18TH ANNUAL GDBBS DSAC STUDENT RESEARCH SYMPOSIUM

Wednesday, February 24th-
Friday, February 26th, 2021

DIVISION STUDENT ADVISORY COUNCIL

GRADUATE DIVISION OF BIOLOGICAL AND BIOMEDICAL SCIENCES

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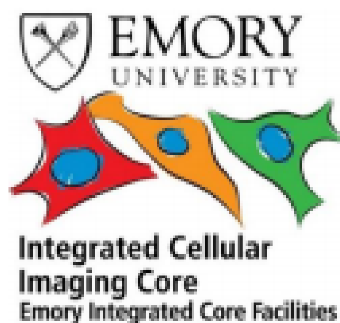

Graduate Division of Biological and Biomedical Sciences

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The 18th Annual GDBBS DSAC Student Research Symposium

Wednesday, February 24th –
Friday, February 26th, 2021

Wednesday, February 24th

10:00-11:15AM

Session 1: Genetics and Genomics

10:00AM -- Brenda Antezana (MMG)
Efficient, non-conjugative dissemination of an integrative and conjugative element, Tn*2009*, that confers multi-drug resistance in *Streptococcus pneumoniae*

10:15AM -- Meghan Wynne (NS)
Mitochondria regulate expression of the Alzheimer's risk gene APOE

10:30AM -- Darian Williams (MSP)
Stable flow-induced Kallikrein-Related Peptidase 10 inhibits endothelial inflammation and atherosclerosis

10:45AM -- Yijian Fan (CB)
Developing distant metastasis models of LKB1-mutant lung cancer

11:00AM -- Brent Allman (PBEE)
Estimating viral fitness from longitudinal *in vivo* studies for pathogens undergoing cellular coinfection

11:15AM-1:00PM - Break

1:00-2:30PM - **Flash Talks**

2:30-3:00PM - Break

3:00-4:00PM

Session 2: Therapeutic Strategies

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3:30PM -- Gianna Branella (CB)
Novel non-genotoxic ligand-based CAR T conditioning in the context of hematopoietic stem cell transplantation and treatment of myeloid-leukemia

3:45PM -- Timothy Hoang (IMP)
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Thursday, February 25th

10:00-11:15AM

Session 3: Epigenetics and Nucleic Acid Modifications

10:00AM -- Dylan Holder (GMB)
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10:15AM -- Anna Kania (GMB)
H3K27me3 demethylases restrain B cell differentiation

10:30AM -- Liz Dreggors (BCDB)
Regulation of rRNA modification and translation by snoRNP assembly factor Hit1 in a yeast model of PEHO syndrome

10:45AM -- Zane Laughlin (BCDB)
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11:00AM -- Izabela Suster (BCDB)
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11:15AM-1:00PM - Break

***1:00-2:30PM* - Flash Talks**

2:30-3:00PM - Break

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Session 4: Aging, Human Disease and Development

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3:15PM -- Nourine Kamili (IMP)
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3:30PM -- Erica Modeste (MSP)
A proteomic network approach for elucidating racial differences in Alzheimer's disease

3:45PM -- Courtney Christian (BCDB)
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4:00PM -- Jennifer Truong (BCDB)
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Friday, February 26th
10:00-11:15AM

Session 5: Receptors and Signaling

10:00AM -- Trisha Lala (NS)
Detection of phosphatidylserine externalization by the synaptic receptor BAI1

10:15AM -- Chad Camp (MSP)
Loss of *Grin2a* Promotes Parvalbumin Interneuron Dysfunction and Synaptic Mistargeting in the Developing Hippocampus

10:30AM -- Carolina Montanez (MSP)
Human RGS14 and NHERF1 regulate PTH1R-G protein signaling events linked to phosphate uptake in kidney

10:45AM -- Stephanie Foster (NS)
Behavioral and molecular assessment of the role of noradrenergic galanin during opioid withdrawal

11:00AM -- Emily Legan (BCDB)
Conformational dynamics and mechanoregulation govern the hemostatic response of von Willebrand factor

11:15AM-1:00PM - Break

***1:00-2:30PM* - Flash Talks**

Session 1:

Genetics and Genomics

Wednesday,
February 24th
10:00AM

Brenda Antezana, MMG	**10:00AM**

Efficient, non-conjugative dissemination of an integrative and conjugative element, Tn*2009*, that confers multi-drug resistance in *Streptococcus pneumoniae*

Brenda S. Antezana[1,2], Yih-Ling Tzeng[2], Sarah Lohsen[2], Xueqing Wu[3], Jorge E. Vidal[4], David S. Stephens[2]

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[4]Department of Microbiology and Immunology, University of Mississippi Medical Center, Jackson, MS, USA

Multi-drug resistance in *Streptococcus pneumoniae* (*Spn*) has been attributed to the dissemination of integrative and conjugative elements (ICEs). Specifically, Tn*916*-like ICE, Tn*2009* (23.5kb), confers tetracycline and macrolide resistance via *tetM* and *mefE*/*mel* expression, respectively. Although prototype Tn*916* (18.0kb) has been shown to disseminate by conjugation in other bacterial species via circular intermediate (CI) formation and integration by site-specific recombination, the mechanism for Tn*916*-like ICE transfer in *Spn* remains undefined. *In silico* analysis detected putative conjugative genes within Tn*2009* that share 99-100% identity with respective Tn*916* conjugative genes. Consistent with previous reports, a sub-lethal concentration of tetracycline induced Tn*916* CI formation, resulting in a ratio of CI to chromosome of 10^{-2} in *Bacillus subtilis*; however, only a minimal Tn*2009* CI formation was induced in *Spn* (ratio of 10^{-8}) Correspondingly, Tn*2009* conjugative gene expression was minimally induced upon tetracycline exposure. Tn*2009* transfer from a donor *Spn* GA16833 to a recipient *Spn* D39 occurred within nasopharyngeal biofilm consortia in a bioreactor at a recombination frequency (rF) of 10^{-4}, which is not affected by a mutation in the essential conjugative gene, *ftsK*, in Tn*2009*. However, DNaseI addition prevented Tn*2009* transfer (rF<10^{-7}) in the biofilm consortia. Deletions of competence genes (*comC*/*D*/*E*) in the recipient D39 resulted in significant reductions in rF, ranging from 10^{-8}-10^{-6}. Whole genome sequencing of four D39 recombinants revealed variably-sized GA16833 DNA fragments integrated into the recombinant genomes, ranging from ~32–67kb that includes intact Tn*2009*. Thus, *Spn* Tn*2009* dissemination is not facilitated by conjugation, but mediated via transformation and homologous recombination.

Meghan Wynne, NS	10:15AM

Mitochondria regulate expression of the Alzheimer's risk gene APOE

Meghan Wynne[1,2], Oluwaseun Ogunbona[3], Avanti Gokhale[1], Erica Werner[1], Stephanie Zlatic[1], and Victor Faundez[1]

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[2]Graduate Program in Neuroscience, Emory University, Atlanta, GA
[3]Department of Pathology, Emory University, Atlanta, GA

Apolipoprotein E (APOE), the primary carrier of lipids and cholesterol in the brain, plays a crucial role in the pathogenesis of Alzheimer's disease (AD). Indeed, the APOE4 allele is the strongest genetic risk factor for AD while the APOE2 allele confers protection against AD. AD has also been associated with mitochondrial dysfunction that is worsened in the presence of the APOE4 allele. Thus, the prevailing model of AD pathogenesis places mitochondria downstream of APOE-dependent processes. Here, we argue that mitochondria also act upstream of APOE by upregulating APOE gene expression. Using RT-qPCR and plate-based immunoassays, we show that mutations in genes encoding proteins residing in the inner mitochondrial membrane, including the SLC25A mitochondrial transporter family and components of the electron transport chain, cause elevations in APOE transcript and protein levels. Importantly, this APOE upregulation extends to cells lacking NDUFS3, an electron transport chain gene recently identified as an AD risk locus. We also show through immunoprecipitation that members of the SLC25A transporter family and electron transport chain proteins, including NDUFS3, physically interact. Together, our data provide support for a novel model where mitochondria regulate APOE expression through mechanisms that depend on the SLC25A transporter family. Thus, we propose that the SLC25A family of mitochondrial transporters can influence APOE-dependent processes in AD pathogenesis.

Darian Williams, MSP **10:30AM**

Stable Flow-Induced Kallikrein-Related Peptidase 10 Inhibits Endothelial Inflammation and Atherosclerosis

Darian Williams[1*], Marwa Mahmoud[2*], Aitor Andueza[2], Sandeep Kumar[2], Dong-Won Kang[2], and Hanjoong Jo[1,2]

[1] Department of Molecular Systems and Pharmacology, Emory University, Atlanta, GA
[2] Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA
*These authors contributed equally.

