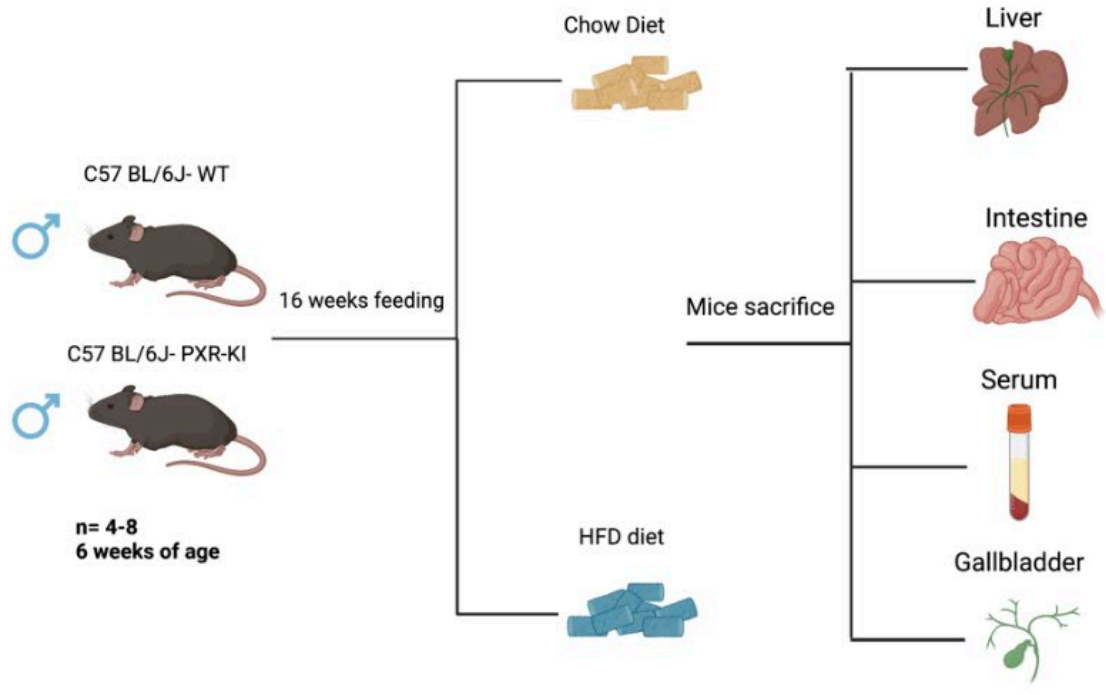


Investigating the Effects of the Nuclear Receptor PXR-KI in the Regulation of Bile Acid Homeostasis, Hepatic functions, and the Development of MASH in Mouse Models

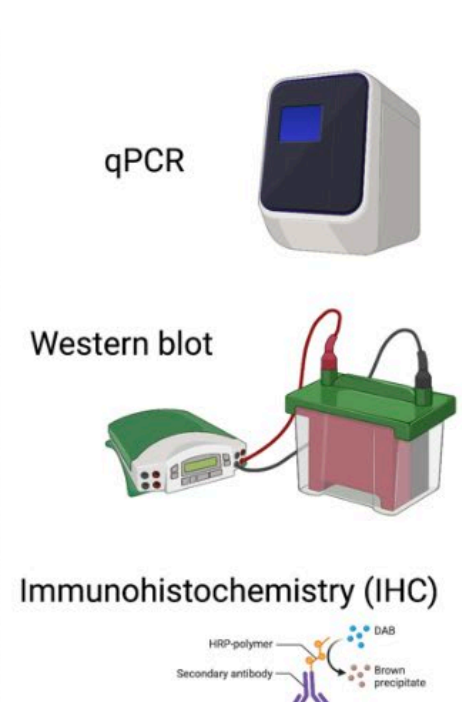
Stephanie Mera, Veronia Basaly, Rulaiha Taylor, Grace L. Guo
Rutgers, The State University of New Jersey

Metabolic-associated fatty liver disease (MAFLD) is the most common chronic liver disease characterized by hepatic steatosis. MAFLD can progress to metabolic associated steatohepatitis (MASH), fibrosis, cirrhosis, and even hepatocellular carcinoma. MAFLD affects 30% of the world's population, especially prevalent in countries like the United States. Pregnane X receptor (PXR) is a ligand activated transcription factor that is highly expressed in the liver and intestine of humans. PXR regulates the expression of genes involved in phase 1 and 2 drug metabolizing enzymes and transporters. PXR plays an important role in regulating glucose, lipid, cholesterol, and bile acid metabolism, making it an interesting target for therapeutics for MASH. PXR activation has been shown to induce lipid and triglyceride accumulation in mouse liver. We hypothesize that blocking of the phosphorylation site of PXR Serine 347 to Alanine (S347A KI) can modify PXR activity, increase bile acid activity, promote liver steatosis, and inflammation, potentially intensifying the development of MASH. To investigate the role of the Ser347 phosphorylation site in the development of MASH, six-week-old male wild-type (WT), and PXR-KI mice, both on the C57BL/6J genetic background, were fed either a chow diet, or HFD diet (40 Kcal% palm oil fat, 20 kcal% fructose, and 2% cholesterol) for 16 weeks. Samples from liver, intestine, blood, and gallbladder were collected to run qPCR to quantify gene expression. We found that PXR-KI mice on HFD demonstrated higher relative mRNA levels of hepatic metabolism-related genes such as Srebp-1c, Cd36, Fasn, and Cyp4a10. Similarly, the PXR-KI mice on HFD showed an increase in relative mRNA levels of hepatic inflammation- and fibrosis-related genes: Lcn2, Timp1, and Col1a1. In conclusion, the results suggest that blocking the Ser347 phosphorylation site of PXR in mice is associated with more liver damage during MASH development, indicating the importance of this site to regulate PXR functions in maintaining lipid and inflammation homeostasis. Moving forward with this project, other techniques will be used to confirm targets related to inflammation and steatosis in MASH. Funding: NIH R25ES20721 and the ASPET SURF Program.

