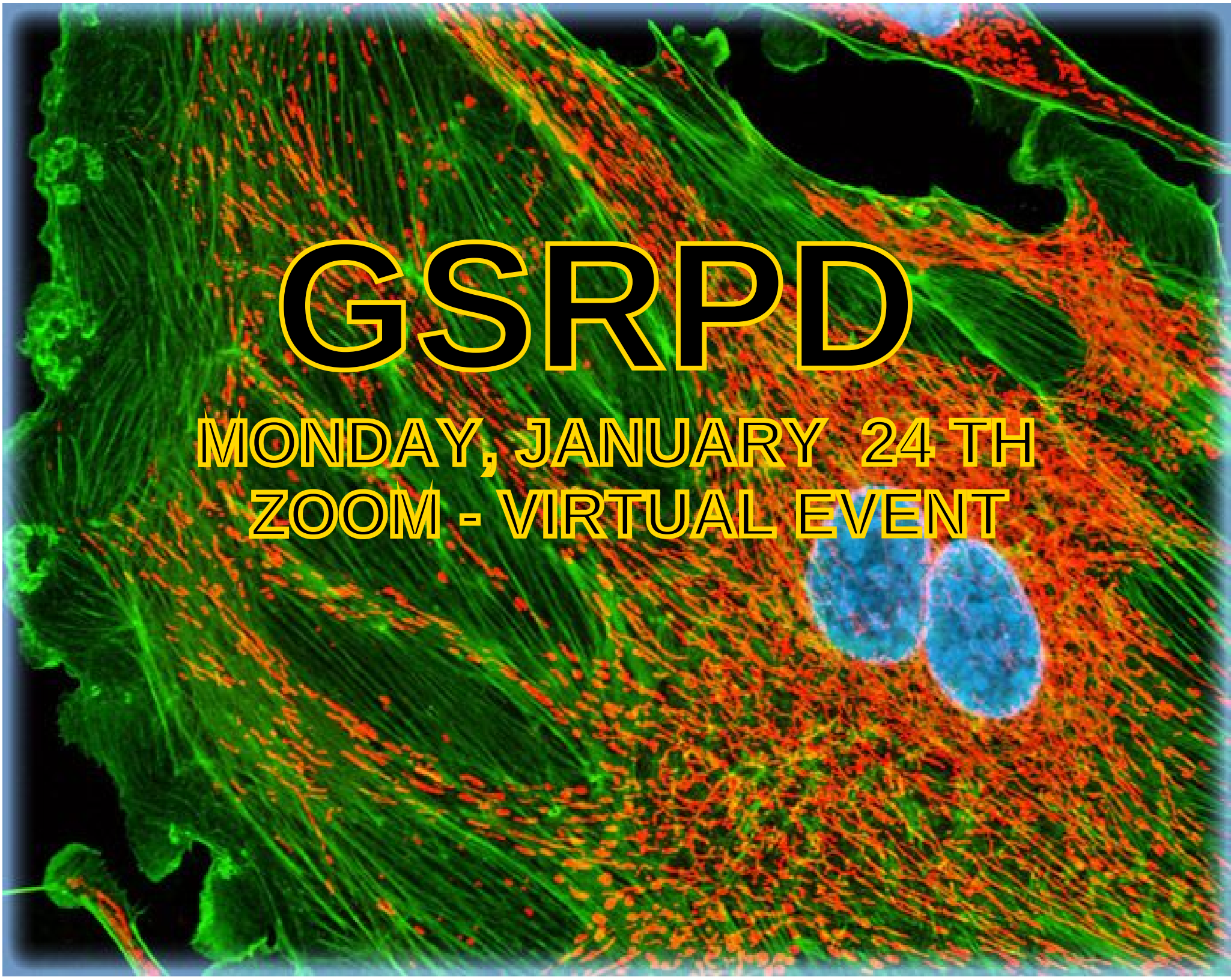

25th Annual

CHUAN-PU LEE, PH.D.
Endowed Graduate Student
Research Presentation Day

WAYNE STATE UNIVERSITY

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IN MEMORIAM: C.P. LEE

Dr. Chuan-Pu Lee (known as “C.P. Lee” by her friends), passed away on July 20, 2016. C.P. Lee was the strongest advocate for graduate students of Wayne State University. She reminded students that diligence and determination were the only limits to achieving their success. C.P. Lee also generously offered pre- and post-doctoral travel awards to aid the cost of national and international conferences. Before her passing, C.P. Lee worked with the GSRPD committee to set up cash prize endowments for exceptional presentations each year at GSRPD. Her life and memory will continue to serve graduate students through this much-anticipated annual event:

The Chuan-Pu Lee, PhD Endowed
Graduate Student Research Presentation Day.

SCHEDULE OF EVENTS

EVENT	TIME	SPEAKERS
WELCOMING REMARKS	8:00 am	GSRPD 2021 Co-Chairs Dr. Linda Hazlett, PhD WSU Vice Dean of Research and Graduate Programs
COFFEE TALK WITH SPEAKER	8:30 am	Informal breakout sessions with keynote speaker
KEYNOTE SPEAKER	9:00 am	Dr. Greg Semenza, MD-PhD Johns Hopkins University School of Medicine
POSTER SESSION I	10:15 am	15, 3-min Rapid fire poster presentations with 2-min Q&A
ORAL SESSION I	11:45 am	5, 10-min presentations with 5-min Q&A
BREAK	1:15 PM	Lunch break with opportunity to ask questions with keynote
POSTER SESSION 2	1:45 pm	15, 3-min Rapid fire poster presentations with 2-min Q&A
ORAL SESSION II	3:15 pm	5, 10-min presentations with 5-min Q&A
CLOSING REMARKS	4:45 pm	Dr. Dan Walz, PhD WSU Associate Dean of Research and Graduate Programs

WELCOME MESSAGE

Welcome to the 25th Annual Graduate Student Research Presentation Day at the Wayne State University School of Medicine! GSRPD is a student-run event that showcases biomedical research across a variety of disciplines. Due to the COVID-19 pandemic, we have had to adapt this event to a virtual format for the second year. Despite current hardships, we have chosen to view the current situation as a welcomed challenge and see our faculty and peers' participation as evidence of the enthusiasm we have for scientific progress as a community! We are excited to feature 40 presentations given by graduate students from both the medical and main campuses at Wayne State University and appreciate those which, due to time constraints, we could not experience this year.

We are delighted to have you socially-distanced with us and hope you can see as many research presentations as possible. Additionally, we extend wishes for the health and safety of all involved and hope that we are able to experience this event with you again next year, regardless of the format.

ACKNOWLEDGMENTS

We would like to thank all faculty members who volunteered their time and experience, as well as the student presenters who shared with us their research. Furthermore, we extend our greatest appreciation to the Office of Graduate Scholars at the Wayne State University School of Medicine for their endless efforts to help execute the procedural aspects of this event. Lastly, we thank all the graduate students who presented their exciting research to the Wayne State community. GSRPD would not have been possible without Wayne State's finest researchers. We look forward to seeing you next year!

Special thanks to Dr. Daniel A. Walz and Deanna Dona for all their support and help.

KEYNOTE SPEAKER

Dr. Gregg Semenza,
MD- PhD

'Hypoxia-inducible factors and cancer progression'

Dr. Semenza is professor of genetic medicine, with joint appointments in pediatrics, radiation oncology, biological chemistry, medicine and oncology, at the Johns Hopkins University School of Medicine. He serves as director of the Vascular Program at the Johns Hopkins Institute for Cell Engineering.

Dr. Semenza's lab made the paradigm shifting discovery of hypoxia-inducible factor 1 (HIF-1), which is a transcription factor that controls the expression of thousands of genes in response to changes in oxygen availability in virtually all metazoan species. The finding, for which he received the 2019 Nobel Prize in Physiology or Medicine, has far-reaching implications for understanding and treating many common diseases, including anemia, ischemic cardiovascular disease, chronic lung diseases, and cancer.

Dr. Semenza received his bachelor's degree from Harvard University and his medical degree and doctorate (in genetics) from the University of Pennsylvania. He completed pediatrics residency training at Duke University Medical Center and postdoctoral training in medical genetics at Johns Hopkins. He joined the Johns Hopkins faculty in 1990.

Dr. Semenza's current research interests include investigating the molecular mechanisms of oxygen homeostasis, the role of HIF-1 in cancer progression, and the development of novel HIF inhibitors for the treatment of cancer and blinding eye diseases. He has authored more than 450 research articles and book chapters, and his work has been cited by other scientists more than 160,000 times.

Dr. Semenza is a founding fellow of the American College of Medical Genetics and Genomics, and was elected to the Association of American Physicians and the National Academy of Sciences in 2008, and the National Academy of Medicine in 2012. He serves as deputy editor of The Journal of Clinical Investigation. He has been honored as a Fellow of the Society for Redox Biology and Medicine (2017), Distinguished Scientist of the American Heart Society (2020) and a Fellow of the AACR Academy (2020).

In addition to the Nobel Prize, Dr. Semenza has received numerous other awards, including the Albert Lasker Basic Medical Research Award (2016), Wiley Prize in Biomedical Sciences (2014), Lefoulon-Delalande Grand Prize from the Institut de France (2012), and Canada Gairdner International Award (2010).