Atherosclerosis is a chronic inflammatory disease of the arterial blood vessels that underlies the occurrence of heart attack, peripheral artery disease, and ischemic stroke; the leading causes of death worldwide. It is well-known that atherosclerosis preferentially occurs in branched and curved regions of the vasculature exposed to disturbed blood flow, while straight regions of the vasculature exposed to stable blood flow are protected from developing atherosclerosis. Dysfunction of the endothelial cells in lesion-prone areas is an important contributor to the development of atherosclerosis, however, the underlying mechanisms by which blood flow regulates endothelial dysfunction and atherosclerosis are still unclear. We have sought to understand the molecular mechanisms of flow-dependent atherosclerosis in order to develop novel therapeutics. Interestingly, our group previously identified over a thousand endothelial genes that change in response to blood flow that may act as novel therapeutics, termed flow-sensitive genes.
Here we look to characterize one of the most flow-sensitive genes in endothelial cells, Kallikrein-Related Peptidase 10 (*KLK10*), and its effects on atherosclerosis. KLK10 is a secreted serine protease that has high expression in endothelial cells under stable flow, while being dramatically reduced under disturbed flow conditions both *in vivo* and *in vitro.* Additionally, treatment of human aortic endothelial cells with recombinant KLK10 is able to inhibit endothelial cell inflammation and permeability in response to disturbed flow. We hypothesize that KLK10 inhibits EC inflammation, permeability, and subsequent atherosclerosis through regulation of the NFκB inflammatory signaling pathway.

Yijian Fan, CB	**10:45AM**

Developing an inducible-caspase 9 cancer cell line to study brain metastasis

Yijian Fan[1], Xiuju Liu[1], Rui Jin[1], Lei Zhu[2], Lily Yang[2], Wei Zhou[1]

[1]Department of Hematology and Medical Oncology, School of Medicine; Winship Cancer Institute, Emory University, Atlanta, GA

[2]Departments of Surgery, Emory University School of Medicine, Atlanta, GA

The brain is one of the most common destinations of lung cancer metastasis, but there are few experimental models to study brain metastases of lung cancer. The cell line WRJ388, which was derived from a cervical lymph node metastasis in our $KRAS^{LSL-G12D/+}$/$LKB1^{fl/fl}$/$Luc^{LSL-Rosa26}$ genetically engineered mouse model, was able to form distant metastases in clinical-relevant organs including brains, livers, and kidneys according to BLI imaging after injected subcutaneously, intravenously, or intracardially. However, the life duration of mice injected with WRJ388 cells is 2~4 weeks, which presented a challenge for brain metastases to reach the size for histology confirmation. To prolong the life of the mice while allowing the growth of brain metastasis of WRJ388, we developed an inducible caspase-9 system in WRJ388 cells by expressing an FKBP12-caspase-9 fusion protein. AP-20187, a bioinert homodimerizer with a molecular weight of 1482.75, can activate caspase-9 to induce cell death, but cannot cross the blood-brain barrier (BBB). 99% of WRJ388-iC9 cells were eliminated by AP-20187 after 4 days at the dose of 0.4nM *in vitro*. After tail-vein injection of the WRJ388-iC9 cells into the nude mice, administration of 1mg/kg AP-20187 was able to reduce the whole-body BLI signal and signals in individual organs if the compound was given on the same day of the tumor injection but failed to control the tumor burdens if given three days after. Future studies will explore the possibility to reduce the tumor burden while sparing brain metastases by coupling with other agents to enhance the delivery of AP-20187.

Brent Allman, PBEE	**11:00AM**

Estimating viral fitness from longitudinal *in vivo* studies for pathogens undergoing cellular coinfection

Brent Allman*[1], **Julie Zhu***[1], Katia Koelle[1]

[1]Department of Biology, Emory University, Atlanta, GA
*These authors contributed equally to the work

Deep-sequencing of longitudinal within-host samples allow for characterization of viral genetic diversity over the course of a host's infection. Recent empirical studies using model animals such as ferrets have used sequencing of longitudinal viral samples to look for the emergence and spread of mutations that may be more well-adapted to the host. These studies have identified mutants that can not only replicate more efficiently within hosts but that can also transmit more efficiently between hosts. Thus within-host evolutionary dynamics have clear consequences at the between-host level. While methods have been developed to quantify the fitness effects of mutations using observed changes in allele frequencies over an infection, these methods do not account for the possibility of cellular coinfection, which may impact fitness inferences by 'diluting' the fitness advantage of a mutant when it occurs in the cellular context of wild-type virions. Here, we extend previous statistical work to quantify the fitness effects of mutations by generalizing the work to allow for cellular coinfection dynamics. After testing deterministic and stochastic iterations of our model on simulated data, we then apply our approaches to empirical longitudinally-sampled H5N1 sequence data from ferrets. Our results indicate that previously inferred fitness values may underestimate the fitness effects of beneficial mutations. These findings underscore the importance of integrating the effect of cellular coinfection when quantitatively assessing the fitness effects of mutations in viral infections that are characterized by high cellular multiplicities of infection.

Flash Talks

Wednesday,
February 24th
1:00-2:30PM

Natalie Alvarez, MMG	**1:00PM**

Structural and molecular nature of SAMHD1 and protein phosphatase 2A

Natalie Alvarez[1,2], Si'Ana Coggins[3], and Baek Kim[2,4]

[1]Microbiology and Molecular Genetics Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA
[2]Department of Pediatrics, School of Medicine, Emory University Atlanta, GA
[3]Uniformed Services University of Health Sciences, Bethesda, Maryland
[4]Center for Drug Discovery, Children's Healthcare of Atlanta, Atlanta, GA

SAMHD1 can suppress HIV-1 replication kinetics in non-dividing cells by lowering dNTP levels. Protein phosphatase 2A (PP2A) activates SAMHD1 through dephosphorylation at the T592 site. The PP2A-B55α phosphatase holoenzyme has three different subunits: the scaffolding subunit A, the B55α regulatory subunit, and the C catalytic subunit. To study the structural nature of both wildtype and phosphomimetic SAMHD1, the proteins were expressed in *E. coli* and purified using a GST column. For the PP2A-A subunit, *E. coli* was also used to express the protein and was purified using a GST column. The baculovirus system was used for the B55α and C subunit and was purified using a nickel column and gel filtration. The cryo-EM structure of the SAMHD1 and PP2A complex has not yet been determined and is one of the goals of this project. To investigate the molecular nature of SAMHD1 and PP2A I will use the purified proteins to perform a binding assay using nickel affinity. Since SAMHD1 should remain enzymatically active for dNTP depletion and HIV-1 restriction in non-dividing macrophages, I hypothesize that PP2A binds to SAMHD1 and keeps SAMHD1 unphosphorylated in order to restrict HIV-1 replication in macrophages. Recently, our lab has observed the SAMHD1 interaction of B55α subunit of PP2A in a SAMHD1 pulldown experiment from non-dividing THP-1 macrophages, supporting our hypothesis.

Matthew Armstrong, GMB

Tet1/Tet2 mediated modulation of Alzheimer's disease

Matthew J Armstrong[1], Peng Jin[1]

[1]Department of Human Genetics, Emory University, Atlanta, GA

Alzheimer's disease (AD) is the 6th leading cause of death in the US. AD is characterized by the progressive decay of neuronal connections and death of neurons, resulting in severe cognitive decline and death. The causes of AD are largely unknown; however, our current understanding indicates genetic, epigenetic, and environmental factors contribute to disease. As epigenetic modifications are influenced by environmental factors and influence gene expression, they serve as a mediator between an individual's genetic composition and phenotypic characteristics. Thus, epigenetic modifications hold promise to explain a significant portion of the missing heritability of AD, and several links between the epigenome, TET2, and AD have already been uncovered. Here we examine how the knockout of Tet1/Tet2 in the 5xFAD model system lead to dysregulation of the transcriptome and contribute to AD pathogenesis. Our cognitive and behavioral data (Morris water maze, tail suspension test, etc.) indicates knockout of TET enzymes alter cognitive/behavioral profiles in WT and FAD mice. Through analyses of the transcriptome, we have identified 17 genes (FDR <0.01) with consistent differential expression in FAD, FAD/Tet1$^{+/-}$, and FAD/Tet$^{+/-}$ relative to WT mice. Among this list are hallmark AD-associated genes, Trem2, Ctsd, and Mpeg1, which are involved in the processing of APP protein and immune response regulation. Furthermore, when compared to WT mice, our study identified 103 differentially expressed genes shared between FAD/Tet1$^{+/-}$ and FAD/Tet2$^{+/-}$ mice, and 131 and 108 genes exclusive to FAD/Tet1$^{+/-}$ and FAD/Tet2$^{+/-}$, respectively (FDR <0.01). Our data suggests TET1/TET2 modulate AD-associated gene expression and augment AD pathology.

Britton Barbee, MSP

Isoform-selective PI3-kinase inhibition confers resilience to cocaine cessation-induced anxiety-like behavior

Britton R. Barbee[1,2,3], Shannon L. Gourley[1,2,3,4]

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[2] Department of Pediatrics, Emory University School of Medicine
[3] Yerkes National Primate Research Center, Emory University
[4] Department of Psychiatry, Emory University School of Medicine

Phosphoinositide 3-kinase (PI3K) is a multi-subunit signaling complex that phosphorylates phosphoinositides, membrane-embedded second messengers that are critical for synaptic and structural plasticity of neurons. Cocaine potentiates PI3K-Akt-mTOR cascade activity. It also triggers anxiety-like behavior in humans and rodent models, and anxiety can be a causal factor in relapse. Broad-spectrum PI3K inhibition mitigates anxiety-like behavior following alcohol cessation, however, it is unknown if PI3K silencing affects anxiety-like behavior following cocaine cessation, and if so, whether specific subunits could be targeted to ameliorate anxiety-like behavior. To address this concern, we used viral-mediated gene silencing to selectively reduce expression of the p110β PI3K isoform in the dorsomedial prefrontal cortex (dmPFC), a brain region involved in mood regulation. Isoform-selective PI3K inhibition mitigated cocaine cessation-elicited anxiety-like behavior. Interestingly, a history of repeated cocaine exposure occluded this resilience, allowing us to then compare immediate-early gene expression between cocaine-vulnerable and cocaine-resilient mice. Resilient mice displayed lower immediate-early gene expression in the claustrum and lateral hypothalamus. Future studies will investigate whether neuronal activity in these regions causally contributes to anxiety-like behavior. Our findings suggest that isoform-selective PI3K inhibition mitigates cocaine cessation-elicited anxiety-like behavior, likely via coordinated brain regions and circuits.