Study Design



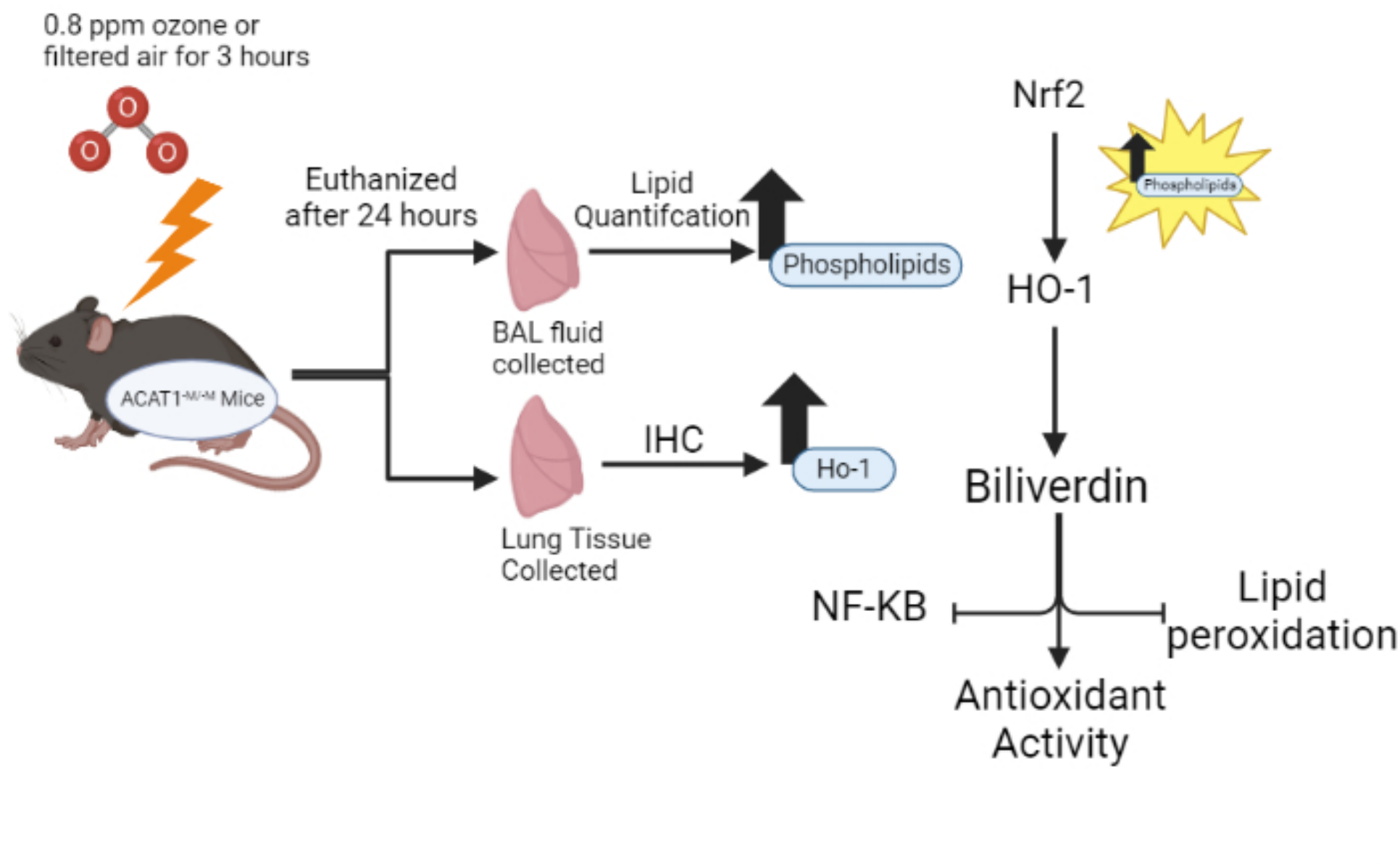
Methods



Deletion of Acyl-Coenzyme A Acyltransferase in Myeloid Cells Enhances Oxidative Stress And Dyslipidemia in the Lung Following Ozone Inhalation

Kyle Yuncza, Carol Gardner, Debra Laskin
Rutgers, The State University of New Jersey

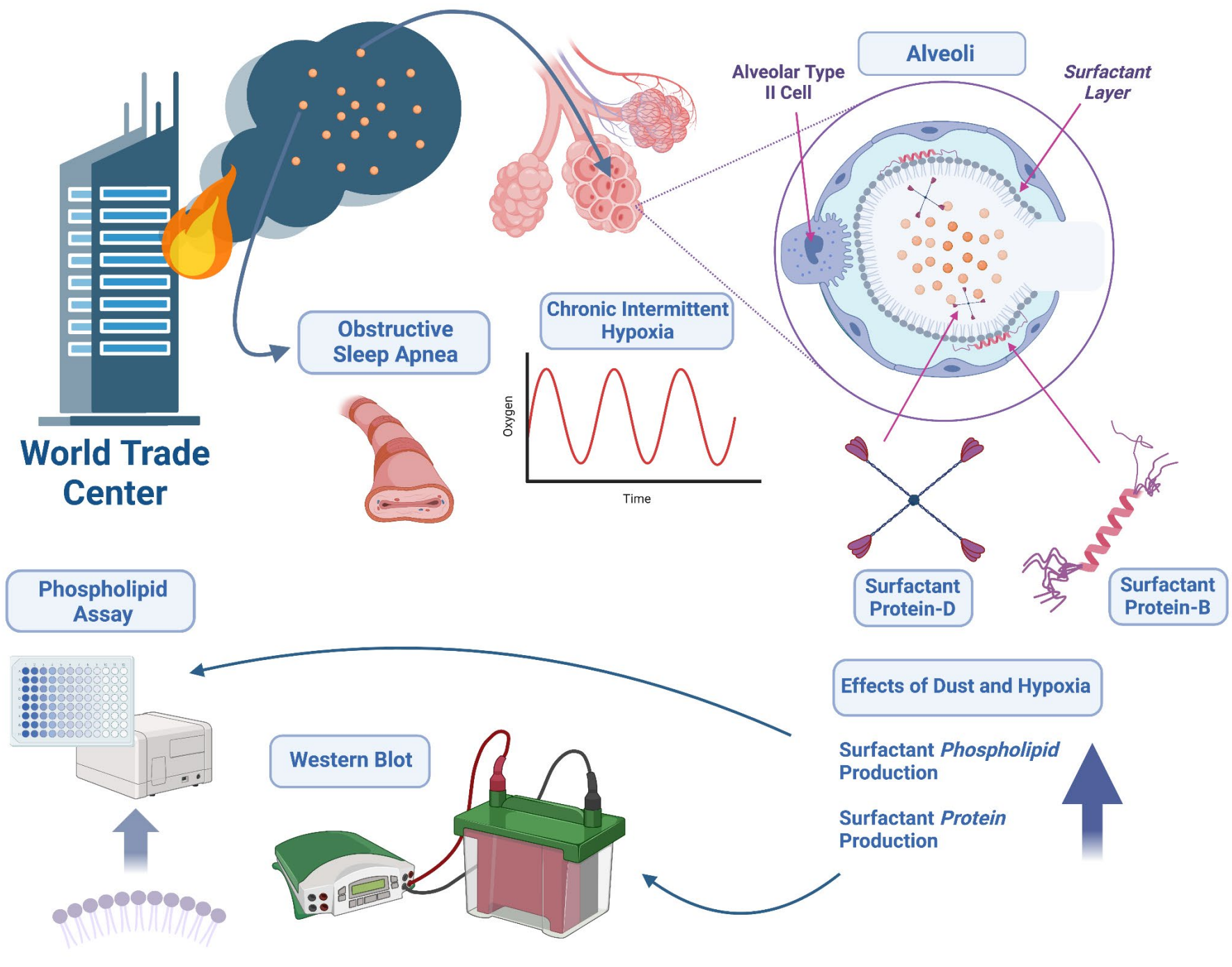
Exposure to ozone, an air pollutant known to cause oxidative stress, has been shown to alter lipid handling in the lung. Acyl-coenzyme A acyltransferase 1 (ACAT1) is responsible for esterification and sequestration of cholesterol and is a major isoenzyme present in alveolar macrophages. ACAT1 has been implicated in the formation of foam cells due to dysregulated lipid metabolism in macrophages. The role of ACAT1 in ozone (O3) toxicity is unknown. We hypothesized that loss of ACAT1 will reduce macrophage activation and injury. To test this, myeloid specific ACAT1 knockout mice (ACAT1-M/-M) and C57BL/6 wild type (WT) mice were exposed to filtered air or O3 (0.8 ppm) for 3 hours. Mice were euthanized 24 hours later and bronchoalveolar lavage (BAL) fluid and lung samples collected. Following exposure of ACAT -M/-M mice to O3, BAL protein levels significantly increased when compared to air exposed ACAT -M/-M mice. By comparison, there was a greater increase in protein in BAL of O3 exposed WT mice suggesting more robust epithelial leakage. BAL fluid from O3 exposed ACAT -M/-M mice also contained significantly higher levels of phospholipids relative to O3 exposed WT mice indicative of dyslipidemia. Loss of ACAT1 in myeloid cells was also correlated with a significantly increased expression of heme oxygenase-1 (HO-1) in lung macrophages indicating exaggerated oxidative stress. These data suggest activation of the Nrf2 pathway resulting in the up-regulation of HO-1 and potentially other anti-oxidants; further studies are in process to explore this possibility. Supported by NIH Grants R01ES004738 and P30ES005022.