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25th Annual

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POSTER SESSION I

1. Characterization of the Response of 9L Orthotopic Brain Tumors to 3D Conformal Radiation Therapy Using Contrast Enhanced MRI

O. Grahm Valadie1,2, S. Brown1, K. Farmer1, N. Tavarekere1, G. Cabral1, S. Shadaia1, S. Panda1, A. deCarvalho1, G. Divine1, R. Knight1, I. Lee1, J. Dolan1, M. Joiner2, J. R. Ewing1,3

1Henry Ford Healthy System, 2Wayne State University, 3Oakland University

Purpose: As a first step to evaluate early predictors of tumor radiation response, the response of 9L orthotopic brain tumors were measured following MRI guided planning of stereotactic radiotherapy to determine the dose to cure 50%, TCD50.

Methods: 118 Fischer-344 rats were implanted with rat 9L cells in 10µl. Tumors either grew untreated or were treated with 20 or 25 Gy to the 95% isodose. Around 8 days post-implantation, rats were imaged with contrast enhanced MRI (MRI- CE) and irradiated using a Small Animal Radiation Research Platform (SARRP) from Xstrahl Inc. (Suwanee, GA) operating at 220 kV and 13 mA with an effective energy of ~70 keV and dose rate of ~2.5 Gy/minute. Rat orientations were assessed using cone-beam CT; tumor locations were co-registered using the MRI-CE images. Treatment planning using MuriPlan software from Xstrahl Inc. allowed delivery of four non-coplanar arcs with an identical isocenter. Rats were monitored for physical and behavioral abnormalities. Study endpoints were animal survival at 200 days with no weight loss exceeding 20%.

Results: The TCD50 was established from Kaplan-Meier survival analysis as between 20 and 25 Gy. Animals with large tumors assessed from MRI-CE on the day of treatment did not survive long, as expected. Cox Proportions-Hazards modeling did not suggest an effect of sex with the caveat of wide confidence intervals.

Conclusion: The radiation response of orthotopic 9L tumors was characterized using clinically relevant treatment techniques. One future direction is to evaluate the predictive value of pre-treatment vascular measures on radiation response.

2. The role of endoplasmic reticulum-associated degradation in spermatogenesis.

Nusrat Jahan Tushi[1], Zhibing Zhang[2], Shengyi Sun[1]

[1]Department of Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI [2]Department of Physiology, Wayne State University School of Medicine, Detroit, MI

Endoplasmic reticulum (ER)-associated degradation (ERAD) plays a critical role in maintaining ER homeostasis and various physiological processes by mediating the degradation of misfolded ER proteins. The SEL1L-HRD1 protein complex represents the most conserved branch of ERAD in mammals, yet its function in regulating the ER homeostasis and differentiation of male germline cells remains unknown. Hence, we aim to investigate the physiological function of SEL1L-HRD1 ERAD in spermatogenesis. We generated spermatocyte-specific Sel1L-deficient mice (SPKO) by crossing Sel1Lflox/flox female mice with males expressing spermatocyte-specific Stra8-iCre. Surprisingly, compared to wild-type or heterozygous littermates, SPKO mice did not show abnormalities in fertility as demonstrated by the litter sizes in breeding assays, as well as sperm count and motility. Histological examination using spermatocyte-specific marker DDX4 and acrosome marker Lectin PNA revealed no abnormalities of spermatogenesis in SPKO mice. Mechanistically, consistent with previous observations in other cell types, SEL1L deficiency indeed led to the loss of HRD1 protein in the round and elongating spermatids. However, the loss of SEL1L-HRD1 ERAD complex did not disturb ER homeostasis, as demonstrated by a lack of activation of ER stress sensors IRE1α and PERK. Together, our results indicate that the SEL1L-HRD1 complex, the most conserved ERAD machinery, is dispensable for ER homeostasis and male fertility in the testis. This finding suggests alternative ERAD machineries mediating a testis-specific ER protein quality control during spermatogenesis.

3. Insight into TCDD-induced disruption of spermatogenesis via single cell RNA-seq

Alex Haimbaugh[1,] Katherine Gurdziel2, Danielle Meyer[1], Camille Akemann[1], and Tracie Baker[1,3]

[1]Department of Pharmacology, School of Medicine, Wayne State University, Detroit, MI 48207, USA [2]Applied Genome Technology Center, School of Medicine, Wayne State University, Detroit, MI 48207, USA [3]University of Florida, Department of Environmental and Global Health, Water Institute, Gainesville, FL, USA

Background: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent and environmentally persistent endocrine disrupting chemical released during waste incineration and fossil fuel combustion. Our previous work demonstrated the latent reproductive effects of early-life TCDD exposure in zebrafish. Zebrafish acutely exposed to low, environmentally relevant levels of TCDD during sexual differentiation in development were later infertile, even years after exposure. Due to the highly heterogeneous cell- type and -stage landscape of the testes, we hypothesized the various cell types in the testes contribute markedly different profiles towards the pathology of TCDD exposure. Methods and results: To investigate the contributions of the diverse cell types in the adult zebrafish testes to TCDD-induced pathology, we turned to single cell RNA-seq and the 10x Genomics platform. The method successfully captured every stage of testicular germ cell development from spermatogonial stem cells to spermatozoa. We found that the testes of adult fish exposed during sexual differentiation to TCDD contained sharply decreased populations of late spermatocytes, spermatids and spermatozoa. Spermatogonia and early spermatocyte populations were, in contrast, enriched following exposure. Pathway analysis of differentially expressed genes supports previous findings that TCDD exposure resulted in male infertility, and suggested this outcome is due to apoptosis of spermatids and spermatozoa, even years after exposure cessation. Apoptosis of mid to late germ cells was confirmed by immunohistochem-istry. Conclusions: This suite of disruption at the cellular and subcellular level provides support for the environmental explanation of idiopathic male infertility. To our knowledge, this is the first study to apply scRNA-seq to zebrafish testes.

4. Parental Leave Utilization at a Major Urban University in the United States

Maurgan Lee, Julie Crego, Lucki Word, Mayra Shafique, Leah Robinson, Anil Aranha, Beena Sood

Obstetrics, Gynecology, Family Medicine, Pediatrics, Internal Medicine, Professional Development, Diversity and Inclusion

Background and Purpose: The United States is the only industrialized nation that does not have a mandated nation-wide paid parental leave for all new parents. When parents properly bond and adjust to the demands of a growing family, it impacts the overall health of mother and child, industry growth, paternal and familial bonding, and increases workplace mobility for women. The purpose of the study was to examine how parental leave policies can better meet the needs of employees and support work/life balance. Methods: Retrospective analysis of human resources data from a major urban university. Data included: socio-demographics, duration of parental leave, and leave frequency. Data was analyzed using SPSS and statistical significance was established at $p < .05$. Results: A total of 5,484 employees were included in the study. Among them, 349 (6.4%) took parental leave, 297(85.1%) female, disproportionately Caucasian (57.9%), and married (79.7%), with a mean income of $68,500. Both men and women used parental leave. However, women took longer parental leave in comparison to men ($p < .05$). Gender, professional, and citizenship status had an impact on parental leave duration and obtaining parental leave. Conclusion: Our study demonstrates that multiple socioeconomic factors are associated with securing parental leave and its duration. Financial and professional repercussions may influence the decision to take parental leave and its duration, and the desire to become parents. These issues can be addressed by formulating institutional policies that include parental leave benefits in the workplace and educating the employers and employees about them.

5. The Endocrine Disrupting Activities Associated with Liquid Crystal Monomers and their Mixtures

Samantha Heldman[1] and Christopher Kassotis[1]

[1]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI 48202

Background: Mixtures of liquid crystal monomers (LCMs) are an essential part of liquid crystal devices such as televisions, computers, and smartphones. Several LCM biphenyls and analogues are predicted to be persistent and bio-accumulative and have been reported in household dust and other environmental samples, suggesting potential human exposure. Recent research exposed chicken embryonic hepatocytes cells to a mixture of LCMs and reported significant modulation of several genes associated with adipogenesis. Methods and Results: To determine potential mechanistic effects, we investigated the activity of a subset of LCMs on hormone receptors involved in adipogenesis. 10 LCMs (5 fluorinated, 5 non-fluorinated) as well as two mixtures containing: all fluorinated (F) and all non-fluorinated (NF) LCMs, were tested at environmentally relevant concentrations for either agonism or antagonism of thyroid receptor beta (TRβ), peroxisome proliferator activated receptor gamma (PPARγ), retinoid X receptor alpha (RXRα), androgen receptor (AR), estrogen receptor alpha (ERα), progesterone receptor B (PR-B) and glucocorticoid receptor (GR) using a transient transfection, luciferase reporter gene assay in human embryonic kidney cells (HEK-293T/17), human hepatoma cells (HEPG2), or human endometrial adenocarcinoma cells (Ishikawa). Several LCMs individually and in mixtures exhibited significant agonism, additive agonism, or antagonism of key receptors involved in adipogenesis. Conclusion: These results suggest that LCMs deserve further investigation as potential contaminants of concern, particularly given likely co-occurrence with diverse mixtures of other endocrine active contaminants. Ongoing research is assessing disruption of other hormone receptors and determination of adipogenic activity using *in vitro* pre-adipocyte models.