Kelsey Bennion, CB

Therapeutically targeting FcγRIIB on highly activated effector $CD8^+$ T cells to potentiate immune checkpoint blockade in metastatic melanoma

Kelsey B. Bennion[1], Marvi Tariq[2], Mandy L. Ford[1]

[1]Department of Surgery, Emory University, Atlanta, GA
[2]Department of Surgery, University of Alabama-Birmingham, Birmingham, AL

As the success of immune checkpoint blockade (ICB) in the clinic increases, variability in patient response underscores the need to identify factors leading to differential therapeutic success. The effector $CD8^+$ T cell response has been characterized as a critical factor to the success of patient response, namely in PD-1 therapy.
We recently showed that the inhibitory receptor, FcγRIIB, functions in a cell-intrinsic manner to temper the highly activated $CD8^+$ T cell response to antigen *in vivo*. As ICB utilizes Fc-containing antibodies, we hypothesize that these antibodies could bind FcγRIIB on $CD8^+$ T cells and elicit counterproductive negative signaling. We asked if FcγRIIB blockade in combination with PD-1 blockade could better augment the antitumor response compared to PD-1 blockade alone. In a B16- hgp100 mouse melanoma model, we observed a significant increase of splenic CD44hi antigen-specific $CD8^+$ T cells in mice given dual blockade compared to PD-1 blockade alone.
To understand the implications of FcγRIIB on $CD8^+$ T cells in humans, healthy human PBMCs were incubated with nivolumab, an αPD-1 antibody. We saw a characteristic proliferative burst in $FcγRIIB^-$ $CD8^+$ T cells, but not in $FcγRIIB^+$ $CD8^+$ T cells, suggesting that $FcγRIIB^+$ $CD8^+$ T cells are nonresponsive to αPD-1 antibody *in vitro*. In stage IV melanoma patients receiving nivolumab, we observed a decrease of $FcγRIIB^+$ $CD8^+$ T cells after the first round of nivolumab, suggesting that nivolumab may contribute to the apoptosis of these cells. Targeting FcγRIIB signaling may potentiate highly activated $CD8^+$ T cells to improve patient response to ICB.

Christine Bowen, BCDB

Kv1.3 regulation of mouse microglial cell (MMC) inflammatory response

Bowen, Christine[1]; Bitarafan, Sara[2]; Wood, Levi[2]; Seyfried, Nicolas[1]; Rangaraju, Srikant[1]

[1]Center for Neurodegenerative Disease, Emory University, Atlanta, GA
[2]Department of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA

Microglia, the enactors of immune responses in the brain, can adopt a pro-inflammatory state in neurodegenerative diseases which is characterized by increased of proinflammatory cytokine production, a bioenergetic shift toward glycolysis, and detrimental responses. Our lab has identified Kv1.3 potassium channel as a marker and regulator of pro-inflammatory responses in Alzheimer disease (AD). We aimed to optimize a cellular model to investigate Kv1.3 channel-dependent pro-inflammatory immune signaling and metabolic responses using the immortalized Mouse Microglia Cell (MMC) line. A flow cytometry assay of functional cell-surface Kv1.3 channels confirmed that lipopolysaccharide (LPS) and interferon gamma (IFNγ) induced dose-dependent increases in Kv1.3 channel expression without increased gene expression indicating post-transcriptional regulation. We used Luminex to measure phospho-proteins in the mitogen-activated-protein-kinase (MAPK) and Akt/mTOR pathways in MMC lysates which showed that Kv1.3 channels regulate early activation of p53, which lies downstream of the p38 MAPK pathway, without effects on Akt/mTOR signaling. We also optimized the Seahorse mitochondrial stress test for MMC cells which will be utilized to define Kv1.3-dependence of microglial bioenergetic shifts following immune activation. The future directs of this project will involve including Amyloid-β oligomers and fibrils to better emulate AD-relevant immune activation *in vitro* using both primary microglia cultures and MMCs. We are also validating a novel microglia-specific Kv1.3 conditional deletion mouse model to determine how regulation of Kv1.3 *in vivo* regulates the disease conditions of AD.

Kathryn Crotty, MSP

Alcohol-induced hyaluronic acid dysregulation impairs alveolar macrophage mitochondrial bioenergetics and phagocytosis

Kathryn Crotty[1], Samantha M. Yeligar[1,2]

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In the lungs, there is a greater risk of respiratory infections and acute respiratory distress syndrome following alcohol misuse. Alveolar macrophages (AM) are the first line of defense against pathogens in the lower respiratory tract. Alcohol-induced oxidative stress and mitochondrial redox imbalance in AM impairs their ability to phagocytose pathogens. Oxidative stress alters the molecular dynamics of the extracellular matrix polysaccharide, hyaluronic acid (HA), implicated in pulmonary inflammation and immunity. We hypothesized that alcohol-induced alveolar macrophage immune dysfunction occurs through HA dysregulation and impaired mitochondrial bioenergetics. *In vitro* experiments were performed using a mouse AM cell line treated ± 0.08% ethanol (EtOH) for 3 days. Phagocytic index of MH-S cells was assessed by internalization and clearance of *S. aureus* fluorescently labeled bacteria. Mitochondrial bioenergetics were measured using an extracellular flux bioanalyzer. RNA was isolated to determine HA Synthases 1-3, Hyaluronidases 1 and 2, and HA binding protein mRNA levels by qRT-PCR. RNA targets that were altered by EtOH-treatment were evaluated by western blot. AM demonstrated diminished phagocytic ability and mitochondrial bioenergetics when exposed to EtOH. Further, AM showed increased HAS2, HAS3, and Hyal2 expression following EtOH treatment. Our studies suggest that EtOH impaired phagocytic ability, mitochondrial function, and HA metabolism in AM. Since EtOH has been shown to induce oxidative stress in AM and oxidative stress alters HA dynamics, these findings support a critical role for HA in modulating AM immune function. Therapeutic strategies to target HA regulation may mitigate the risk of pulmonary infections in individuals with alcohol misuse.

Julia de Amorim, BCDB

Identification of RNA exosome cofactors in neuronal cells to probe disease mechanism

***Julia L. de Amorim*[1,2]**, Sara W. Leung[1], Ambro van Hoof[3], Anita H. Corbett[1,2]

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[3]Department of Microbiology and Molecular Genetics, University of Texas Health Science Center-Houston, Houston, Texas.

The RNA exosome is a 10-subunit complex that mediates both RNA processing and degradation. This complex is ubiquitously expressed, essential, and critical for fundamental cellular functions, such as ribosomal RNA processing. Recent studies have linked mutations in genes encoding multiple subunits of the complex to tissue-specific human disease. For example, missense mutations in the human *EXOSC3* gene, which encodes an RNA exosome subunit, cause Pontocerebellar Hypoplasia type 1b (PCH1b), a disease characterized by atrophy of the pons and cerebellum. The mutations encode single amino acid changes in conserved regions of the EXOSC3 protein. A decrease in the interaction of the RNA exosome complex with cofactors could explain the distinct disease phenotypes. However, most RNA exosome cofactor studies have been carried out in budding yeast and thus, do not provide insight into whether tissue-specific cofactors could exist. Biochemical experiments that employ cultured N2A cells were used to identify RNA exosome-interacting proteins in both the nucleus and the cytoplasm. Preliminary results from this analysis reveal an association between the RNA exosome and other decay-related proteins. We are extending these studies to explore the possibility of tissue-specific cofactors by immunoprecipitating EXOSC3 and analyzing co-purifying proteins from the mouse cerebellum (affected in disease) and cortex (unaffected). We are also exploring whether amino acid changes that are linked to disease alter cofactor interactions. These studies will provide insight into both the functional consequences of amino acid substitutions in the RNA exosome that cause disease and the role of cofactors in conferring RNA target specificity.

Brian Dobosh, IMP

Nanoflow cytometry identifies an imbalance of epithelium- and neutrophil-derived extracellular vesicles in the airway environment of pediatric cystic fibrosis patients

Brian Dobosh, Vincent Giacalone, Milton Brown, Lucas Silva, Lokesh Guglani, Rabindra Tirouvanziam

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[2]Center for CF and Airways Disease, Children's Healthcare of Atlanta, Atlanta, GA, USA

Progressive lung disease is the leading cause of mortality in cystic fibrosis (CF), a chronic condition characterized by recruitment of neutrophils (PMNs) into the airways. Newly arrived PMNs are exposed to extracellular vesicles (EVs) from the epithelium and other PMNs. These EVs are necessary and sufficient to induce pathological changes including reduced bacterial killing and immunosuppressive activities toward macrophages and T-cells. However, children with CF do not always show a high PMN presence in their airways, which suggests that the balance between PMN recruitment and the activity of other cells is still in flux in early disease.

We utilized spectral nanoflow cytometry to profile the EV content of the bronchoalveolar lavage fluid (BALF) from CF children. EVs were stained with Di-8-ANEPPs, and anti-EpCAM,-CD66b and -CD115 (epithelial, PMN, macrophage origins, respectively). Violet side scatter and/or fluorescence threshold triggering were used for EV detection.