The Synergistic Effects of World Trade Center Dust and Chronic Intermittent Hypoxia on Pulmonary Surfactant

Andrew Jelinsky, Sungjae Lee, Elena Abramova, Kinal Vayas,
Andrew Gow, Jag Sunderram
Rutgers, The State University of New Jersey and Virginia Tech

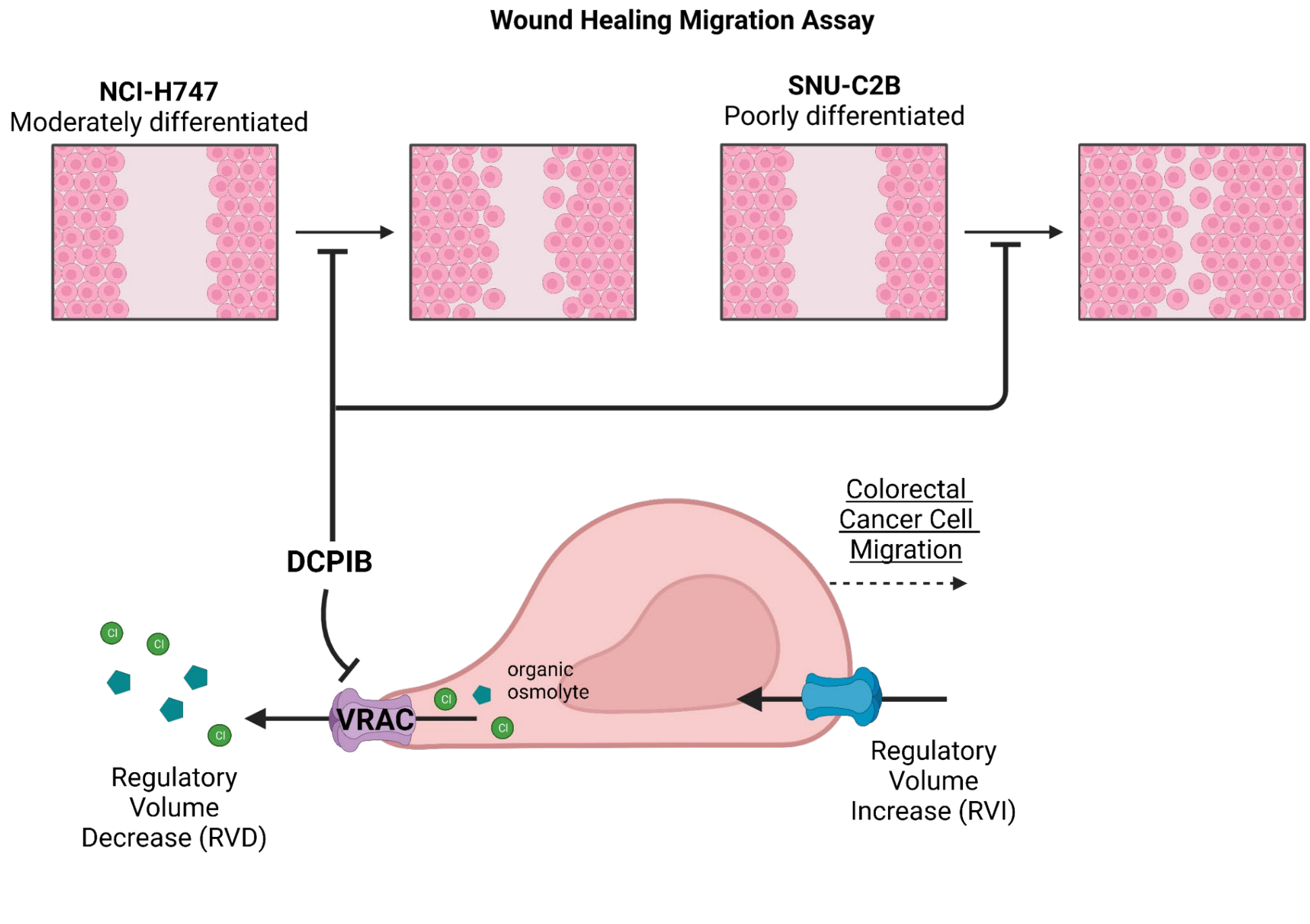
Exposure to dust created by the collapse of the world trade center (WTC dust) resulted in a number of pulmonary complications. There is a high prevalence of obstructive sleep apnea (OSA) within this population as well. It is unknown how these two insults interact in terms of lung function. In this study, we used WTC dust installation and chronic intermittent hypoxia (CIH), a model of OSA, to examine the effects on surfactant production. Surfactant regulates surface tension and immune function in the lung lining. It consists of phospholipids and surfactant proteins (SPs). Here, we examined phospholipid and SP-D levels in response to CIH (for 5, 14, & 28d) and WTC dust. CIH was simulated by cycling the inhaled gas between room air and a FiO2 of 5% 20 times per hour for 8 hours a day. WTC dust was intratracheally administered in saline. Western blot analysis was conducted on bronchoalveolar lavage (BAL) fluid to test for SP-D. Phospholipid within the BAL samples was also measured. CIH at 5, 14, & 28d resulted in an increase in phospholipid, irrespective of dust exposure. This increase was greatest at 5d. WTC in the absence of CIH produced a significant loss of phospholipid. Increased SP-D production was seen at 5 and 14d but not at 28d, indicating a greater acute than chronic effect. These results show an increase in surfactant production in response to CIH and immune function with WTC dust. Supported by the NIH R25ES020721 and U01OH012072 Grants, and the ASPET SURF Program.