6. Structural and biochemical characterization and drug design of the SARS-CoV-2 3CL protease

Michael Muczynski1

1Dept. of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI

A study produced by a collaboration of data gathering sources states that COVID-19 has claimed the lives of 5.31 million people. COVID-19 has caused immense distress to medical systems around the world. SARS-CoV-2, the virus related to COVID-19 has mutated and decreased vaccine efficacy substantially since its arrival, requiring alternate methods of attack such as antiviral drug therapy. The SARS-CoV-2 3CL protease cleaves the coronavirus polyprotein at eleven conserved sites; this is an essential step in the viral replicative cycle making it an attractive target for drug design. Currently, our lead compound, RS-3294, has been shown to have nanomolar inhibition through the use of FRET-based enzyme assays screening against SARS-CoV-2 3CL protease. In-silico screening also provides for identification of other potentially strong inhibitors of the SARS-CoV-2 3CL protease. Utilizing Schrodinger's (2021-3) virtual screening workflow and the MCULE database we were able to identify compounds with strong inhibitory potential.

7. Female rats demonstrate higher locomotor response to cocaine than males

Taylor N. Takla[1], Nareen Sadik[2], Cameron J. Davidson[2], Srini Kallakuri[2], Sezar Kachel[3], Majd Yahya[3], Sarah Durack[3], Shane A. Perrine[2,4]

[1]Translational Neuroscience Program, WSU SOM; [2] Department of Psychiatry and Behavioral Neurosciences, WSU SOM; [3] College of Liberal Arts and Sciences, WSU; [4] John D. Dingell VA Medical Center

Understanding sex differences in drug abuse behaviors is of immense research interest. Prior research indicates that females are more sensitive to stimulant drugs than males. To explore these purported differences, male and female rats were assessed for differences in locomotor activity (LMA) following repeated cocaine exposure. We hypothesized female rats would have higher LMA than males and show greater levels of LMA sensitization. Male and female rats were subjected to chronic, noncontingent saline (control, 0.2 mL females, 0.3 mL males) or cocaine exposure (10 mg/kg intraperitoneal) once daily for 10 consecutive days. Then they underwent a forced withdrawal for 21 days followed by cocaine or saline challenge. LMA was measured on days 0 (baseline), 1 (acute), 10 (chronic), and 31 (challenge) in an open field chamber. Animals exposed to chronic and acute cocaine demonstrated higher LMA than the control groups in both sexes, with females locomoting significantly more than males. Counter to our hypotheses, neither sex showed psychomotor sensitization via LMA during chronic cocaine administration. Although our data validated the impact of cocaine on LMA in females, it was counter to prior work on behavioral sensitization following chronic cocaine administration. These differences could be attributed to an increase in stereotypy behavior (repetitive grooming and rearing). Further steps for this investigation include analyzing stereotypy behavior as the dependent variable and exploring the role of biomolecular condensates in the brain. Specifically we are interested in activator of G-protein signaling 3 (AGS3) and disshevled 2 (DVL2) signaling as mediators of the cocaine's actions.

8. Characterization of caffeine response regulatory variants in vascular endothelial cells

Carly Boye[1], Cindy Kalita[1], Anthony Findley[1], Adnan Alazizi[1], Roger Pique-Regi[1,2], Francesca Luca[1,2]

[1]Center for Molecular Medicine and Genetics, [2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI

Non-coding regions in the human genome contain gene regulatory sequences. Variants within these regions can contribute to phenotypes by modulating gene expression and are also key mediators of the response to environmental stimuli. Gene-environment (GxE) interactions contribute to complex traits such as cardiovascular disease (CVD). In particular, caffeine is the most widely consumed stimulant and is known to produce a vascular response. Though results on the role of caffeine in cardiovascular health have been conflicting, previous GxE studies have confirmed the importance of caffeine in coronary artery disease risk. To further investigate GxE interactions for caffeine, we treated vascular endothelial cells with caffeine to measure allele-specific effects (ASE) on gene regulation using the massively parallel reporter assay Biallelic Targeted STARR-Seq (BiT-STARR-Seq).

We performed 6 BiT-STARR-seq replicates and investigated 34,420 genetic variants. We found 2,601 variants with significant ASE (FDR<10%) across both the caffeine and control treatments. We investigated interactions between genetic regulatory variants and caffeine treatment through conditional ASE (cASE). We identified significant cASE (FDR<10%) for 357 variants. These variants were enriched in binding sites for caffeine response factors identified through ATAC-seq (OR=2.10, p=1.8e-4. In particular, variants in the vitamin D receptor binding sites disproportionately showed cASE (p=4.04e-6). Variants with ASE and cASE recapitulate previously known genetic regulatory variants in artery tissues. Our results emphasize the importance of studying both the genetics and GxE interactions in vascular endothelial cells, uncover some of the mechanisms underlying GxE for caffeine and are relevant to the understanding of CVD risk.

9. Investigating the Co-localization of a Fos Neuronal Ensemble and HDAC5 in the Rat Nucleus Accumbens Following Reinstatement to Cocaine-Seeking

Trombley, A.T.[1,2], Kallakuri, S.[1,3], Sadik, N.[1,3], Durack, S.[1], Khan, S.J.[1], Perrine, S.A.[1,2,3].

[1]Department of Psychiatry and Behavioral Neurosciences, [2]Translational Neuroscience Program, [3]John D. Dingell VA Medical Center, Detroit, MI

Fos-expressing neuronal ensembles in the rat nucleus accumbens (NAc) encode associations between cocaine-paired contextual cues and reinstatement of cocaine-seeking following cocaine withdrawal. Histone deacetylase 5 (HDAC5), an epigenetic regulator protein, controls drug-seeking at the transcriptional level. Its overexpression in the NAc attenuates context-induced reinstatement of cocaine-seeking following withdrawal. Whether HDAC5 acts specifically in the corresponding NAc ensemble to weaken cue-drug associations is not known. To examine this, female and male Fos-LacZ transgenic Wistar rats underwent 9-10 intermittent intravenous cocaine self-administration sessions (0.3 mg/kg/infusion) followed by a 21d withdrawal period. Animals were then administered a priming injection of cocaine (15mg/kg, intraperitoneal), tested for cocaine-seeking behavior, and sacrificed. The harvested brains were processed for assessing colocalization of Fos and HDAC5 in the NAc by immunofluorescence microscopy. We hypothesize that HDAC5 is co-localized with Fos, modulating the ensemble to weaken drug-context associations and regulate cocaine-seeking.

10. Progression of thoracic aortic aneurysm due to increased inflammation: infiltrated CD11b+ immune cells to vessel media

Lucynda Pham[1], Shichao Wu[2], Donghong Ju[2], Li Li, PhD[1,2]

[1]Center Molecular Medicine Genetics, Wayne State University School of Medicine, Detroit, MI; [2]Dept. of Internal Medicine, Wayne State University School of Medicine, Detroit, MI.

Background: Marfan Syndrome can lead to the development of thoracic aortic aneurysm subject to rupture/ death and has been linked to mutations in extracellular matrix protein fibrillin-1 (FBN1). It is unknown how FBN1 mutations cause aneurysm formation and progression. There is increasing evidence that inflammation plays a role in aneurysm progression. However, i) the different subtypes of immune cells present are unknown and ii) the signal that recruits the immune cells to the media is unknown. Aim: to evaluate the immune cell subtypes and role of inflammation in aneurysm progression. Methods and results: Results from the RNAseq data comparing thoracic aorta of our Marfan aneurysm mouse model (Q24m/m) show inflammation as the top biological process and significant increase in ITGAM (CD11b) expression. Immunofluorescent (IF) staining (Figure 1a) of tissue sections and western blot of aortic lysates (Figure 1b) was performed to confirm the expression of CD11b. The increased CD11b expression in Q24m/m compared to WT suggests infiltration of immune cells to the media of the vessel wall. The subtype of CD11b+ immune cells was done by IF staining using different cell markers (Figure 2). Monocytes and macrophages were present in the media of the Q24m/m vessel and the ratio of M1 vs M2 macrophages was evaluated. The beta integrin subunit (CD18) was shown by IHC staining (Figure 3a) and ICAM-1 expression was shown by IF staining (Figure 3b). There was increased expression of both CD18 and ICAM-1 in Q24m/m. Conclusion: Infiltration of CD11b+ immune cells contribute to progression of aneurysm.