The ratio of neutrophil- to epithelial-derived EVs in CF BALF correlated with the percentage of PMNs that are present in the airways ($p = 0.003$, Spearman's $\rho = 0.689$). This ratio also correlated with the PRAGMA disease score, which quantifies airway damage by chest computed tomography ($p = 0.001$, $\rho = 0.857$).

Using a method to quantify EVs from specific cell types in vivo, we demonstrated the ratio of PMN- and epithelial cell-derived EVs tracks with airway damage and neutrophil influx, suggesting an interplay between these cells in early CF disease. This EV-focused method can be applied to other diseases in which sampling cells is difficult.

Kristen Easley, BCDB

Alcohol decreases the barrier function of airway epithelial cells through the TGFß1 pathway

Kristen Fowler Easley[1], Ashish J. Mehta[1], Bashar S. Staitieh[1], and Michael Koval[1]

[1]Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322

Alcohol use disorder causes 88,000 deaths each year in the United States. Chronic alcohol use significantly increases people's risk of developing lung infections and acute respiratory distress syndrome (ARDS). This increased sensitivity to injury is a condition known as alcoholic lung syndrome. Little is known about how alcohol impacts epithelial cells in the conducting airway, i.e. the trachea and bronchi, which are the first lines of defense against infectious pathogens in the lung. We hypothesize that alcohol exposure causes a decrease in barrier function of airway epithelial cells through a TGFß1-dependent mechanism. We cultured primary airway epithelial cells on Transwells and differentiated them in vitro at air liquid interface (ALI), exposed them to 60mM or 100mM ethanol for 2 weeks and measured barrier function by transepithelial electrical resistance (TER). We found that ethanol caused a decrease in barrier function compared to control cells. However, cells treated with ethanol and a TGFß1 inhibitor had barrier function similar to control cells. Additionally, we cultured primary airway epithelial cells isolated from healthy and alcoholic patients to determine if in vivo exposure caused long-term changes to tight junction protein levels. Our results show that alcohol-exposed cells have lower JAM-A protein expression compared to healthy cells. Rat airway cells exposed to ethanol in vitro also show a decrease in JAM-A at both the RNA and protein level. Together, these results indicate that chronic alcohol exposure detrimentally alters the conducting airway epithelial cell barrier potentially through the loss of JAM-A.

Cody Elkins, IMP

High-fat-diet-induced obesity reduces the adipose-tissue-associated regulatory T cell compartment by altering cholesterol homeostasis in male mice

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Fat-associated regulatory T cells (Tregs) play an important role in suppressing adipose inflammation and maintaining metabolic homeostasis. It has been shown that chronic high-fat-diet (HFD)-induced obesity leads to a significant depletion of Tregs within the epididymal visceral-adipose tissue (VAT) of male mice. This reduction is associated with an increase in pro-inflammatory immune cell populations, increased levels of inflammatory cytokines within the VAT, and a reduction in systemic insulin sensitivity. However, the mechanisms underlying the reduction in VAT Tregs under chronic HFD conditions is unclear. Thus, we utilized a T cell receptor (TCR) transgenic (tg) mouse model (vTreg53-TCR-tg), in which Tregs preferentially accumulate in VAT, to assess how HFD affects the Treg compartment. Mice were fed on HFD or normal chow diet (NCD) for 8 or 16 weeks, and VAT Tregs were isolated by fluorescence-activated cell sorting for bulk RNAseq analysis. Gene set enrichment analysis revealed a significant reduction in Treg transcripts associated with cholesterol homeostasis and metabolism within the HFD-fed group compared to NCD-fed controls. Additionally, CRISPR-mediated ablation of *Srebf2*, a master regulator of cholesterol biosynthesis and uptake, significantly reduced enrichment of TCR-tg Tregs in the VAT, but not in the spleen of NCD-fed recipient mice following adoptive transfer. These results suggest that VAT Tregs critically depend on cholesterol metabolism, and perturbation of this pathway could be a major driver of obesity-induced dysregulation of VAT Tregs and metabolic homeostasis.

Ramona Haji-Seyed-Javadi, GMB

Identifying HELZ as a novel DNA double-strand break repair protein

Ramona Haji-Seyed-Javadi[1], Allyson E. Koyen[1], Matthew Z. Madden, David S. Yu[1]

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Lung cancer is one of the leading causes of death with an estimated 228,150 new cases diagnosed each year. A major cause of unsuccessful cancer therapy is resistance driven by cancer cells' repair mechanisms. A rationale-driven therapeutic approach is to exploit specific DNA repair pathways to improve the efficacy of DNA-damaging chemotherapeutic agents such as etoposide. We have identified Helicase with Zinc finger (HELZ) as a novel mediator of etoposide resistance in small cell lung cancer (SCLC). HELZ is a putative RNA helicase with no prior link to DNA repair. Our preliminary data indicate that HELZ depletion in SCLC cells causes hypersensitivity to etoposide and ionizing radiation (IR). HELZ localizes to distinct DNA repair foci and its depletion impairs homologous recombination (HR). HELZ depletion also causes spontaneous induction of R-loops, implying that HELZ may promote resolution of R-loops in mediating DNA double-strand break repair. Moreover, mass spectrometry analysis of purified HELZ in cells identified PRP19 and DDX1 as interacting partners and this was confirmed by co-immunoprecipitation analyses. Together, these data suggest that HELZ may function directly in mediating genome integrity by promoting HR at DNA double-strand sites. Uncovering the mechanisms by which HELZ participates in DNA repair pathway will provide new insights into how HELZ promotes active genome maintenance, and how HELZ may be exploited as a novel therapeutic target for chemotherapy resistant SCLC.

Nicholas Harbin, MSP

Regulator of G-protein signaling 14 (RGS14) alters hippocampal pathology following excitotoxic injury due to status epilepticus

***Nicholas H. Harbin*[1]**, Daniel J. Lustberg[2], David Weinshenker[2], John R. Hepler[1]

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Regulator of G-protein signaling 14 (RGS14) is highly expressed in pyramidal cells of area CA2 of the hippocampus and interacts with multiple signaling pathways to suppress calcium influx, neuronal excitability, and synaptic plasticity. In contrast to neurons of neighboring CA1 and CA3, CA2 pyramidal neurons are highly resistant to excitotoxic injury (e.g. seizure activity), although the mechanisms governing this resilience are unknown. Because RGS14 blocks calcium influx and downstream signaling in neuronal spines, we hypothesize that RGS14 may regulate hippocampal response to excitotoxic injury caused by prolonged seizure activity (i.e. status epilepticus). Testing this hypothesis, we evaluated kainic acid (KA)-induced behavioral responses and hippocampal pathology in wild-type (WT) and RGS14 knockout (RGS14 KO) mice. Although we observed no difference in behavioral response to KA between genotypes, we found a significant upregulation of RGS14 five and seven days after KA treatment in area CA1 of WT mice, as measured by immunohistochemistry. Using microtubule associated protein 2 (MAP2) as a proxy for neuronal integrity, we observed a significant loss of MAP2 expression in area CA1 of KA-treated RGS14 KO mice but not WT mice at the same time points. Evaluating microgliosis and astrogliosis using ionized calcium binding adaptor molecule (IBA1) and glial fibrillary acidic protein (GFAP) expression as markers, respectively, we found the abundance of both proteins were upregulated following KA-induced seizures in WT mice but were unchanged in RGS14 KO mice. Together, these results suggest that RGS14 plays an essential role in regulating pathological response to seizure injury in the hippocampus.

Sherod Haynes, NS

The extended amygdala gates the affective consequences of chronic psychological stress

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There has been a sharp increase in the prevalence of Major Depressive Disorder (MDD), in part, as a result of compounding psychological effects of the Global Coronavirus pandemic. Recent advances in psychiatric research have identified key brain structures implicated in the display and subsequent treatment of depressed mood. Yet, very little is known regarding how MDD initially develops, and particularly how stress impacts MDD development in some people and not others. Using Chronic Social Defeat Stress (CSDS), an ethologically-relevant social stressor that utilizes male-male territorial aggression, we observed the eventual development of depressive-like behavior in mice over a period of 10 days. We identified a nucleus in the extended amygdala, the Bed Nucleus of the Stria Terminalis (BNST) as playing a critical role in determining which mice went on to develop depression-like behavior. As an initial response to social stress, an increase in firing rates were observed selectively in a small subset of Corticotropin-Releasing Factor (CRF) neurons in the anteriolateral BNST. However, we observed that over time, only the mice that sustained increases in firing rates went on to develop resiliency, whereas the majority of mice (~70%) that did not, went on to develop depressive-like behavior. Using cell-type specific chemogenetics, we mimicked the neuroadaptation using activating Gq-DREADDs and CNO-drinking water construct to sustain firing rates over the course of days. Additionally, In vivo Fiber photometry and optogenetics further demonstrated the critical role of CRF BNST neurons in affecting the emotional consequences of chronic stress. Altogether, the findings provide a compelling cellular mechanism for why some persons succumb to depression compared to others despite shared experience of chronic psychological stress.