Inhibition of Volume-Regulated Anion Channel Suppresses Migration of Colorectal Cancer Cells

Brian Chan, Jordan Lee, Darin Mak, Steven An
Rutgers, The State University of New Jersey

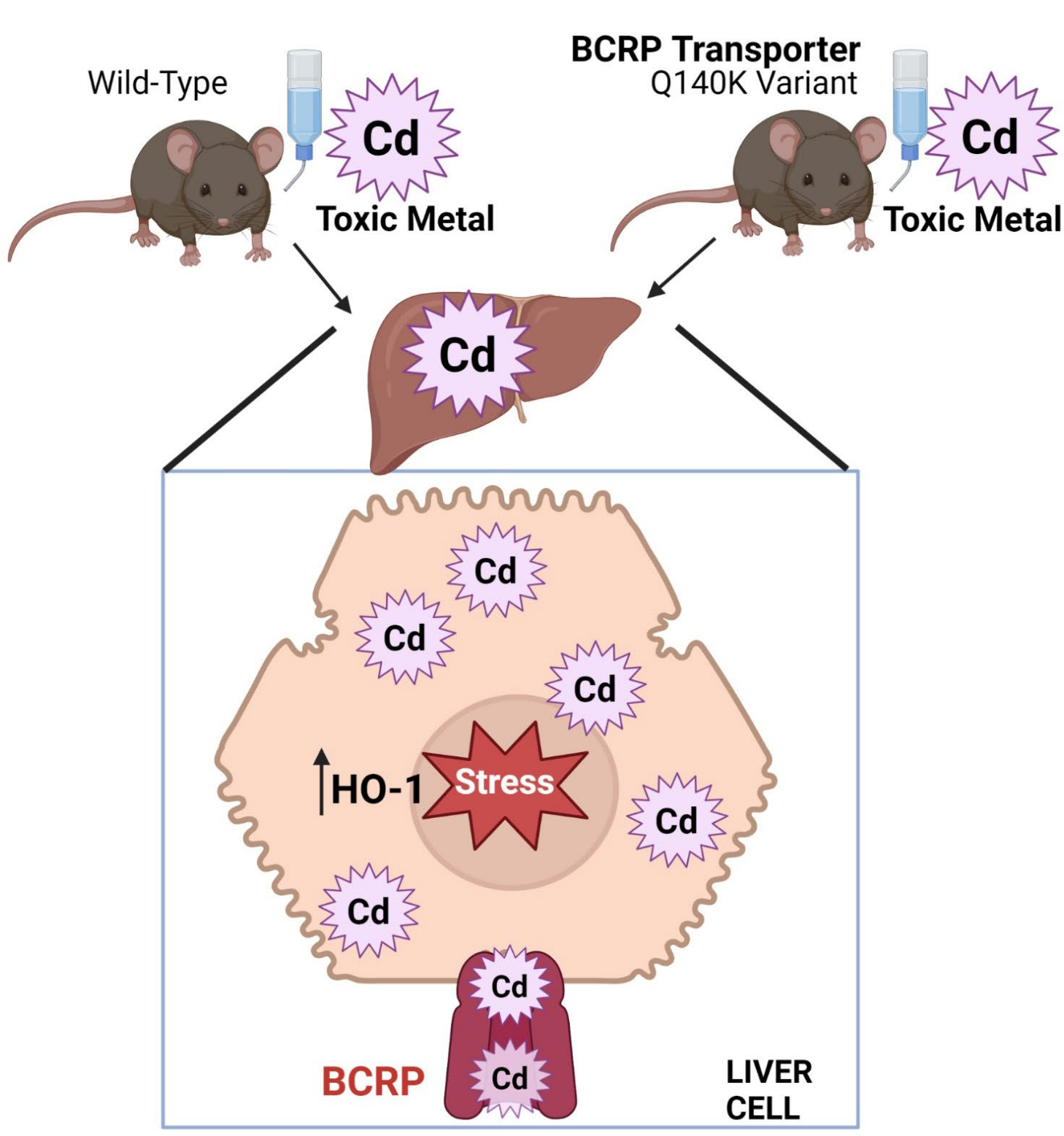
Cell volume homeostasis is fundamental to cell functions, including proliferation, migration, and growth. Previous studies have identified LRRC8 (leucine rich repeat containing 8 subunits) as the bona fide volume-regulated anion channel (VRAC, ICl) essential for the regulatory volume decrease in response to cell swelling (ICl, swell). Here we explored the role of LRRC8 in colorectal cancer (CRC) cell migration. Cellular migration of CRC cell lines harboring different KRAS mutations were visualized and quantified, in real-time, with a live cell microscopy in the presence and absence of DCPIB, a selective blocker of VRAC. For CRC cell line derived from cecum, poorly differentiated SNU-C2B (G12D mutant) exhibited faster cellular migration than moderately differentiated NCI-H747 (G13D mutant); cellular migration corroborated increased cell stiffness as measured by magnetic twisting cytometry and faster remodeling of the underlying cytoskeleton (CSK) network as measured by spontaneous tracer motions. For CRC cell line derived from colon, however, HCT-15 harboring G13D mutation migrated faster than SW-480 and SW-837 harboring G12V and G12C mutations, respectively. Interestingly, cellular migrations and the rate of CSK remodeling ranked in order with KRAS mutations (G12D>G13D>G12V>G12C). For all KRAS mutants, DCPIB significantly inhibited cellular migrations. Further work is warranted to investigate the role of LRRC8 on the material properties of CRC. In conclusion, investigating the effect of pharmacologic agents on modulating the cytoskeleton and VRACs may serve as novel therapeutic targets for CRC metastasis. Supported by NIH R25ES020721, P01HL114471, R01HL164404, and R01DK100483 grants.



Efflux Transporter Genetics and Heavy Metal Toxicity in Mice

Erica Acox, Xia Wen, Lauren M. Aleksunes
Delaware State University and Rutgers, The State University of New Jersey

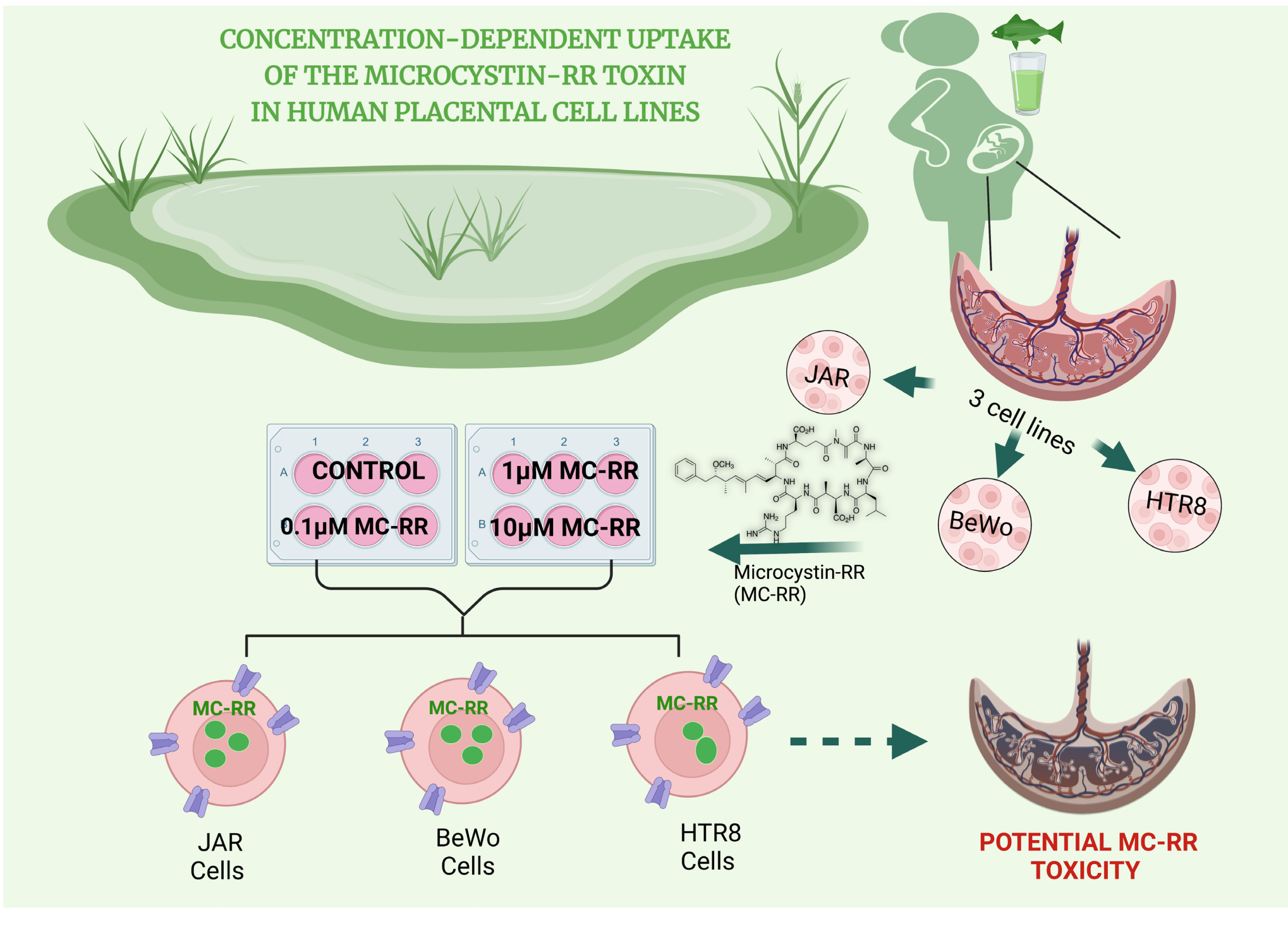
Cadmium is a highly toxic, naturally occurring metal classified as a probable human carcinogen by the U.S. Environmental Protection Agency. Cadmium can accumulate in the liver and cause organ toxicity. Breast Cancer Resistant Protein (BCRP) is an efflux transporter that removes toxins from the body and protects organs from injury. A reduced function polymorphism in BCRP (Q141K in humans; Q140K in mice) can alter BCRP transport activity and in turn, xenobiotic disposition. The purpose of this study was to evaluate the ability of BCRP to influence cadmium concentrations and toxicity in the liver using transgenic mice with the Q140K polymorphism. Adult, male wild-type and Q140K mice were exposed to regular drinking water or water containing 50 ppm cadmium chloride for 14 days. Liver tissues were then collected and homogenized for analysis for protein expression by western blotting. We observed that compared to wild-type mice, those with the Q140K variant had 1) ~50% reduced BCRP expression and function leading to higher cadmium levels and 2) a 35% greater up-regulation of cellular stress protein HO-1. These studies provide insight into how genetic variation in BCRP can alter the susceptibility of the liver to cadmium-induced toxicity. Supported by the NIH R25ES020721 Grant and the Society of Toxicology Intern Program.