11. Sevoflurane enhances high-frequency oscillation output, phase-amplitude coupling, and effective connectivity in patients with drug-resistant focal epilepsy

Ethan Firestone[1,2], Masaki Sonoda[1,3], Keiko Wada[4,5], Kazuki Sakakura[1,6], Naoto Kuroda[1,7], Yutaro Takayama[3,8], Tomoyuki Miyazaki[4,9], Masaki Iwasaki[8], Eishi Asano[1,10]

1. Department of Pediatrics, Children's Hospital of Michigan, Detroit Medical Center, Wayne State University, Detroit, MI 48201, USA 2. Department of Physiology, Wayne State University School of Medicine, Detroit, MI 48201, USA 3. Department of Neurosurgery, Yokohama City University Graduate School of Medicine, Yokohama 2360004, Japan 4. Department of Anesthesiology, National Center Hospital, National Center of Neurology and Psychiatry, Kodaira, Tokyo 1878551, Japan 5. Department of Anesthesiology and Critical Care, Yokohama City University, Yokohama, 2360004, Japan 6. Department of Neurosurgery, University of Tsukuba, Tsukuba, 3058575, Japan 7. Department of Epileptology, Tohoku University Graduate School of Medicine, Sendai 9808575, Japan 8. Department of Neurosurgery, National Center Hospital, National Center of Neurology and Psychiatry, Kodaira, Tokyo 1878551, Japan 9. Department of Physiology, Yokohama City University Graduate School of Medicine, Yokohama 2360004, Japan 10. Department of Neurology, Children's Hospital of Michigan, Detroit Medical Center, Wayne State University, Detroit, MI 48201, USA

Rationale. Surgical treatment for drug-resistant focal epilepsy is often a grueling two-stage process that involves days of extra-operative, intracranial EEG (iEEG) recording. Thus, there is great need to develop techniques capable of localizing the epileptogenic zone during surgery, to reduce this diagnostic burden and optimize postoperative seizure control. Since sevoflurane anesthesia reversibly activates epileptiform iEEG discharges, we aimed to determine if sevoflurane-augmented phase-amplitude coupling (PAC) between high-frequency oscillations (HFO: 80-300 Hz) and delta waves (3-4 Hz), as well as their inter-regional effective connectivity measured by transfer entropy (TE), can intraoperatively track seizure foci.Methods. This is an observational study of eight pediatric patients with drug-resistant focal epilepsy who achieved seizure freedom following a two-stage resective surgery under sevoflurane anesthesia. We retrospectively analyzed the above intraoperative iEEG features at an oxygen baseline, three time points as sevoflurane dynamically increased from 0 to 2 minimum alveolar concentration (MAC), and another at 2 MAC.**Results.** HFO amplitude, PAC, and TE increased as a function of sevoflurane concentration, and this effect was greatest in the resected (epileptic) brain sites just prior to- and after- the sevoflurane concentration reached 2 MAC. In contrast, delta amplitude and TE were increased most in the preserved sites, at the same anesthetic stage.Conclusions. Increasing sevoflurane concentration may elevate HFO spectral output, PAC, and TE-defined effective connectivity preferentially within epileptic sites. This enhancement may be permitted via hypersynchronization with delta activity, which provides a window of disinhibition when delta is reduced immediately prior to achieving 2 MAC of sevoflurane.

12. Defense mechanisms against oxidative damage in metastatic prostate cancer: adipocyte-mediated modulation of mTOR and ferroptosis pathways

Alexis Wilson[1]*, Mackenzie Herroon[2], Shane Mecca[2], Laimar Garmo[2], and Izabela Podgorski[1,2]

Wayne State University School of Medicine Department of Cancer Biology1, Pharmacology2

Prostate cancer (PCa) is the most common cancer amongst males and becomes incurable once it advances to the secondary site. The most prevailing site of metastasis from PCa is the bone marrow, composed of metabolically active cells called adipocytes. Our lab has shown that adipocytes promote PCa progression and therapy evasion through modulation of tumor metabolism and activation of pro-survival signaling; however, the molecular mechanisms behind tumor-promoting effects of fat cells are not understood. It is known that high iron levels within the tumor microenvironment help the growth of PCa cells. However, iron overload can cause an increase in harmful reactive oxygen species, and tumor cells have developed defense mechanisms to protect them from this oxidative damage. Our data show that expression of iron storage protein ferrtin is reduced in PC3 and ARCaP(M) cells upon exposure to adipocytes. Low ferritin levels have been associated with induction of ferroptosis, a form of iron-mediated cell death. However, our results demonstrate that interaction with adipocytes increases the levels of ferroptosis gate-keeper protein GPX4 in PCa cells, suggesting an adipocyte-mediated defense mechanism against ferroptosis. Interestingly, an additional consequence of adipocyte-tumor cell cross-talk is altered mTOR signaling in both the PCa cells and the fat cells, and the knockdown of GPX4 in PCa cells affects the expression of downstream mTORC1 proteins, 4EBP1 and p70S6K, pointing to an interplay between mTOR and ferroptosis. Understanding the mechanisms of adipocyte contribution to dysregulation of the mTOR pathway and escape from ferroptosis may have therapeutic implications for metastatic PCa.

13. Effects of per- and polyfluoroalkyl substances on bone marrow microenvironment: Potential implications for bone metastatic cancers

Laimar C Garmo[1], Mackenzie K Herroon[1], Shane Mecca[1], Alexis Wilson[2], Michael C Petriello[1,3], and Izabela Podgorski[1,]

[1]Center Molecular Medicine Genetics, Wayne State University School of Medicine, Detroit, MI; [2]Dept. of Internal Medicine, Wayne State University School of Medicine, Detroit, MI.

Per- and polyfluoroalkyl substances (PFAS) are a group of industrial chemicals that are widely used in everyday products. PFAS have incredibly long half-lives, bioaccumulate in bone and potentially disrupt bone homeostasis; however, their specific effects on bone marrow microenvironment remain understudied. Bone is a site of metastasis from several tumor types including prostate cancer (PCa) and previous studies from our lab have shown that alterations in bone microenvironment accelerate metastatic progression of PCa tumors in bone. We hypothesized that bone microenvironment altered by PFAS exposure can contribute to skeletal progression of PCa tumors. We show that exposure of mice to a cocktail of 5 PFAS chemicals (PFOS, PFOA, PFNA, PFHxS, GenX) increases the number of bone marrow adipocytes and augments expression of adipogenesis-associated genes. RNAseq analyses of tibia from PFAS-exposed mice indicate changes in bone metabolism, supported by in vitro osteoclastogenesis assays. Mass spectrometry analyses of bone extracts from PFAS cocktail-exposed mice reveal that out of 5 PFAS compounds studied, PFHxS accumulates in bone the most. Interestingly, our in vitro assays employing bone marrow derived mesenchymal cells show that exposure to PFHxS promotes marrow adipogenesis. Studies examining the direct impact of long-term PFAS exposure on in vitro PCa cultures and progression of intratibially implanted PCa tumors are currently ongoing. Our findings to date indicate that changes in bone marrow microenvironment induced by PFAS have a potential to disrupt bone equilibrium, creating a microenvironment conducive to tumor growth and survival.

14. Loss of Flavin Containing Monooxygenase 3 (FMO3) protects Mice Against Dioxin-like Polychlorinated Biphenyl (PCB)-induced Inflammation

Manisha Agarwal[1]* and Michael Petriello[1,2]

[1]Pharmacology Department, School of Medicine, Wayne State University, Detroit MI 48202 [2]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI 48202

Introduction: Dioxin-like pollutants (DLPs) are persistent organic lipophilic compounds that bioaccumulate in the adipose tissue and are shown to be associated with cardiometabolic disorders. Toxicity of these compounds is shown to be linked to aryl hydrocarbon receptor (AhR) activation, through mechanisms related to cytochrome P4501a1 induction. We have recently shown that induction of another xenobiotic detoxification enzyme, FMO3, and its major enzymatic product, trimethylamine-N-Oxide (TMAO) may also be critical for DLP-induced inflammation and systemic toxicity. Meat based diets rich in Choline and carnitine, gets converted to trimethylamine (TMA) by the gut microbiota. This TMA is then oxidized to TMAO by FMO3.

Hypothesis: Loss of FMO3 protects mice against DLPs induced toxicity through either decreased AhR activation and/or changes in gut microbiota composition.

Methods: Male C57BL/6 or FMO3 knockout mice were treated with vehicle or PCB126 (dose-1umol/kg) by oral gavage at weeks 2 and 4 of a 12-week study. We performed hepatic RNA-seq analysis and 16S rRNA sequencing on cecum samples.

Results: Hepatic RNA-seq analysis showed significant differentially regulated genes with log2-fold change with FDR values <0.05 between C57BL6 and FMO3-/- mice after PCB126 exposure. GO pathway analysis showed significantly more differential enriched pathways for C57BL6 than FMO3-/- mice due to PCB exposure. For example, glutathione transferase activity pathway was enriched only in C57BL6 mice. We saw marked differences in beta diversity of FMO3-/- gut microbiota compared to WT mice due to genotype.

Conclusion: Absence of FMO3 leads to completely different response to dioxin-like pollutants in liver and gut microbiota

15. Cbf-β is required for development and differentiation of murine mucosal-associated invariant T cells

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Mucosal-associated invariant T (MAIT) cells primarily reside in mucosal tissues and tissues exposed to the environment to act as a defense against microbial threats. Additionally, they are also involved in a broad spectrum of diseases, including infection, cancer, allergy, and autoimmunity. Although some transcriptional factors and micro-RNA have been recently identified to regulate MAIT cell development and differentiation, a significant gap in our knowledge of MAIT cell regulation still exists. The core binding factor subunit beta (Cbf-β) is a non-DNA binding protein forming a heterodimer with RUNX family proteins to regulate diverse signaling pathways which maintain homeostasis of a wide range of immune cells. To understand the role of Cbf-β in MAIT cell development and function, we generated T cell lineage specific Cbf-β knockout mice (CD4cre+Cbf-β KO) and timely inducible Cbf-β knockdown mice (UBC-cre+Cbf-β KO). We found that Cbf-β deficiency impaired thymic MAIT cell development and interrupted MAIT1 and MAIT17 differentiation. Additionally, inducible knockdown of Cbf-β reduced activation and cytotoxicity in peripheral MAIT cells. Our work suggests that Cbf-β is required for thymic MAIT cell development and MAIT1 and MAIT17 commitment in addition to playing a role in MAIT cell activation and cytotoxicity. Overall, Cbf-β may serve as a novel target to modulate MAIT cells in MAIT cell-based immunotherapies.