Elizabeth Heaton, NS

Striatal melanocortin-4 receptor controls behavioral flexibility in mice

Elizabeth C. Heaton[1-3], Gracy Trinoskey-Rice[1-3], Shannon L. Gourley[1-3]

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Goal-directed action refers to behaviors that are dynamic, sensitive to unexpected events, and require the dorsomedial striatum (DMS). Meanwhile, habitual behaviors, which are reflexive and unchanging, rely on the dorsolateral striatum (DLS). Molecular factors underlying an organism's ability to flexibly shift between goal-directed and habitual behavior are incompletely understood. We recently discovered that striatal melanocortin-4 receptor (MC4R*)* level correlates with this behavioral flexibility in adult male and female mice. To identify functional consequences, we used viral-mediated gene silencing to reduce *Mc4r*. *Mc4r* knockdown in the DMS enhanced the ability of mice to select actions based on reward likelihood and value, while reduction in the DLS facilitated habit formation. Thus, inhibiting MC4R appears to enhance the putative functions of distinct striatal subregions in decision making. Changes in *Mc4r* expression is thought to modulate activity of medium spiny neurons, leading to the hypothesis that chemogenetic manipulation of *Mc4r*+ DMS neurons would impact expression of behavioral flexibility. Stimulation of *Mc4r*+ DMS neurons via Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) facilitated animals' ability to select actions based on reward likelihood. Meanwhile, inhibition via Gi-DREADDs rendered animals insensitive to changes in reward likelihood, promoting habits. These results reveal that striatal MC4R may be a key factor in sustaining *versus* "breaking" habits, and thus could serve as a target for treating maladaptive habits that contribute to neuropsychiatric disease.

Emily Hill, GMB

Modeling reactive gliosis using human cortical organoids

***Emily Hill*[1], ** Steven Sloan[1]

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Astrocytes are the most abundant glial cells in the human brain, and provide many critical functions, such as secretion of neurogenic and synaptogenic factors, blood brain barrier support, and response to brain injury and neurodegenerative disease, known as reactive gliosis or astrogliosis. Astrogliosis is characterized by secretion of either trophic factors or inflammatory cytokines by astrocytes that can be protective or detrimental to neurons, resulting even in neuronal death and a worsening of disease phenotypes. Although astrocyte reactivity is a critical driver of neurological pathophysiology, little is known about how alterations in genomic organization during astrogliosis underly changes in gene expression and lead to a shift in cell state. To answer this, I will induce reactivity in cortical brain organoids derived from human induced pluripotent stem cells. Cortical organoids are a 3D *in vitro* model that reflect many architectural and cellular features of human brain development. I treated cortical organoids with inflammatory cytokines secreted by activated microglia to induce astrogliosis. I confirmed that reactive astrocyte genes have higher steady state levels in organoids treated with inflammatory cytokines, as well as upregulation of several chromatin modifier genes of interest. Additionally, I performed RNA-sequencing on these organoids to profile gene expression changes. Next, I will use ATAC-sequencing to investigate changes in chromatin accessibility between reactive and non-reactive astrocytes. Profiling reactivity at the genomic organization level will help determine major transcriptional and epigenetic regulators of the reactive cell state and provide insight into how reactivity may be controlled to promote better clinical outcomes.

Kenyaita Hodge, GMB

CellDMC identifies CpGs significantly associated with post-menstrual, post-natal, and gestational age among infants born preterm in epithelial and immune cells

Kenyaita M. Hodge[1], Stefan H. Graw[1], Amber A. Burt[1], and Todd M. Everson[1]

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Approximately 1 in 10 babies are born prematurely each year in the United States. These preterm infants have varying degrees of neonatal development and prematurity at birth, which can give rise to a range of complications. We are particularly interested in the neonatal period as this is one of the most dynamic periods in human development, and changes in the epigenetic landscape during this critical period of development can impact health throughout the life-course. As such, we are investigating age associated changes in DNA methylation and cell type specific effects.
In the Neonatal Neurobehavior and Outcomes in Very Preterm Infants (NOVI) study, buccal cell tissue was collected from 542 very preterm infants (born < 30 weeks gestational age) at NICU discharge, and DNA methylation levels were profiled with the Infinium MethylationEPIC BeadChip. We used CellDMC to identify differentially methylated CpGs associated with post-menstrual age (PMA), post-natal age (PNA), and gestational age (GA) as well as the specific cell type(s) driving the differential methylation. Adjusting for sex, study site, and batch, our preliminary findings show ~24,000 CpGs associated with PMA and PNA, respectively, and <10,000 CpGs associated with GA driven by epithelial cells. There are <4,000 CpGs associated with PMA, PNA, and GA driven by immune cells.
It is vital to identify CpGs and their associated genes that undergo dynamic changes in regulation during early development, in addition to determining which cell types are contributing to differential methylation to gain insight into early life aging.

Lauren Hodkinson, GMB

Determinants of context-specific transcription factor function

***Lauren J. Hodkinson*[1]**, Juila Gross[2], Erica N. Larschan[3], and Leila E. Rieder[1]

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Despite a cell's crowded nuclear environment, transcription factors locate and bind *cis* elements across the entire genome while performing context-specific functions. To explore this phenomenon, we broadly aim to understand how transcription factors read both *cis* sequence and genomic context to function uniquely at different binding sites, which is critical for development and disease. One example of a context-specific transcription factor in the genetic model *Drosophila melanogaster* is the Chromatin-Linked Adapter for MSL Proteins (CLAMP) which targets GA-rich *cis* elements but performs several distinct functions throughout the genome. CLAMP primes the male X-chromosome for dosage compensation, regulates promoters genome-wide, and promotes formation of the conserved histone locus body (HLB), which regulates expression of the replication-dependent histone genes. Although CLAMP targets similar *cis* elements in all three contexts, it recruits very different locus-specific transcription factors. Here we investigate how the function of CLAMP at the histone locus is impacted by the origin of its *cis* binding elements. We engineered flies to carry a transgenic histone locus in which we replaced the natural *cis* elements with CLAMP-recruiting GA-rich elements from different genomic origins. We assessed how these *cis* elements impacted HLB formation by staining third instar larval polytene chromosomes with antibodies specific to HLB proteins as well as proteins found in other CLAMP binding contexts. In the future, we will assess CLAMP binding using ChIP-qPCR and histone gene transcription using qRT-PCR to further evaluate locus function. Together, these data will determine how much genomic origin contributes to histone locus specific CLAMP function.

Session 2:

Therapeutic Strategies

Wednesday, February 24th 3:00PM

James Ackley, CB	**3:00PM**

Stromal-derived protection against FASL induced cell death in multiple myeloma

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Multiple myeloma is the second most common hematological malignancy and, despite advancements in therapy, remains incurable. BCMA-targeted Chimeric Antigen Receptor T-cell (CAR-T) therapy is becoming a powerful tool in the treatment of multiple myeloma with patient response rates over 80%. These responses are short lived however with median progression free survival being 15 months in the best of cases. Understanding how target cells evade apoptosis after exposure to CAR-T therapy and targeting vulnerabilities within these mechanisms with rational drug combinations will enhance the depth of patient responses and prolong patient remission. We have previously demonstrated that stromal-derived IL6 confers resistance to the BCL-2 family antagonists Venetoclax and ABT-737 by altering the binding of BCL-2 family proteins. This effect was reversible through IL6 and IL6R antagonism. CAR-T cells utilize this same apoptotic machinery to induce FASL/CD95 dependent cell death. Using the HS-5 stromal cell line which constitutively produce high levels of IL6, and a version of these cells deficient in IL6 production, I have examined the effects of stromal conditioned media and IL6 on the ability of FASL to induce target cell death. Here we show that HS5 conditioned media, IL6 alone, and HS5 conditioned media deficient in IL6 can all protect myeloma cell lines from FASL induced cell death. These effects are cell line specific and can be compounded to enhance protection against FASL induced cell death. IL6 inhibition used in conjunction with CAR-T therapy represents a promising mechanism through which responses to CAR-T therapy can be enhanced.

Mycobacterium tuberculosis modulates DC-T cell cross-talk and Th17 polarization by dampening Notch signaling

Ana B. Enriquez[1], Jonathan K. Sia[1], Hedwin K. Dkhar[1], Shu Ling Goh[1], Melanie Quezada[1], Jyothi Rengarajan[1,2]

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Tuberculosis (TB) is among the leading causes of death worldwide. While IFN-γ and CD4+ Th1 T cell responses are necessary to mount an immune response to Mycobacterium tuberculosis (Mtb), the causative agent of TB, they are not sufficient to provide protection. Studies from several groups, including our own, have highlighted an important role for IL-17 and Th17 responses in immunity to Mtb infection. Previous research from the laboratory has shown that Mtb restricts Th17 responses by dampening dendritic cell (DC) responses and has identified CD40-mediated co-stimulation as critical for generating Th17 responses in response to Mtb. We showed that exogenous CD40 engagement on Mtb-infected DCs enhances Th17 polarization and reduces Mtb burden in the lungs in a vaccination model. However, the DC mechanisms that mediate CD40-dependent Th17 polarization and protection have not been defined. Here we show that Notch signaling in DCs modulates DC-T cell crosstalk and influences T cell polarization during infection. Engaging the CD40 pathway on Mtb-infected DCs increases the mRNA and protein expression of Notch ligands DLL4 and Jagged1 over the course of infection. Blockade of Jagged1 during a DC-T cell co-culture lowers Th1 responses while blockade of both DLL4 and Jagged1 significantly limits Th17 polarization. These results reveal that during infection, Mtb restricts expression of Notch ligands, which impedes Th17 responses during TB. Insights from this study could be applied to designing more efficacious TB vaccines and adjuvants.