Concentration-Dependent Uptake of the Microcystin-RR Toxin in Human Placental Cell Lines

Mokshitkumar Patel, Michael Jeffrey Campbell, Shuo Xiao,
Xia Wen, Lauren M. Aleksunes
Rutgers, The State University of New Jersey

The incidence of harmful algae blooms (HAB) is increasing and as a result, populations are being exposed to cyanotoxins produced by the blue-green algae cyanobacteria. Two of the most prominent cyanotoxins in freshwater are microcystin-LR (MC-LR), and microcystin-RR (MC-RR). Microcystins enter cells through organic anion-transporting peptides (OATP) and inhibit protein phosphatases leading to cellular damage to the liver and brain. The placenta also expresses OATP transporters that could facilitate the uptake of microcystins leading to potential toxicity. We hypothesize that microcystin-RR will enter placenta cells. To test this, two human cytotrophoblast cell lines (JAR, BeWo) and one human extravillous trophoblast cell line (HTR8/SVneo) were incubated with microcystin-RR at concentrations of 0, 0.1, 1, and 10µm. The uptake of microcystin-RR was assessed by western blotting of cell lysates using an antibody that recognizes proteins bound by microcystin-RR. Significant uptake of MC-RR in all 3 cell lines was observed at a concentration of 10µm. Furthermore, only JAR cells exhibited significant uptake of MC-RR at a concentration of 1µm as well. Recognizing that MC-RR can actively enter placenta cells, we suspect that cyanotoxins can cause toxicity to the placenta. Moving forward, we will examine the ability of MC-RR to injure trophoblast cells as well as identify the specific OATP transporters responsible for entry into the placenta. Funded by Rutgers University Foundation and NIH R25ES020721.

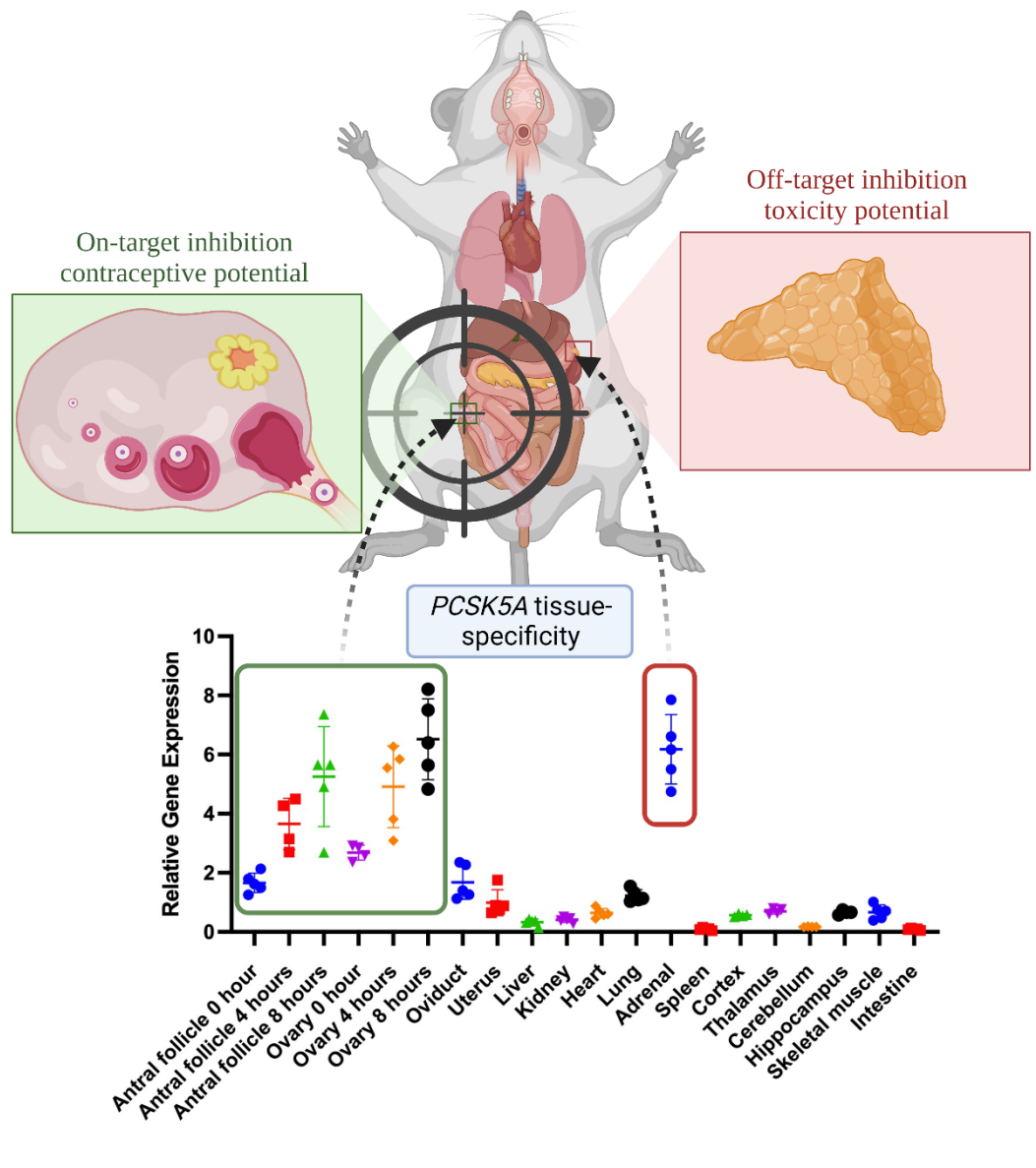


Investigating the Ovary Specificity of Proprotein Convertases to Evaluate for their Non-hormonal Contraceptive Candidacy