ORAL SESSION 1

1. Isoforms of transcriptional cofactor SIN3 differentially regulate genes necessary for energy metabolism and cell survival

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Temporal and spatial gene expression is necessary for cell viability and organismal development. Gene activity is regulated by histone modification patterns and positioning. SIN3 is an essential transcriptional regulator that acts as a scaffolding protein for a histone deacetylase (HDAC) complex. Two major isoforms of SIN3 exist in *Drosophila*, SIN3 220 and SIN3 187, which differ in their C termini. These isoforms have distinct expression patterns and perform overlapping as well as distinct functions. Using *Drosophila* S2 cells expressing either SIN3 220 or SIN3 187, we identified isoform specific binding sites and differential gene expression patterns mediated by the isoforms. Analyzing gene expression patterns in cells expressing either one of the SIN3 isoforms, we noted that compared to SIN3 220, SIN3 187 expressing cells repress various cell cycle and mitochondrial maintenance genes and activate several pro-apoptotic genes. Here, we are interested in studying the regulation of energy metabolism and survival by the SIN3 isoforms. We observed that cells expressing SIN3 187 exhibit higher sensitivity to oxidative stress as compared to cells expressing the SIN3 220 isoform. We also determined that SIN3 187 expressing cells have lower oxygen consumption capacity, indicating possible mitochondrial dysfunction. We are interested to further analyze the link between the differential gene expression patterns and cell physiology. Together, these studies will help us to understand why SIN3 187 expressing cells exhibit a disadvantage in terms of cell survival. Results from these studies are expected to identify molecular mechanisms used by the SIN3 isoforms to control cell viability.

2. Growth hormone receptor signaling in hypothalamic AgRP neurons controls body core temperature in a Sex-Specific Manner

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Evidence for hypothalamic regulation of energy homeostasis and thermoregulation in brown adipose tissue (BAT) during aging has been well recognized, yet the molecular mediators involved in this process are poorly understood. During aging, BAT loses its thermogenic capacity and adaptation to cold temperatures thus reducing its ability to maintain normal energy homeostasis and body temperature late in life. The arcuate hypothalamus (ARC) is a brain region that controls energy homeostasis, reproduction, and neuroendocrine regulation of GH (growth hormone) secretion through molecularly distinct cell types. To determine the significance of GHR signaling in AgRP neurons in thermoregulation, we inactivated GHR specifically in AgRP neurons by crossing a well-established AgRP-ires-cre with $GHRP^{lox/lox}$ mice ($AgRP^{\Delta GHR}$). We show that neuronal specific deletion of GHR in AgRP neurons impairs energy homeostasis and core body temperature in a sex specific manner, affecting $AgRP^{\Delta GHR}$ female but not male mice. Additionally, $AgRPP^{\Delta GHR}$ females were completely resistant to cold exposure and exhibited significant reduction in mitochondrial uncoupling protein 1 (Ucp1) and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (Pgc1α) in the BAT under cold exposure. We observed no differences in the body core temperature or responses to cold in male $AgRPP^{\Delta GHR}$ mice compared to control littermates. These studies demonstrate the novel role for GHR signaling in the hypothalamus in control of thermoregulation. GHR expression is significantly reduced in aged hypothalamus, thus, revealing mechanisms by which GHR signals modulate adaptive thermogenesis, will provide insight into the brain systems that are critical for the thermogenic vitality of the elderly.

3. Disruption of a Cargo Transport System for Sperm Formation by a Single Amino Acid Mutation in Mouse PACRG Protein

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Interaction between mouse meiosis-expressed gene 1 (MEIG1) and Parkin co-regulated gene (PACRG) is essential for sperm formation and male fertility. PACRG recruits meiosis-expressed gene 1 (MEIG1) to the manchette for normal spermiogenesis, and the key amino acid on mouse MEIG1 that mediates interaction with PACRG has been identified. MEIG1/PACRG interaction is conserved in humans. The structure of the human MEIG1/PACRG complex has been resolved, and the key amino acids on human PACRG that mediate interaction with MEIG1 have also been identified and are conserved in mice. Mutations of these amino acids, particularly H121, significantly reduced MEIG1/PACRG interaction. To study the role of H121 *in vivo,* we mutated H121 of mPACRG using CRISPR/cas9 system and phenotyped the mutant mice. Although grossly normal, all homozygous mutant males analyzed were completely infertile, accompanied with severe reduction in sperm count. Motility of the sperm cells were severely reduced, and all sperm were morphologically abnormal. Histological examination of the testis demonstrates impaired spermiogenesis in mutant mice. Electron microscopy of the testes revealed severe sperm flagellar disruption in the mutant mice. Western blot analysis indicated that the protein levels of PACRG, MEIG1 and SPAG16L, a cargo protein of the MEIG1/PACRG complex, were not changed in the mutant mice. However, immunofluorescence staining revealed that MEIG1 and SPAG16L were no longer present in the manchette of the mutant sperm. These findings demonstrate that *in vivo*, H121 is a key amino acid mediating the interaction between PACRG and MEIG1 and is crucial for downstream sperm flagella formation.

4. Overcoming Venetoclax Resistance in Acute Myeloid Leukemia (AML)

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Most AML patients are older adults who have an especially dismal prognosis due to limited therapeutic options. Recently, the Bcl-2 inhibitor, venetoclax (VEN), was approved for use in combination with hypomethylating agents [azacitidine (AZA) or decitabine] in AML patients who are ≥75 years old or ineligible for intensive chemotherapy. The combination of VEN and AZA has become a new standard of care for older patients, but resistance results in a 1-year overall survival rate of only 30-40% and the median overall survival following treatment failure is < 3 months. Previous studies have found VEN resistance can be overcome by targeting mitochondria and the antiapoptotic protein, Mcl-1. Our studies on ONC213, a novel imipridone, have found that it has potent antileukemic activity, reduces Mcl-1 and targets mitochondria of AML cells. The combination also targets AML progenitor cells and shows *in vivo* efficacy. To determine if ONC213 could overcome acquired resistance to VEN and AZA, we developed VEN and AZA resistant AML cell lines. We found ONC213 could resensitize resistant cells to VEN and its combination with VEN showed highly synergistic antileukemic activity against these cell lines. ONC213 alone and in combination with VEN suppressed oxidative phosphorylation (OXPHOS) and Mcl-1 in AML cells, including those with inherent or acquired resistance to VEN+AZA. Here we demonstrate that resistance to VEN+AZA in AML can be addressed through dual targeting of mitochondria and Mcl-1 using the novel imipridone, ONC213, and that ONC213 offers a potential approach to treat VEN+AZA resistant AML.

5. Macrophage capacity for antibody-dependent cellular phagocytosis genetically linked to host capacity to effectively response to targeted monoclonal antibody therapy

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Targeted monoclonal antibody (mAb) therapies have been in wide-use for several decades. Trastuzumab targets the tumor associated antigen (TAA) epidermal growth factor 2 (HER2), and has rose to prominence as a first-line treatment for $HER2^{+}$ malignancies. Despite the success garnered by targeted immunotherapeutics, differential response among patients treated with trastuzumab suggest that host genetics may contribute to response to treatment. Using a F1 cross of diversity outbred (DO) mice and BALB/c mice (n=126), we orthotopically implant syngeneic tumor (TUBO) expressing rat NEU (HER2 homolog). Treatment with αNeu (7.16.4) mAb therapy illustrates that the differential response seen in humans is mirrored in genetically diverse mouse populations. With the help of genetic linkage analysis, we show several loci across the murine genome contribute to a robust response. Collaborative Cross (CC) mouse strains containing/lacking these loci in a similar F1 cross were used to confirm these loci *in vivo*. While these loci cannot fully predict a robust response, these efforts have yielded experimental mouse strains capable of ~90% response to treatment, as well as strains wherein only ~10% of mice successfully eliminate tumor. Canonical mechanisms of targeted immunotherapy were evaluated using tissues collected from these mice as well as the original DOxBALB/c F1 cohort. Here we show that tumor doubling time (TDT) in treated mice of diverse background correlates with bone marrow derived macrophage (BMDM) capacity to phagocytize opsonized tumor in coculture. QuantSeq 3’ transcriptomic analysis yields additional insights into tumor infiltrating immune cell populations that may impact host response to therapy.

POSTER SESSION II

1. Role of γC4 Protocadherin in survival and self-avoidance in the mouse retina

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Cell adhesion molecules (CAMs) are diverse cell surface molecules involved in an array of developmental functions, including neural circuit formation. One family of CAMs, the γ-Protocadherins, consists of 22 protein isoforms hypothesized to play a role in defining neuronal identity. The γ-Pcdhs regulate many essential processes during CNS development including neuronal survival, synapse formation, dendritic self-avoidance, and the formation of dendritic arbors. How exactly the γ-Pcdhs mediate these processes is not well understood. Recently one isoform, γC4, has been identified as essential for postnatal viability and neuronal survival in mice, where mice lacking this isoform die soon after birth. This study seeks to understand the unique function and mechanism of the γC4 isoform in the processes of neuronal survival and self-avoidance in the mouse retina. We **hypothesize**: 1) the γC4 isoform is the only necessary and sufficient isoform for promotion of neuronal survival in the retina due to its unique protein sequence. 2) γC4 alone cannot promote self-avoidance in starburst amacrine cells of the retina, also as a due to unique protein sequence. 3) γC4 has specific binding partners which promote neuronal survival. The **methods** used to investigate these hypotheses include the use of reduced diversity mouse lines to ask whether γC4's role in these two processes is truly unique among isoforms, and how that role is mediated. Preliminary **results** indicate loss of γC4 leads to decreased density of amacrine and retinal ganglion cell types, and that the presence of only γC4 in starburst amacrine cells results in disrupted self-avoidance.