Gianna Branella, CB **3:30PM**

Novel non-genotoxic ligand-based CAR T conditioning in the context of hematopoietic stem cell transplantation and treatment of myeloid-leukemia

Gianna M. Branella[1,2]**,** Jasmine Y. Lee[1,2], Andrew Fedanov[1], Christopher B. Doering[1], H. Trent Spencer[1]

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To date, there are no known leukemia-specific cell-surface antigens that distinguish healthy from malignant myeloid-lineage cells, making the translation of cell-based immunotherapies challenging for myeloid malignancies. While there has been great success in the implementation of chimeric antigen receptor (CAR) T therapy for the treatment of B cell malignancies, similar strategies cannot be used for the treatment of other hematological malignancies, such as acute myeloid leukemia (AML), as myeloid depletion cannot be managed similar to B-cell aplasia. As hematopoietic stem cell transplantation (HSCT) is often regarded as the only chance of cure for patients with advanced AML, combining cancer treatment with pre-transplant conditioning can lessen the use of harmful genotoxic therapeutics and enhance therapeutic benefit. Therefore, we have developed a CAR to dually target both malignant and healthy hematopoietic tissue through interaction with CD117 utilizing its cognate ligand, stem cell factor (SCF). Herein, we show successful generation of an anti-CD117 CAR through binding to the CD117 receptor, causing subsequent T cell activation. Additionally, anti-CD117 CAR T cells become specifically activated when co-cultured with CD117-positive AML cell lines using a non-cytotoxic T cell model. Further, we show specific cell lysis of CD117-positive AML cell lines when co-cultured with human primary T cells expressing the anti-CD117 CAR. Through these studies, we aim to advance the use of CAR T therapy for patients with AML and elucidate the broader application of a combination cancer and bone marrow pre-transplant conditioning treatment.

Timothy Hoang, IMP	**3:45PM**

Baricitinib lowers inflammation and pathology in SARS-CoV-2-infected rhesus macaques

Timothy N. Hoang[1,13], Maria Pino[1,13], Arun K. Boddapati[2,13], Elise G. Viox[1], Carly E. Starke[3], Amit A. Upadhyay[2], Sanjeev Gumber[4,5], Michael Nekorchuk[3], Kathleen Busman-Sahay[3], Zachary Strongin[1], Justin L. Harper[1], Gregory K. Tharp[2], Kathryn L. Pellegrini[2], Shannon Kirejczyk[5], Keivan Zandi[6], Sijia Tao[6], Tristan R. Horton[2], Elizabeth N. Beagle[2], Ernestine A. Mahar[1], Michelle YH Lee[1], Joyce Cohen[7], Sherrie M. Jean[7], Jennifer S. Wood[7], Fawn Connor-Stroud[7], Rachelle L. Stammen[7], Olivia M. Delmas[1], Shelly Wang[1], Kimberly A. Cooney[8], Michael N. Sayegh[8], Lanfang Wang[8], Peter D. Filev[9], Daniela Weiskopf[10], Guido Silvestri[1,4], Jesse Waggoner[8], Anne Piantadosi[4,8], Sudhir P. Kasturi[1], Hilmi Al-Shakhshir[11], Susan P. Ribeiro[11,†], Rafick P. Sekaly[11,†], Rebecca D. Levit[8], Jacob D. Estes[3,12], Thomas H. Vanderford[1], Raymond F. Schinazi[6], Steven E. Bosinger[1,2,4], Mirko Paiardini[*,1,4]

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[13]These authors contributed equally;

The emergence of SARS-CoV-2 and COVID-19 pandemic has placed an excessive burden on healthcare systems with over 2,000,000 deaths worldwide. Thus, therapeutic approaches aimed at mitigating disease severity are urgently needed. Immunological features of COVID-19 progression include an influx of innate and adaptive immune cells to the lung, with severe cases of COVID-19 having elevated levels of pro-inflammatory cytokines and chemokines. Baricitinib is an oral, selective inhibitor of JAK1/2 with potent anti-inflammatory activity approved for treatment of patients with active rheumatoid arthritis and predicted to have anti-SARS-CoV-2 effects based on *in silico* modeling.

8 rhesus macaques (RMs) were infected with $1.1x10^6$ PFU SARS-CoV-2; at 2 days post infection (dpi), 4 of the 8 RMs began daily baricitinib treatment (4 mg/day). Baricitinib was found in plasma and lungs of all treated RMs and was safe and well tolerated. Viral replication dynamics measured from nasal/throat swabs, bronchoalveolar lavages and tissues at necropsy was not reduced with baricitinib. Innate Type-I IFN antiviral responses and SARS-CoV-2-specific T-cell responses remained similar between the two groups. Importantly, RMs treated with baricitinib showed reduced inflammation, T-cell immune activation and proliferation, reduced neutrophil NETosis activity, and more limited lung pathology, with decreased type-II pneumocyte and peribronchiolar hyperplasia. Baricitinib treated animals had a rapid and potent suppression of alveolar macrophage production of cytokines and chemokines responsible for a pro-inflammatory environment and recruitment of neutrophil and pro-inflammatory monocytes. Altogether, these data support a beneficial role for the use of baricitinib as a frontline treatment for inflammation induced by SARS-CoV-2 infection.

<u>Session 3:</u>

Epigenetics and Nucleic Acid Modifications

Thursday,
February 25th
10:00AM

Dylan Holder, GMB	**10:00AM**

Investigating the role of H2A.Z in the establishment of facultative heterochromatin using INTACT-CUT&Tag

Dylan Holder[1,2]**,** Roger Deal, PhD.[1,2]

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The epigenome is a vast and dynamic network of chromatin interactions that give rise to the unique cell types of multicellular organisms. Therefore, the dynamics of a specific cell type's epigenome should be considered unique. However, understanding *in vivo* chromatin dynamics has been limited by the resolution of available techniques. Epigenetic profiling via ChIP-seq typically requires a large starting cell population due to its incomplete extraction efficiency. This requirement for a high starting cell count limits most experiments to a heterogeneous population of cells where observations are potentially confounded by unique cells types with distinct epigenetic profiles. This limitation is especially apparent in the study of histone variant H2AZ-mediated transcription in *Arabidopsis thaliana*. Traditional profiling techniques reveal H2AZ as both a transcriptional activator and repressor in different contexts. Therefore, there is no model proposed to sufficiently account for the genome wide relationship between H2AZ and transcriptional regulation. As such, there is a clear need for both increased cell type resolution and temporal resolution to understand the order of events that take place during an H2AZ dependent response. In this talk I introduce Isolation of Nuclei in specific Cell Types followed by Cleavage Under Targets and Tagmentation (INTACT-CUT&Tag) to capture chromatin profiles of H2A.Z and H3K27me3 specifically in root-hair cells during a phosphate starvation response. I show that applying this innovation in chromatin profiling to INTACT recapitulates ChIP-seq chromatin profiles in as few as 50,000 root-hair nuclei. The speed and efficiency of this technique will allow us to study *in vivo* chromatin dynamics at a resolution unattainable by previous methods.

Anna Kania, GMB	**10:15AM**

H3K27me3 demethylases restrain B cell differentiation

Anna K. Kania[1], Madeline J. Price[1], Christopher D. Scharer[1], and Jeremy M. Boss[1]
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The humoral immunity relies on robust differentiation of naïve B cells into antibody secreting plasma cells (PC) in response to foreign, but not self-antigens. To ensure proper immune responses, B cell differentiation is regulated through processes that remodel the epigenome. The histone modification H3 lysine 27 trimethylation (H3K27me3) is associated with a repressive chromatin state and gene silencing. This histone modification is deposited by EZH2, which has been shown to regulate all stages of B cell differentiation leading to PC. However, a role for the two demethylases UTX and JMJD3 that remove H3K27me3 in B cells is still to be elucidated. To determine how these enzymes modulate B cell differentiation, we crossed *Utx*$^{fl/fl}$*Jmjd3*$^{fl/fl}$ mice onto the *Cd19*$^{Cre/+}$ background (dKO mice). Stimulation of *Cd19*$^{Cre/+}$ (CreCtrl) and dKO mice with the T cell independent antigens, LPS or NP-Ficoll, led to a significant increase in PC in dKO mice. This phenotype was attributed to enhanced differentiation of marginal zone (MZ), but not follicular B cells (FoB). UTX- and JMJD3-deficient PC upregulated genes associated with oxidative phosphorylation metabolism and resulted in a significantly higher spare respiratory capacity. Integrative analysis of changes in gene expression and chromatin accessibility in dKO PC revealed a number of candidate transcription factors which are predicted to cooperate with UTX and JMJD3 to restrain B cell differentiation. Taken together, our data place H3K27me3 demethylases as critical epigenetic regulators that restrict the magnitude of PC formation.