Astha Adroja, Jiyang Zhang, Pawat Pattarawat, Inhyeok Hwang,
Brittany Goods, Francesca Duncan, Shuo Xiao

Rutgers, The State University of New Jersey, Dartmouth College,
and Northwestern University

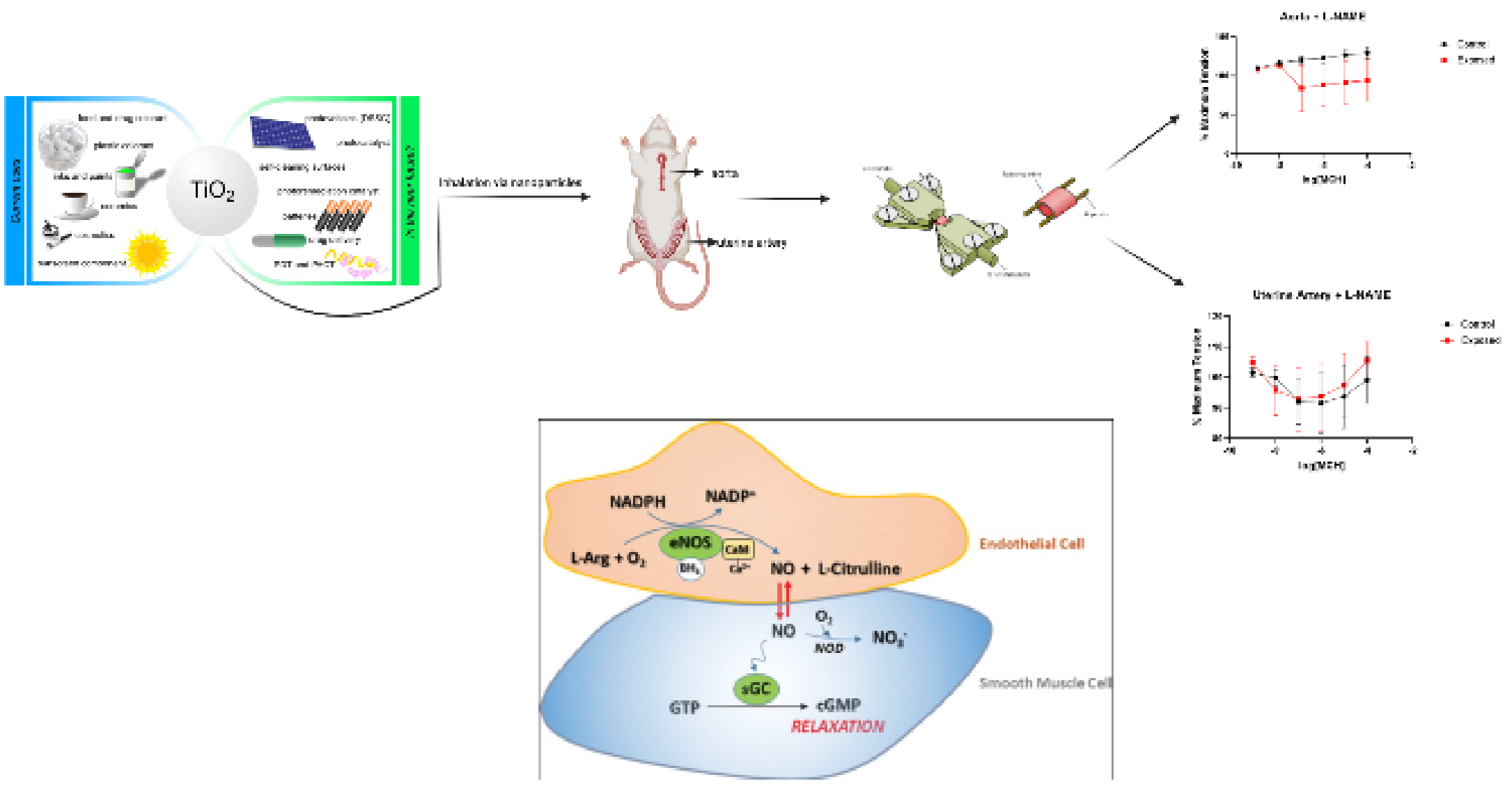
Traditional hormone-based female contraceptives can cause undesired side effects including depression, stroke, obesity, and hormone-related cancers for susceptible populations. *Pcsk3, Pcsk5A, Pcsk5B*, and *Pcsk6*, members of the proprotein convertase (PC) family, have recently emerged as potential targets for developing nonhormonal contraceptives. Our RNA-sequencing analysis revealed that they are continuously upregulated in ovulating follicles. Inhibitors of PCs were found to dose-dependently inhibit ovulation without affecting secretion of estradiol, testosterone, and progesterone. However, the high abundance of PC expression across tissues poses questions about potential toxicity of their inhibitors. The purpose of this experiment was to establish the expression profiles of the 4 mentioned PC isoforms in various mouse tissues to determine their tissue specificity. Mouse tissue samples were collected including oviduct, uterus, liver, kidney, heart, lung, adrenal, spleen, brain, skeletal muscle, and intestine. Ovary and antral follicle samples, stimulated to ovulate with Human chorionic gonadotropin (hCG), were also collected at 0-, 4-, and 8-hours post-stimulation for total RNA extraction and reverse transcription quantitative polymerase chain reaction (RT-qPCR). The results of RT-qPCR showed that *Pcsk5A* expression was highly abundant in the antral follicle and ovary at 8-hours post stimulation, while there was also comparably high expression of *Pcsk5A* in the adrenal gland. With a necessity to determine adrenal related toxicity in future in vivo studies, *Pcsk5A* presents as a promising target for hormone-independent contraception due to its ovary specificity. Supported by the NIH R25ES020721 Grant, the Bill and Melinda Gates Foundation, and the American Society for Pharmacology and Experimental Therapeutics SURF Program.