2. Is there a placental microbiota? A meta-analysis of published placental microbiota datasets

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The existence of a human placental microbiota is under debate. Traditionally, the human placenta was considered sterile since microbial colonization was associated with adverse pregnancy outcomes. Yet, recent DNA sequencing studies report a microbiota in human placentas from uncomplicated term pregnancies. However, the placental microbiota is reportedly low biomass, and therefore could be confounded by background DNA contamination. If a placental microbiota exists, then patterns or consistency in placental bacterial DNA signals should be evident across studies. Methods/Results: Using all publicly available 16S rRNA gene datasets with sufficient metadata for sample discrimination, the data were re-analyzed for consistency. 16S rRNA gene Amplicon Sequence Variants (ASVs) identified as *Lactobacillus* were among the top five relatively abundant ASVs for eight of fifteen studies. However, it was clear that the prevalence of *Lactobacillus*, a typical vaginal bacterium, was driven primarily by vaginal delivery contamination and secondarily by background DNA contamination. After removal of background DNA contaminants by DECONTAM, *Lactobacillus* ASVs were ranked among the top five relatively abundant ASVs in one of five studies for which data analysis could be restricted to placentas from term cesarean deliveries. A sub-analysis of six studies targeting the 16S rRNA gene V4 hypervariable region demonstrated that bacterial DNA profiles of placental samples clustered primarily by study origin and mode of delivery, and that placental samples and technical controls share principal bacterial ASVs. **Conclusion:** Placentas from normal term pregnancies do not share a consistent DNA signal across studies and therefore contemporary evidence supports sterility in the placenta.

3. Inhibition of the SARS-CoV-2 Papain-Like protease

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) continues to devastate the world. Papain-like protease (PLpro) and 3-chymotrypsin like protease (3CLpro) are two SARS-CoV-2 protease antiviral targets. PLpro is an attractive target because it plays a vital prerequisite role in the cleavage and maturation of coronaviral polyproteins, disturbance of host responses, and assembly of the replicase-transcriptase complex. Here we report several effective inhibitors of PLpro through biochemical studies. This collection of compounds inhibit the peptidase activity of PLpro *In vitro*. Further studies such as structural studies, viral replication studies, and *In vivo* studies will accelerate structure-based drug design efforts targeting PLpro for the identification of high-affinity inhibitors of high clinical value.

4. The Associations of *Megasphaera* and Virulent *Gardnerella* Species with Clinical Outcomes to Oral Metronidazole Therapy

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Bacterial vaginosis (BV) is a state of vaginal dysbiosis in which an array of BV-associated species displaces dominant species of *Lactobacillus*. It elevates risk for complications such as HIV and pre-term birth. There is no consensus on causes of BV. Standard of care treatment is oral metronidazole; however, therapy has high rates of recurrence. Our previous study of recurrent BV patients (3+ episodes/year) found a strong association of *Gardnerella_Gsp07, G.swidsinskii,* and *G.leopoldii* with refractory and recurrent responses, and a more robust recovery of *Lactobacillus* species among remission patients. My follow-up study analyzed a subset of the initial patient samples by next generation sequencing of the V4 domain of the 16S ribosomal RNA. My goal was to identify key species present in pre-treatment microbiota, that could predict post-treatment refractory or remission responses, and prognostic species at post-treatment, that predict later recurrence versus long-term remission. I found that *Gardnerella_Gsp07*, *G.swidsinksii,* and *G.leopoldii* were elevated in pretreatment refractory patients, that *Megasphaera_1* was elevated in remission patients, and that combinations of small numbers of species had positive and negative predictive values above 80% for refractory and recurrent responses at post-treatment. We propose a model that offers testable hypotheses about the causes of these responses. We also found indirect evidence that refractory responses may be associated with inactivation or sequestration of metronidazole. If validated in a larger study, these results could direct new therapeutic strategies and deepen our understanding of the causes of recurrent BV.

5. Understanding the role of epigenetics in the regenerating zebrafish retina

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According to the World Health Organization, greater than 2.2 billion people suffer from vision impairment worldwide. However, humans and other mammals possess limited regenerative ability following retinal injury and disease. In contrast, zebrafish, a vertebrate sharing the same retinal architecture with humans, maintain a profound capability for retinal regeneration. Following complete ablation of photoreceptors utilizing high intensity light, zebrafish Müller glia (MG) re-enter the cell cycle to produce a pool of progenitor cells that will regenerate new photoreceptors. We recently identified a window of time between 5-10 days post injury (dpi) that we believe to be critical to the differentiation of new photoreceptors. We hypothesized that DNA methyltransferase (DNMT) enzymes could play a role during this process based on their established role in neuronal differentiation during retinal development. Specifically, we hypothesized that loss of DNMT function during 5-10 dpi would lead to inhibited differentiation and unchecked proliferation of stem cells. Therefore, we first profiled the gene expression of all 6 *dnmt* paralogues and found that *dnmt3a* was the only paralogue shown to increase in expression from 5-10 dpi. Next, we designed splice-blocking morpholinos to disrupt DNMT3a function during this 5-10 dpi window. Preliminary results from immunohistochemical analysis of cone precursors in the DNMT3a-inhibited retinas suggested a delay in regeneration. Continued studies are aimed at determining the epigenetic consequences of this inhibition. Ultimately, the long-term goal of understanding the role of epigenetics in regeneration is to aid us in potentially “unlocking” evolutionarily silenced regeneration pathways in the human retina.

6. Higher risk of lead exposure predicts higher anxiety symptoms In Detroit youth

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Michigan ranks third in the nation in lead contamination in children, and youth living in urban areas, such as Detroit, are at higher risk for lead exposure due to aging and/or deteriorating homes. Lead is a known neurotoxic metal with well-documented adverse effects on cognitive and behavioral development in youth. However, less is known about the impacts on *mental health*. This study examined the effects of lead exposure risk on anxiety in youth living in Detroit vs. surrounding suburban areas. Methods and Results: Sixty-five adolescents (37 female, 10-17 years) completed an online survey about mental health. We split the sample into two groups: youth living in the city of Detroit (n=19) and those living outside the city but <50 miles of Detroit (n=46). Youth self-reported on their anxiety symptoms using the Screen for Child Anxiety Related Emotional Disorders. Lead exposure risk was determined using data from Data Driven Detroit to estimate the percent of children who tested positive for elevated blood lead levels in each participant's zip code (2017-2019). Linear regression was used to test the impact of lead exposure risk on anxiety across the entire sample and in Detroit vs. non-Detroit subgroups, separately, controlling for gender. Overall, lead exposure risk did not significantly predict anxiety across the entire sample. Lead exposure risk was, however, a significant positive predictor of anxiety symptoms in Detroit (but not non-Detroit) youth. **Conclusion**: Our results revealed that youth living in Detroit may be at higher risk of mental health consequences of lead exposure.

7. Use of fear-potentiated startle in the development of a rodent model of PTSD

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Individuals exposed to psychological trauma can develop significant personal and professional impairments, and have greater likelihood to develop posttraumatic stress disorder (PTSD), depression, anxiety, and substance use disorders (SUD). For this reason, it is important that we investigate neurobehavioral differences that underlie one's risk versus resilience for developing PTSD and its most commonly co-morbid disorders.We have developed a translational rodent model, combining Pavlovian fear learning with an innate reflexive response in mammals, the acoustic startle response. These methods are easily translated across species because of the highly conserved neural pathways involved. An investigative paradigm called fear potentiated startle (FPS) makes use of this pathway to study and empirically model fear-related symptoms of PTSD.FPS is defined as the increase in the innate startle response to sudden noise (e.g., 40ms, 100dB white noise burst) when the reflex is elicited during states of heightened fear. One such state is during and following the repeated pairing of a neutral stimulus (CS; e.g., visual cue like a light) with an aversive unconditioned stimulus (US, e.g., electric shock). We have reliably shown that after acquisition of the conditioned fear, rats have a significantly greater startle responses in the presence of the CS vs. baseline startle to the noise burst alone. Extinction of this conditioned fear through repeated unpaired CS presentations involves a new learning process and serves as the basis for clinical exposure therapies. Our plan is to use these FPS paradigms in tandem with established laboratory models of SUD and other psychiatric comorbidities.