Liz Dreggors, BCDB 10:30AM

Regulation of rRNA modification and translation by snoRNP assembly factor Hit1 in a yeast model of PEHO syndrome

Liz Dreggors[1,2], Sohail Khoshnevis[1,3], Homa Ghalei[1,2]

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Regulation of protein synthesis is critical for control of gene expression in all cells. Ribosomes are RNA-protein machines responsible for translating all proteins, and defects in ribosome production, function, or regulation result in serious human diseases. One such disease is progressive encephalopathy, with edema, hypsarrhythmia and optic atrophy (PEHO) syndrome. PEHO syndrome is a devastating neurodevelopmental disorder caused by mutations in the *ZNHIT3* gene, which encodes a nuclear protein required for producing ribosomes. Although little is known about the function of ZNHIT3, studies of its homolog in budding yeast (termed Hit1) reveal that this protein is critical for formation of complexes that chemically modify ribosomal (r)RNAs by depositing 2'-O-methylations at key ribosome locations. In this work, we assessed the molecular consequences of the two PEHO-causing ZNHIT3 mutations for translational control of gene expression using a budding yeast model system. Our data show that PEHO-linked mutations in yeast result in loss of the Hit1 protein, altered rRNA 2'-O-methylation, and global dysregulation of translation which ultimately affects the fidelity of protein synthesis. We are currently investigating the contribution of reduced ribosome number and altered rRNA 2'-O-methylation to translational defects in the yeast PEHO model. In the future, we plan to examine if the paradigm of Hit1-mediated regulation of translation is conserved in human cells and can be targeted for the treatment of PEHO syndrome and other diseases that arise from dysregulation of ZNHIT3.

Zane Laughlin (BCDB)	10:45AM

Mechanism of bacterial ribosome 50S subunit recognition and modification by *Mycobacterium tuberculosis* TlyA

Zane Laughlin[1,2] and Graeme Conn[1,2]

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The 2'-O-methyltransferase TlyA incorporates two modifications in ribosomal (r)RNA at nucleotides C1392 of 16S rRNA and C2144 of 23S rRNA in the small (30S) and large (50S) ribosome subunits, respectively. These modifications are necessary for optimal binding and activity of the tuberactinomycin antibiotic capreomycin. Here, we used cryo-electron microscopy (cryo-EM) structural studies, mutagenesis and complementary methyltransferase assays to determine TlyA's mechanism of 50S recognition and modification. The 3.4Å-resolution structure of *M. tuberculosis* TlyA bound to the 50S defined the complete TlyA structure for the first time and revealed interactions with 23S rRNA Helix69 where C2144 is located. Specific substrate recognition by TlyA appears to be largely directed by the N-terminal domain (NTD) which recognizes the unique structure formed by the base of Helix69 and adjacent rRNA junction. This interaction positions the CTD and bound SAM analog directly over nucleotide C2144. The role of specific TlyA residues in 23S binding was tested via mutagenesis and measurement of methylation activity using two different *in vitro* assays. Collectively, these studies identify an extended 50S binding surface in TlyA that extends across the NTD and CTD, both of which both contain residues critical for substrate binding and modification. Our 50S-TlyA structure also revealed that base-flipping is not required for C2144 modification by TlyA, in contrast to other antibiotic-resistance methyltransferases that act adjacent to TlyA's other target site in the 16S rRNA. However, whether TlyA uses a distinct mechanism, including base-flipping, to modify the 30S subunit remains to be determined in future studies.

Izabela Suster (BCDB)	11:00AM

Transcription of miR-124-2 in hNPCs drives early human neuronal lineage development

Izabela Suster[1], Peng Teng[1], Yangping Li[2], Zhexing Wen[3], Bing Yao[2], Yue Feng[1]

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MicroRNAs (miRs) are a class of small non-coding RNAs essential for brain development and function, among which miR-124 is a key driver for neuronal lineage establishment by suppressing inhibitors of neuron differentiation. Altered miR-124 expression is found in brain diseases, including Alzheimer's disease and major depressive disorder. Human miR-124 can be derived from primary transcript precursors from three paralogous loci, namely pri-miR-124-1, -2, and -3. However, molecular mechanisms that govern the biogenesis of human miR-124 remain largely unknown. In particular, which miR-124 paralog(s) is responsible for initiating human neuronal lineage development is undetermined. We discovered that pri-miR-124-2 is the primary miR-124 precursor in human iPSC-derived neural progenitor cells (hNPCs), whereas pri-miR-124-1 and pri-miR-3 are negligible, suggesting that pri-miR-124-2 is poised to initiate the onset of miR-124 biogenesis and differentiation of hNPCs. We further demonstrated that robust transcription of the *MIR124-2 Host* Gene (*MIR124-2HG*) is the underlying mechanism for predominant pri-miR-124-2 expression in hNPCs, which is achieved by a complex functional interplay between developmentally regulated chromatin accessibility and transcription factors. Moreover, we identified a human-specific proximal promoter element that acts as a scaffold for transcription activator SP1 and repressor MAFK to regulate *MIR124-2HG* transcription during hNPC development. Our results revealed novel mechanisms that underlie the biogenesis of miR-124 in hNPCs to trigger human neuronal development.

Flash Talks

Thursday, February 25th

1:00-2:30PM

Katherine Hutchinson, BCDB

Defining the regulation and function of an inducible, phase-separated nuclear compartment

Katherine M. Hutchinson[1], Jeremy Hunn[1] & Daniel Reines[1]

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Numerous biological processes involve proteins with low complexity domains (LCD) which often self-assemble into subcellular compartments formed by reversible phase separation. Such a process is the RNA polymerase II transcription cycle which involves initiation, elongation, co-transcriptional modification of nascent RNA and termination. Proteins with LCDs, including RNA polymerase II and accessory factors involved in various stages of the transcription cycle, can condense into nuclear foci to perform these functions. *Saccharomyces cerevisia*e nuclear poly-adenylated RNA-binding protein 3 (Nab3), which is part of the Nrd1-Nab3-Sen1 complex that terminates RNA polymerase II transcription, is one such protein that contains a prion-like domain (PrLD) of low complexity, enabling Nab3 to form amyloid *in vitro* and phase separate *in vivo*. These characteristics are representative of PrLD-containing proteins implicated in certain neurodegenerative diseases. To understand the normal and pathological roles of phase-separating proteins, we need a fundamental understanding of the molecular mechanisms that regulate the process. Our work suggests that Nab3's localization to a nuclear granule is regulated at least in part by the phosphorylation state of its binding partner, Nrd1, and functions as a site for modifications of mature RNAs to stabilize them under stress conditions. This establishes the first documentation for a mechanism by which Nab3's dynamic localization to a nuclear granule is regulated. This work provides fundamental knowledge in understanding the molecular mechanisms that regulate Nab3 granule formation and should be applicable to proteins capable of forming subcellular compartments seen across biology.

Alexa Iannitelli, NS

Transcriptional Profiling of the Locus Coeruleus Following Acute Administration of Noradrenergic-specific Neurotoxin DSP-4

Alexa F. Iannitelli[1], Rachel P. Tillage[1], David Weinshenker[1]

[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA

Degeneration of the locus coeruleus (LC), the major noradrenergic (NE) nucleus in the brain, is ubiquitous in both Alzheimer's disease (AD) and Parkinson's disease (PD), and LC dysfunction is thought to contribute to their symptoms. DSP-4 is a neurotoxin which selectively affects the LC-NE system in rodents. Acute administration of DSP-4 causes LC fiber and terminal damage and loss of NE in LC projection regions such as the hippocampus and prefrontal cortex, while repeated administration leads to frank cell body degeneration. These characteristics support DSP-4 as a suitable model for studying neurodegenerative diseases that also affect the LC disproportionately, including AD and PD. The goal of the present study was to identify the dysregulated genes and pathways in LC neurons as a result of acute DSP-4 administration in rodents. Consistent with the literature, we found that mice administered one 50 mg/kg dose of DSP-4 showed a decrease in NE content and turnover in projection regions such as the hippocampus and prefrontal cortex after one week compared to saline-treated controls. We next employed the Translating Ribosome Affinity Purification (TRAP) method to isolate mRNA from LC cell bodies following saline or DSP-4 administration. Results showed differential gene expression in experimental and control groups, with particular pathways related to noradrenergic function being the most severely dysregulated. This work will help us understand the impact that DSP-4 has on LC functioning on a transcriptional level, leading to a more comprehensive knowledge of the molecular mechanisms underlying LC dysfunction and degeneration in human disease.

Anders Johnson, MMG

Cooperation of proteolytic toxins and superantigens during streptococcal toxic shock syndrome

Anders Johnson[1], Christopher LaRock[1]

[1]Department of Microbiology and Immunology, Emory University, Atlanta, GA

Streptococcal toxic shock syndrome (STSS) is a lethal complication of group A *Streptococcus* (GAS) infection. Symptoms result from severe inflammation induced by Streptococcal pyrogenic exotoxins (Spes), superantigens that non-specifically bind T cell receptors and MHC-II, activating >1000 times more T lymphocytes than typical for an infection. This over-activation results in a pro-inflammatory cytokine storm leading to STSS, a fatal condition if untreated. Unlike SpeA and other superantigen Spes, SpeB is a protease important in the pathogenesis of GAS. We have previously shown SpeB is pro-inflammatory and directly activates the cytokine IL-1β, but how SpeB contributes to STSS remains unclear. Using whole human blood and peripheral blood mononuclear cell models, we aim to examine the role of SpeB in the molecular mechanisms responsible for STSS. We show that SpeB contributes to the induction of proinflammatory cytokines such as IFNγ, hallmarks of superantigen-mediated inflammation. Using anti-inflammatory drugs to inhibit specific targets of SpeB, we also show the importance of these targets for superantigen activity. This work proposes that GAS exotoxins cooperate to promote bacterial pathogenesis. By identifying the molecular mechanisms involved in superantigen-induced STSS, we can further study targeted treatments with host-directed immunomodulatory drugs.