Proportional Impact of Inhibitors on Vascular Reactivity - Unraveling the Effects of Titanium Dioxide on Endothelium-Dependent Relaxation

Pratyush Venkatesh, Talia N. Seymore, Chelsea M. Cary, Phoebe A. Stapleton
Rutgers, The State University of New Jersey

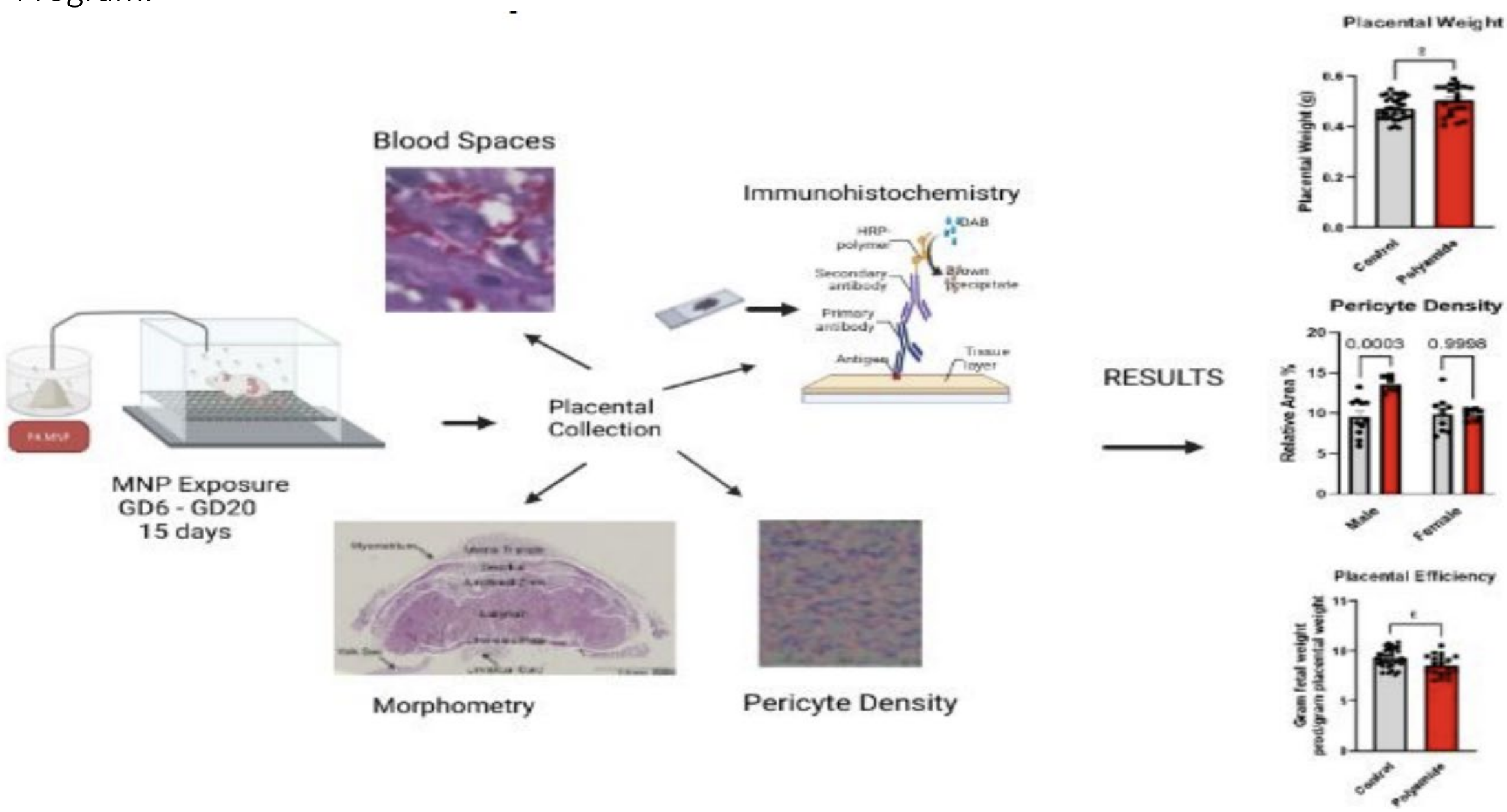
Nano-titanium dioxide (nTiO2), an engineered nanoparticle extensively used in consumer products. Exposure to nanosized particles during pregnancy has been shown to impair maternal health and fetal growth. We have previously identified that maternal inhalation of aerosolized nTiO2 during gestation can impair uterine arterial relaxation, limiting blood delivery to the feto-placental unit, a process that can have dangerous implications for fetal health. The purpose of this study was to identify the mechanism of nTiO2-induced vaso-impairment. We hypothesized that maternal nTiO2 inhalation will impair endothelium-dependent dilation due to reduced nitric oxide (NO) bioavailability. Pregnant rats were exposed to aerosolized nTiO2 nanoparticles (9.48 µg/m3 ± 0.11) from gestational day (GD) 5 to 19. Particle aerodynamic diameter was measured in real-time using a scanning mobility particle sizer (SMPS) (125.26 nm ± 1.82). On GD 20, maternal aorta and uterine artery were isolated, excised, and threaded onto metal wires to evaluate vasoactive responses to increased concentrations (10-9M – 10-4M) methacholine (an endothelium-dependent dilator). These measures were repeated in the presence of N(gamma)-nitro-L-arginine methyl ester (L-NAME; 10-4M), an endothelial nitric oxide synthase inhibitor. Although L-NAME successfully inhibited vasorelaxation, there were no significant differences between the vascular reactivity of control and exposed animals (82.0 ± 7.2 vs 74.8 ± 12.3 percent maximum tension in uterine arteries). By understanding the effects exerted by nTiO2 exposure during pregnancy, we can formulate targeted preventive strategies aimed at preserving maternal and fetal health while mitigating the potential risks associated with nTiO2 exposure during gestation. Supported by NIH R01ES031285 and NIH R25ES020721.



Impairment of Placental Efficiency in Rats Exposed to Micro- and Nanoplastics Throughout Pregnancy

Nacala Gadsden, Chelsea M. Cary, Phoebe A. Stapleton
Lehman College and Rutgers, The State University of New Jersey

Micro- and nanoplastics (MNPs) are ubiquitous environmental contaminants. The increased use and physical degradation of bulk for plastic material leads to the production of micro- and nanoplastics particles, defined as less than 5 mm or 100 nm in diameter, respectively. Human exposure to MNPs often occurs through inhalation, which is supported by the detection of MNPs in human lungs. We investigate the microvascular effects of xenobiotic exposure from a reproductive standpoint and have demonstrated vasomotor functional impairment in uterine radial arteriole exposed to MNPs. Previous studies have demonstrated that particulate inhalation can alter the placental structure, potentially leading to abnormal fetal development. The purpose of these studies were to determine any structural changes to the placental tissue. Herein, we assessed placental development after MNP exposure from gestational day (GD) 6 through GD 20 through the morphometric analysis of H&E-stained placental sections and immunohistochemistry (IHC). Using Image-Pro Premier, the area of the different placental zones (i.e., labyrinth, junctional, and decidua) was acquired. Furthermore, vascular development in the labyrinth zone was quantified by measurement of the maternal and fetal blood spaces. Using IHC we targeted smooth muscle actin to measure trophoblast invasion of vessels in the metrial gland and pericyte density in the labyrinth zone. No significant differences were detected in the area of placental zones, however, exposed males demonstrated a 42% increase in pericyte density ($p<0.05$) in comparison to the naive males. Future studies will assess how markers of vascular development are affected after MNP inhalation during pregnancy. Supported by NIH-R01-ES031285 and NIH R25ES020721, Rutgers-RISE program, and Society of Toxicology Intern Program.