9. Quantifying the effect of the His98Tyr substitution in Autosomal-Dominant Myoglobinopathy on the dynamics of Oxymyoglobin formation using hybrid Monte Carlo- Molecular Dynamics

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Background: Adult-onset autosomal dominant myopathy was recently discovered in fourteen members of six European families and is the first identified genetic disease of myoglobin (Mb). His 94 is a coordinating residue of Fe moeity of heme, and the His94Tyr mutation been extensively characterized in vivo, unlike His98Tyr. Grand canonical molecular dynamics is an advanced simulation technique which inserts or deletes molecules in the simulation according to a Monte Carlo Metropolis Criterion and then the molecules are sampled by molecular dynamics. We hypothesis there is a qualitative and quantitative difference in the paths of oxygen from solvent to heme in mutant and wild-type Mb. **Methods and Results:** Human myoglobin (pdb: 3rgk) was solvated in 20:80 molecular Oxygen:Water. Each simulation was equilibrated in the NVT ensemble in NAMD at 310 K. Dual control volumes were defined in the bulk solvent and on the heme group, and Oxygen insertion/deletion was limited to these two regions while water was inserted anywhere. Py-MCMD was run for 1000 cycles of alternating between 500 Monte Carlo and Molecular Dynamics steps. Time Oxygen spent within Mb from entry to arrival at the heme group differed in mutant and wild type. Additionally, the paths taken varied between mutant and wild type. Certain regions of the Mb molecule which were inaccessible to oxygen in wild type were accesible in mutant Mb. The average number of waters surrounding the heme group differed in mutant and WT. **Conclusion:** His98Tyr Mb has qualitative and quantitative differences in Oxygen dynamics from WT Mb

10. Does consumption of Oreo™ cookies produce psychomotor activating and locomotor sensitizing effects in female and male rats.

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Food addiction is a debated topic in the scientific community. More research is needed, particularly in preclinical models, to determine if consumption of high-fat, high-sugar foods produces similar sensitizing and rewarding behavioral effects as drugs of abuse. Likewise, if the same underlying neurobiological mechanisms as drugs of abuse are being used should be determined. Most preclinical studies have assessed the motivation to seek palatable foods with a focus on cross-sensitization between food and drugs of abuse. However, it is conceivable that behavioral sensitization can occur with exposure to palatable foods, such as high-fat, high-sugar foods. Therefore, we constructed a novel behavioral paradigm in rats to test this *de facto* hypothesis. First, female and male Sprague-Dawley rats were given access to unsalted rice cakes for habituation. Then, rats were given access to either unsalted rice cakes as control or Oreo™ cookies to mimic a high-fat, high-sugar snack once per day (30-min) for 10 days, followed by a 21-day abstinence period with exposure to standard rat chow only. Finally, a challenge day with either rice cakes or Oreos was done. Results show that rats consumed more Oreos™ on challenge day if they had previously received Oreos™ compared to rice cakes or first exposure to Oreos™, indicating sensitization in consumption behavior. Analysis of locomotor activity and sensitization in these rats and potential sex effects is ongoing. We anticipate providing early preclinical evidence showing psychomotor sensitization to a high-fat, high-sugar diet. Finally, we will pursue the neurobiology of this addiction-related behavior to these foods.

11. The epigenetic regulator JMJD2 is required for the maintenance and function of epidermal Langerhans Cells

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Background: Langerhans Cells (LCs) are an extremely unique population of immune cells located within the epidermis. For decades, LCs were thought to be the quintessential dendritic cell (DC), however this is not entirely true. Fairly recent fate-mapping studies have shown that LCs are more ontogenically related to tissue resident macrophages (TRMs) than they are to DCs. Presently, epigenetic regulation of immune cells remains a hot topic of interest in medical research. Considerable amounts of effort have been devoted to investigating the role of epigenetic regulation within classical DCs and TRMs, however there is currently not a single publication available that has investigated the role of epigenetic regulation within LCs. The epigenetic regulator JMJD2 is a family of proteins responsible for demethylating lysine residues located on Histone H3. The JMJD2 proteins are expressed within a multitude of different cell types, including LCs. Methods and Results: By breeding a CD11c-Cre mouse with a JMJD2 floxed mouse we were able to generate a conditional KO mouse in which the JMJD2abc proteins were deleted from LCs during their post-natal differentiation phase. As expected, the deletion of JMJD2abc from CD11c+ cells resulted in the significant decrease of LC frequency in adult mice, as well as P14 mice, when compared to their WT counterparts. LC function was also impacted in JMJD2abc cKO mice, as there was a significant decrease in LC antigen uptake compared to WT mice. Conclusion: Our data highly suggests that JMJD2abc is required for the maintenance and function of epidermal Langerhans Cells.

12. Gestational Benzene Exposure Predisposes Offspring to Metabolic Syndrome through Alterations in Hypothalamic Development

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The hypothalamus is essential in the regulation of metabolism, notably during critical windows of neurodevelopment. An abnormal hormonal and inflammatory milieu during development can trigger persistent changes in the function of hypothalamic neurocircuits, which leads to long lasting effects on the body's energy homeostasis and metabolism (Sadagurski et al, 2015). Benzene, a volatile organic compound, is a known carcinogen capable of crossing the blood-brain barrier. We recently demonstrated that gestational exposure to low concentrations of benzene, induces a severe metabolic imbalance in offspring (Koshko et al, 2021). However, the mechanisms behind these outcomes remain unclear. We hypothesize that gestational exposure to low-dose benzene induces hypothalamic stress, contributing to adverse metabolic effects later in life. In this study, we exposed pregnant C57BL/6JB dams to benzene at 50 ppm or filtered air for 5 days/week (6h/day from gestational day 1 to birth), and analyzed the neural outcomes of young offspring. We assessed neuroinflammation and found increases in the microglia-specific Iba1 marker, in the ARC of the hypothalamus in benzene-exposed male and female P21 offspring. Quantification of the fiber density in the anterior PVH revealed significant decreases in AgRP and α-MSH projections in benzene-exposed offspring indicating impairment in hypothalamic development. Our results imply that severe metabolic syndrome in adult offspring gestationally exposed to benzene (Koshko et al, 2021), might be a consequence of abnormal hypothalamic development and early-life hypothalamic inflammation. Our new data provide the evidence and mechanistic basis that gestational VOC exposure is a potential risk factor for late-life metabolic disorders.

13. Utilizing PET imaging to quantify epigenetic changes following chronic fentanyl administration

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Innovative neuroimaging technologies have rapidly developed throughout the years and are used as a modality to investigate neurochemical and genetic changes. As part of our efforts to elucidate the impact of chronic fentanyl administration on epigenetic mechanisms, a group of female rats were subjected to either fentanyl (20µg/kg subcutaneous) or saline treatment. All animals were assessed for changes in class IIa histone deacetylase (HDAC) activity via positron emission tomography (PET) imaging with [^{18}F]TFAHA, a class IIa HDAC radioligand at two-time points (one week before and after forced withdrawal of two weeks of injections). Imaging results show an increase in [^{18}F]TFAHA distribution volume in the nucleus accumbens and hippocampus in control animals post-injection. However, a decrease in [^{18}F]TFAHA distribution volume was observed in these structures post-chronic fentanyl injection suggesting potential epigenetic modulation. Additional studies with a larger sample of males and females are ongoing.

14. Role of the I148M Gene Variant in Fatty Liver and Cardiovascular Disease

Andrew Butcko

Wayne State University School of Medicine Physiology Department

Non-alcoholic fatty liver disease (NAFLD) is the most common form of liver disease worldwide affecting an estimated 1 in 4 people globally. Interestingly, the majority of individuals living with NAFLD die from cardiovascular related complications, as oppose to liver failure. Scientist have identified a common genetic polymorphism in the PNPLA3 gene, known as the I148M variant, as the greatest genetic risk factor for the development and progression of NAFLD. More recently, a genome wide association study has found a significant association between the I148M gene variant and reduced risk of developing coronary artery disease, implying that the variant may play a cardioprotective role in addition to predisposing individuals for NAFLD. Currently the mechanisms responsible for the phenotype of the I148M gene variant are not well understood but likely involve altered hepatic triglyceride lipolysis. Thus, the goal of the current study is to elucidate altered molecular mechanisms of the I148M gene variant which may provide a cardioprotective effect. Male CD57B6/J mice of approximately 4-months of age were randomly assigned to one of two dietary groups: standard rodent chow (CON) or a high fat NAFLD-inducing diet (NAFLD) for 16-weeks while being housed at thermoneutral temperatures. All mice were given ad libitum access to food and water. Body weights were recorded weekly. Following 16-weeks of dietary treatment and an overnight fast, blood and major organs were harvested from the animals immediately following euthanasia. Preliminary results indicate that mice carrying the I148M gene variant had increased hepatic steatosis.

ORAL SESSION 2

1. Neuroprotective effects of Canagliflozin: lessons from aged genetically diverse UM-HET3 mice

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Background: The aging brain is characterized by a progressive increase in neuroinflammation and central insulin resistance all of which contribute to neurodegenerative diseases and cognitive impairment. Recently, the Intervention Testing Program (ITP) demonstrated that the anti-diabetes drug, Canagliflozin (Cana), a sodium-glucose transporter 2 (SGLT2) inhibitor, led to lower fasting glucose and improved glucose tolerance in both sexes, but extended median lifespan by 14% only in male mice. **Methods and results**: Here we show that Cana treatment significantly improved central insulin sensitivity in the hypothalamus and the hippocampus in aged 30-month-old animals of both sexes. Additionally, the expression of pro-inflammatory cytokines secreted from glia cells was strongly reduced in Cana-treated mice with a stronger response in aged male mice. Histologically, Cana significantly reduced age-associated gliosis in the hypothalamus in aged male mice. Cana-associated decrease in microgliosis was partially dependent on mTOR signaling, resulting in reduced phosphorylation of S6 in microglia in Cana-treated aged male but not female mice. Importantly, Cana treatment improved exploratory, and locomotor activity in 30-month-old male but not female mice. **Conclusion**: Taken together, our findings demonstrate sex-specific neuroprotective effects of Cana and suggest Cana as a potential treatment for neurodegenerative diseases.