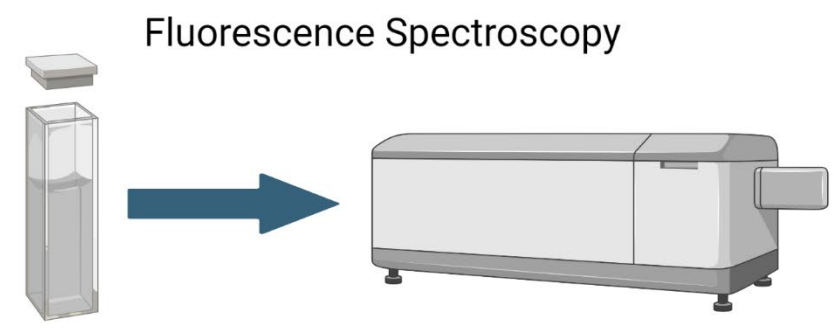


Identification of Compounds that Inhibit the Binding of the FtsZ-Targeting Antibacterial Agent TXA6101 to Serum Albumin

Adrita Dasgupta, Eric Bryan, Daniel Pilch
Rutgers, The State University of New Jersey

Methicillin-resistant Staphylococcus aureus (MRSA) infections are a global public health problem. TXA6101 is a potent inhibitor of the FtsZ protein that in turn disrupts bacterial cell division. However, TXA6101 activity against MRSA decreases in the presence of serum albumin. The purpose of this study is to identify inhibitors of TXA6101 binding to albumin with the potential of enhancing activity against MRSA when used in combination with TXA6101. To this end, fluorescence spectroscopy was used to characterize TXA6101 binding to human and mouse serum albumin (HSA and MSA, respectively) at 37°C. Assays where TXA6101 was titrated into 1.8 µM albumin revealed TXA6101 bound tightly to HSA (Kd = 3.67 µM) and MSA (Kd = 0.89 µM). TXA6101 binding to both HSA and MSA was accompanied by reduction in a fluorescence peak at 340 nm corresponding to the tryptophan residue in site 1 of albumin, and induction of a peak at 430 nm corresponding to albumin-bound TXA6101. Further fluorometric studies probed the impact of two potential albumin binding inhibitors (indomethacin or iopanoic acid) on the binding of TXA6101 to HSA. These studies revealed that both compounds inhibited TXA6101 binding, with the reduction being greater for indomethacin. Future studies will be aimed at determining whether TXA6101 combined with indomethacin or iopanoic acid can potentiate activity against MRSA in the presence of HSA or MSA in culture, and ultimately in vivo.
Supported by the NIH grants R25ES020721 and RO1AI118874, and the American Society for Pharmacology and Experimental Therapeutics SURF Program.

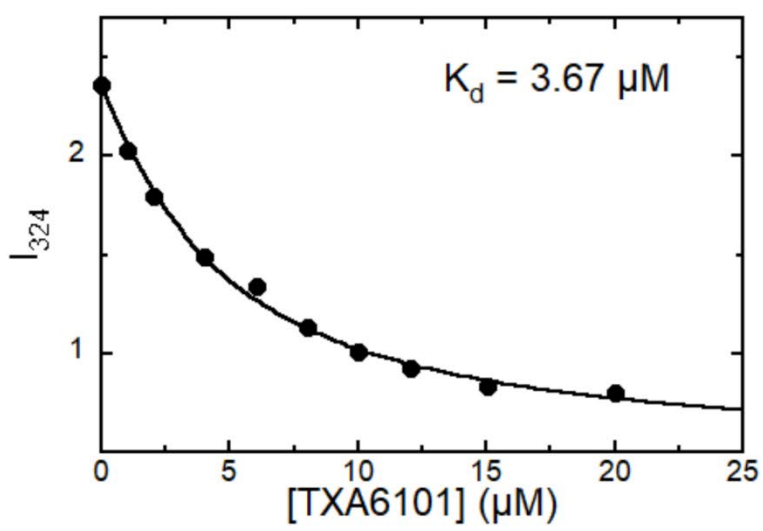
Methods



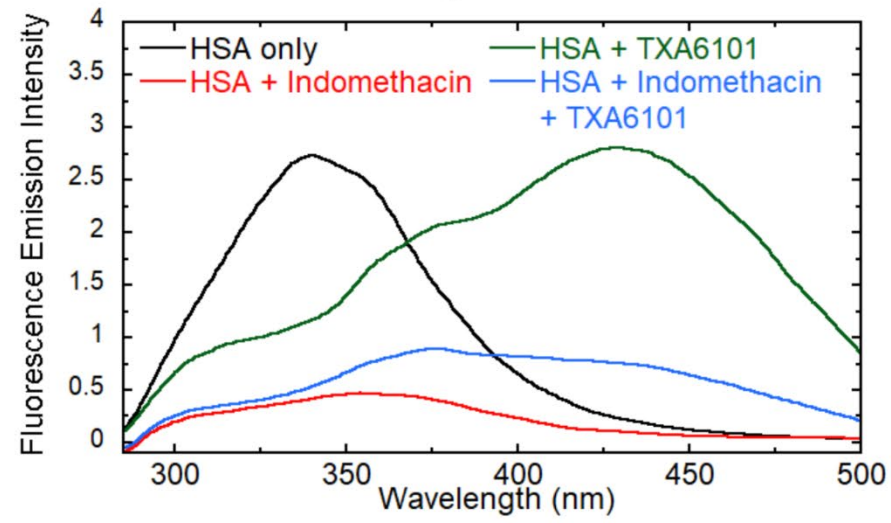
- TXA6101 binding to serum albumin
- Impact of different inhibitors on TXA6101 binding to serum albumin

Results

Binding Curve for the Interaction of TXA6101 with Human Serum Albumin (HSA)



Impact of Indomethacin on TXA6101 Binding to HSA

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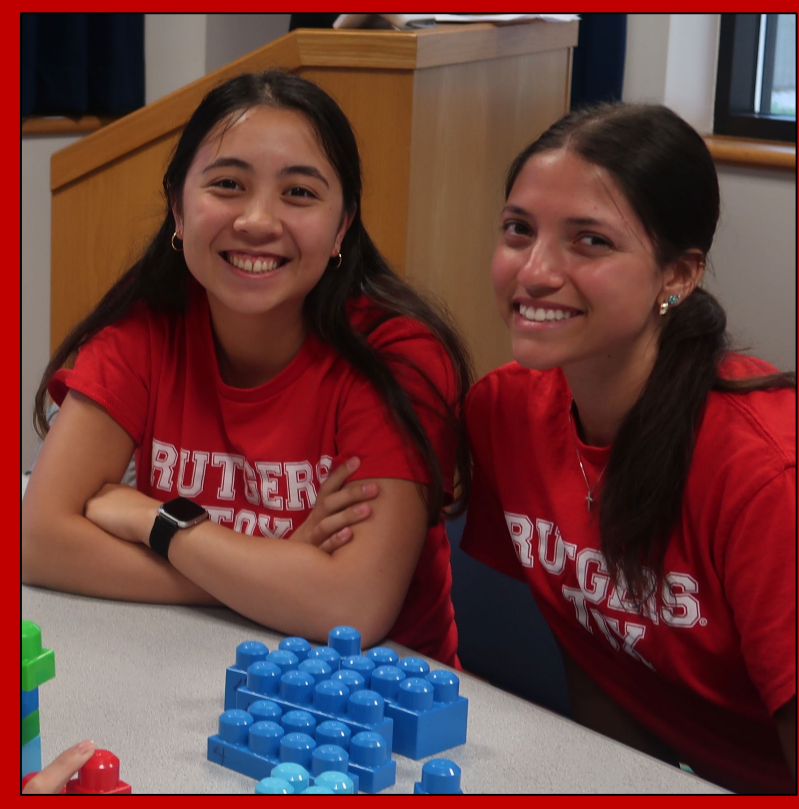

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