2. Mechanism-based Drug Development to Treat Advanced Prostate Cancer

Claire Soave[1], Charles Ducker[2], Nathan Nicely[3], Yanfang Huang[1], Luke Pardy[1], Peter Shaw[2] and Manohar Ratnam[1*]

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As prostate cancer (PC) is generally dependent on the androgen receptor (AR) for growth, androgen deprivation therapy (ADT) is used to treat advanced PC. However, the tumors frequently progress by restoring AR signaling through various mechanisms, leading to resistance. Additionally, ADT has undesirable effects on androgen signaling in differentiated tissues.

We have previously established ELK1 as a AR tethering protein in chromatin, essential for activation of a critical set of androgen/AR target growth genes in PC. The N-terminal A/B domain of AR binds to ELK1 by co-opting the two ERK docking sites on ELK1. The platform antagonist (KCI807) binds to AR (K_d = 7x10^{-8} M), blocking its association with ELK1 and inhibiting PC tumor growth by selectively inhibiting a subset of AR target genes supporting cell growth.

We have identified two ELK1 recognition sites in AR and also the binding site of KCI807. In our working model, KCI807 binds in a cleft adjacent to the downstream ELK1-binding site, displacing an alpha helix which in turn disrupts ELK1 binding. We have developed a new drug-like compound (KCI838) that recapitulates the mechanism of action and target selectivity of KCI807 but is a more fast-acting and potent inhibitor of AR-dependent growth in PCa model cells, including ADT/enzalutamide-resistant cells. KCI838 also has characteristics that greatly reduce the vulnerability to major liver enzymes seen in KCI807. Based on its drug-like characteristics, targeted effects, enhanced potency and metabolic viability, KCI838 warrants investigation as a drug to treat PC that is resistant to current AR-targeted therapies.

3. Adiponectin resistance decreases coronary microvascular angiogenesis: Role of aldehyde dehydrogenase 2

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Adiponectin, a cardioprotective adipocytokine. 4-hydroxy-2-nonenal (4HNE), a reactive aldehyde, is elevated in diabetes that decreases coronary angiogenesis. Aldehyde dehydrogenase 2 (ALDH2), a mitochondrial enzyme, detoxifies 4HNE. Thus, we hypothesize that ALDH2 restores 4HNE-induced downregulation of adiponectin signaling in coronary endothelial cells (CECs) and subsequent coronary angiogenesis in diabetes. To test our hypothesis, we performed three different assays of angiogenesis with 4HNE and/or adiponectin treatments. Adiponectin significantly increased angiogenesis in all three assays. Treatment with disulfiram, an ALDH2 inhibitor, exacerbated 4HNE-mediated decrease in adiponectin-induced increased angiogenesis, whereas pretreatment with alda1, an ALDH2 activator, rescued the effect of 4HNE. Additionally, 4HNE treatment significantly decreased the levels of adiponectin receptor (AdipoR)1, AdipoR2, APPL1, AKT, phospho-AKT, and phospho-AMPK in MCECs. DSF pretreatment exacerbated 4HNE-mediated decrease in AdipoR1, APPL1, phospho-AKT, and AMPK levels, whereas alda1 pretreatment rescued the 4HNE mediated effect. To recapitulate our *in vitro* study in *in vivo* we used control, diabetic (db/db), intrinsic low ALDH2 activity (AL), and diabetic with intrinsic low ALDH2 activity (AF) mice. AF mice significantly increased serum adiponectin levels, whereas decreased cardiac tissue levels of AdipoR1, APPL1, P-AMPK, and P-AKT compared with all the other groups. To confirm AMPK as a downstream target of adiponectin signaling in coronary angiogenesis, we inhibited AMPK with compound C. Compound C completely abrogated adiponectin-induced increased coronary angiogenesis. In summary, 4HNE-induces adiponectin resistance in diabetic mouse heart through downregulating adiponectin signaling in CECs, whereas ALDH2 restores the 4HNE-induced effect. Therefore, ALDH2 can be a therapeutic target to improve coronary microvasculature in diabetic heart.

4. The role of HOCl enhancement and H4B deficiency on NO bioavailability as a component of oocyte quality and aging

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Inducible nitric oxide synthase (iNOS) and myeloperoxidase (MPO) are heme-containing enzymes that have attracted attention for their roles in numerous inflammatory disorders. Chronological aging, which enhances oocyte deterioration, is associated with increased inflammation, and observed incidence of high MPO-HOCl and deficient tetrahydrobiopterin (H4B). In addition, the incidence of NOS monomerization, protein nitration, and oxidative stress is presumably high, impacting oocyte quality. Moreover, we have previously shown NO is important in maintenance of oocyte quality, as it protects the oocyte from aging. We hypothesize that, in aged animals, 1) NO bioavailability will be decreased through HOCl mediated iNOS heme destruction and subunit dissociation accompanied by the release of H4B, and 2) aging-associated deficiency of H4B can be related to NO deficiency due to decreased iNOS dimerization. Using absorbance spectroscopy and gel filtration chromatography, we found that dimer iNOS dissociation between 15 and 100 µM HOCl was accompanied by loss of heme content and NO synthesis activity. There was partial unfolding of the subunits at 300 µM HOCl and above accompanied by loss of reductase activities. Then, using oocytes (n=50) from B6D2F1 mice-young breeders (YB, 8-14 weeks [w]), retired breeders (RB, 48-52w) and old animals (OA, 80-84w), we measured concentrations of H4B and its metabolites through HPLC analysis showing a significant decrease in OA (48%) and RB (73%) compared to YB (100%). Furthermore, based on our previous work, the presence of melatonin plays a crucial role as an MPO inhibitor and HOCl scavenger to protect the oocyte.

5. PDGFRA extrachromosomal DNA (ecDNA) amplification addiction and escape in Glioblastoma

Artem Berezovsky[1,2], Indrani Datta[3], Ruicong She[3], Andrea Transou[2], Susan Irtenkauf[2], Laura Hasselbach[2], Laila Poisson[3], Ana C. deCarvalho[1,2]

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Platelet-derived growth factor receptor alpha (PDGFRA) is amplified in 14% of glioblastoma (GBM), oftentimes presenting as extrachromosomal DNA (ecDNA). Our objective is to identify the molecular contexts in which PDGFRA is a driver of gliomagenesis. We have isolated subpopulations from a patient-derived GBM model (HF3253), harboring two alterations in PDGFRA: internal deletion leading to constitutive activation and ecDNA amplification driving high copy number and heterogeneity. HF3253 symptom-free survival correlated with the initial frequency of PDGFRA ecDNA(+) population implanted; prolonged symptom-free survival was due to selection for initially low-frequency PDGFRA ecDNA(+) clones, based on histology and TaqMan Copy Number. Exploiting intra-tumoral heterogeneity, we have isolated single cell clones that exhibit diploid PDGFRA copy number corresponding to very low levels of PDGFRA mRNA and undetectable PDGFRα protein. Symptom-free survival was substantially increased in 4 ecDNA(-) clones compared to parental ecDNA(+) (Log rank test $p < 0.0001$; $n \geq 4$/group). In contrast to parental HF3253, ecDNA(-) tumors demonstrated diffuse tumor morphology and did not exhibit de novo PDGFRA copy number gains post-implant. We conducted RNA-sequencing on 19 HF3253 ecDNA+/- populations (FDR=0.05). The ecDNA(+) population enriched for known functions of PDGFRA in oligodendrocyte precursor cells. The ecDNA(-) population demonstrated broad metabolic changes coinciding with up-regulation of numerous kinases and Myc. Our data validates PDGFRα as a therapeutic target in glioma, demonstrates that detection of ecDNA-amplified PDGFRA has the potential to be a predictive biomarker of future PDGFRα-targeted therapies, and indicates that targeting compensatory growth factors may prevent the development of adaptive resistance to loss of PDGFRA ecDNA.

JUDGES

Biochemistry, Molecular Biology, Immunology (BMI)

Raghavendar Thipparthi

Cancer Biology

Gen Sheng Wu
Malathy Shekhar
Heather Gibson

Center for Molecular Medicine & Genetics (CMMG)

Lawrence L. Grossman
Maik Huettemann

Ophthalmology, Visual and Anatomical Sciences (OVAS)

Linda Hazlett
Ryan Insolera
Shunbin Xu

Pathology

Rodrigo Fernandez-Valdivia

To our judges this year,

Sincerest of thanks to you for all that you do for Wayne State as a whole, but especially thank you for donating your time and effort to our GSRPD. We appreciate your flexibility and patience while we all adapted to the changes in moving the event to a virtual format. The range of expertise on the panel this year is second to none, and we have all benefitted from your knowledge. The GSRPD would not exist without you, and we cannot thank you enough for helping to make it a reality.

Sincerely,

GSRPD 2021 Committee

THANK YOU TO OUR WONDERFUL JUDGES!

NOTES