Michael Kelberman, NS

Alterations in evoked locus coeruleus activity in a rat model of Alzheimer's disease

Michael Kelberman[1], David Weinshenker[1]

[1]Department of Human Genetics, Emory University, Atlanta, GA

Alzheimer's disease (AD) is the most common neurodegenerative disorder in the world and yet there are no cures or even disease-modifying therapeutics available for patients. The locus coeruleus (LC) is the primary source of the neurotransmitter norepinephrine in the brain, and fires in two distinct patterns: tonic and phasic. Tonic firing consists of 0.5-2Hz pacemaker activity that is associated with arousal, exploration, and behavioral flexibility. Phasic firing is typically marked by 10-20Hz bursts of activity in response to salient environmental stimuli and goal-directed behaviors. In AD, the LC has been identified as one of the earliest regions to develop hyperphosphorylated "pretangle" tau. Hyperphosphorylated tau appears in the LC decades prior to cognitive decline, and parallels the emergence of non-cognitive behavioral impairments such as anxiety, agitation, and sleep disturbances that are consistent with altered LC activity. Although hyperphosphorylated tau has been shown to disrupt firing patterns of excitatory and inhibitory neurons in the forebrain, it is unclear whether it alters LC activity. To address this question, we recorded LC cells from isoflurane anesthetized, 6- and 15- month TgF344-AD rats, which develop endogenous hyperphosphorylated tau in the LC prior to other brain regions, and their wild-type littermates. Tonic activity was recorded for 5-min, and phasic activity was evoked with 0.5ms and 5ms 10mA footshocks applied to the contralateral hindpaw. Preliminary evidence suggests alterations in footshock response with no change in tonic firing patterns. Altered LC firing to salient and/or aversive stimuli may underlie abnormal behaviors evident in prodromal AD.

Tala Khatib, BCDB

Jag1 and IL13ra2 define rare subpopulations within lung adenocarcinoma critical in promoting metastatic disease

Tala Khatib[1], Janna Mouw[1], Adam Marcus[1]

[1]Department of Hematology and Oncology, Emory University, Atlanta, GA

Metastasis accounts for 90% of cancer-related deaths. One primary mode of metastasis is collective invasion, where packs of cells invade into the adjacent stroma while maintaining cell-cell contacts. Cells within the pack are heterogenous, and harbor distinct genetic and phenotypic subpopulations that cooperate to drive collective invasion. Our lab has established a technique to investigate phenotypic heterogeneity in cells, termed Spatiotemporal Cellular Genomic Analysis (**SaGA**). SaGA is an image-guided approach that can isolate and purify live cells based upon *in-situ* phenotypic criteria. We've used this technique to isolate lung adenocarcinoma cells within the collective invasion pack, where cells on the leading edge are 'leaders', and cells within the pack are 'followers'. Transcriptomic analysis of leaders and followers identified Jag1 and IL13ra2 as differentially expressed transmembrane proteins. Jag1 is a ligand in Notch signaling and 45-fold higher in leaders compared to followers. IL13ra2 is a decoy receptor to immunoregulatory cytokine, IL13, and 3000-fold higher in followers compared to leaders. Fluorescence activated cell sorting of Jag1- and IL13ra2-positive subpopulations mimic established leader and follower invasiveness across a panel of lung adenocarcinoma cells. These data suggest that Jag1 and IL13ra2 are biomarkers for invasive and noninvasive subpopulations, providing a molecular basis to digest and immediately sort rare subpopulations from patient lung adenocarcinoma samples. The objective is to present translational evidence of the validity of these biomarkers and determine the molecular mechanisms required to maintain each tumor subpopulation. Establishing the mechanisms that fuel these rare subpopulations is critical to understanding and treating metastatic disease.

Ellen Krall, GMB

Elucidating the Mechanism of Methyl cpg Binding Domain-containing protein 9 (MBD9) Mediated H2A.Z Deposition

Ellen Krall[1,2], Roger Deal[1]

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[2]Program of Genetics and Molecular Biology in the Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA

In the eukaryotic nucleus, histone proteins bind and condense DNA into chromatin. Chromatin influences the accessibility of loci to DNA-binding proteins and is modulated by the cell to coordinate specific gene expression profiles during development or cell stimulus. Protein complexes including histone acetyltransferases (HATs) and chromatin remodelers alter chromatin structure and therefore modulate transcriptional output. One chromatin remodeler, SWI2/SNF2-Related 1 (SWR1), deposits the conserved histone variant H2A.Z, an essential variant for embryonic development in animals and an important variant for plant environmental response. Paradoxically, H2A.Z can both negatively and positively influence transcription, but the mechanism behind this duality remains elusive. Answers may lie in how H2A.Z is deposited initially, but little is known about how SWR1 is targeted to specific loci for H2A.Z deposition. We found that thousands of loci in the model plant organism *Arabidopsis thaliana* are dependent on the protein Methyl-cpg-Binding Domain-containing protein 9 (MBD9) for H2A.Z incorporation. MBD9 complexes with SWR1, but the mechanism of MBD9-mediated H2A.Z deposition remains unclear. I hypothesize that the acetyl binding Bromo domain of MBD9 and local chromatin acetylation are necessary and sufficient for SWR1 targeting and H2A.Z deposition. We found that SWR1 interacts with HAT components, and MBD9-dependent H2A.Z sites are enriched in acetylation. We also identified a conserved residue of the MBD9 Bromo domain that may be essential for its function and assess the suitability of an αMBD9 antibody for future experiments. Ongoing work will further define the effect of the MBD9 Bromo domain and chromatin acetylation on H2A.Z deposition.

Sean Kundinger, BCDB

Phosphorylation in arginine-rich low complexity domains regulates RNA-binding protein oligomerization

Sean R. Kundinger[1], Isaac Bishof[1], Eric B. Dammer[1], Duc M. Duong[1], and Nicholas T. Seyfried[1,2] *

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[2]Department of Neurology, Emory University School of Medicine, Atlanta, GA

Post-translational modifications (PTMs) within arginine (Arg)-rich nuclear RNA-binding proteins, such as phosphorylation, regulate multiple critical steps in RNA metabolism including spliceosome assembly, alternative splicing and mRNA export, among others. However, the identification of PTMs within Arg-rich domains with complete trypsin digestion is extremely challenging due to the high density of Arg residues within these proteins. Here, we report a middle-down proteomic approach coupled with electron transfer dissociation (ETD) mass spectrometry to map previously unknown sites of phosphorylation within the Arg-rich domains of SRSF2 and structurally similar RNA-binding proteins from nuclear extracts of HEK-293 cells. Notably, the Arg-rich domains in RNA-binding proteins are densely modified by phosphorylation compared with the remainder of the proteome, with phosphorylation favoring RSRS motifs. Dephosphorylation of nuclear extracts using calf-intestinal phosphatase (CIP) *in vitro* causes SRSF2 to enrich to the insoluble pellet following sarkosyl fractionation. Reciprocally, phosphorylation of SRSF2 by serine-/arginine protein kinase 2 (SRPK2) *in vitro* drives monomeric SRSF2 species formation, whereas unphosphorylated SRSF2 exists primarily as high molecular weight oligomers by non-denaturing Native PAGE. Inhibition of SRPK2 by SRPIN340 in HEK293 cells regulates an increase in cells harboring SRSF2 cytoplasmic fibrils, imaged by Immunocytochemistry. Furthermore, we show that dephosphorylation may modify interactions between Arg-rich proteins, as SRSF2 has stronger association with fellow Arg-rich proteins U1-70K and LUC7L3 upon dephosphorylation. Collectively, these findings suggest that the degree of phosphorylation within Arg-rich domains may be among the highest in the proteome, and a possible unexplored regulator of RNA-binding protein structure, solubility and protein interactions.

Samantha Lanjewar, GMB

Identifying drivers of human astrocyte development

Samantha Lanjewar[1]**,** Steven Sloan[1]

[1] Department of Human Genetics, Emory University, Atlanta, GA

Astrocytes are the most abundant non-neuronal cells in the central nervous system and play active roles in synapse formation and neural circuit development. Neurons and astrocytes are derived from the same progenitor cells called radial glia, which sequentially produce neurons and then astrocytes. The timing of this transition, termed the "gliogenic switch", is critical for proper brain development. Our goal is to uncover novel extrinsic and intrinsic factors that control human astrocyte development through the use of human cortical organoids. This system recapitulates the timing of the gliogenic switch seen in human fetal development, thus serving as an ideal reductionist model. To identify extracellular factors needed to initiate the gliogenic switch, we are culturing young organoids in conditioned media from mature organoids to test whether proteins secreted from neurons induce astrogenesis. We are also engrafting neurons into organoids to determine whether direct interaction between mature neurons and radial glia initiate precocious astrogenesis. To determine the intracellular changes that drive astrocyte development, we are investigating the possible synergism required between the Notch, BMP, and JAK-STAT pathways, each of which is known to be required individually for astrocyte production. We plan to create stable lines of CRISPRa human induced pluripotent stem cells and activate these pathways combinatorially in young organoids. Ultimately, understanding the extrinsic and intrinsic drivers of the gliogenic switch will provide insight into how perturbations to the timing and production of astrocytes could lead to the development and pathogenesis of neurodevelopmental disorders.

Jasmine Lee, CB

Developing CAR T cell therapeutics for high-risk Neuroblastoma: A strategy to identify cell surface targets and testing of an anti-PTK7 CAR

Jasmine Y. Lee[1,2], Gianna M. Branella[1,2], Andrew Fedanov[1], Victor Maximov[1], Suttipong Suttapitugsakul[3], Kiran K. Parwani[1,2], Christopher B. Doering[1], Ronghu Wu[3], Kelly C. Goldsmith[1,2], H. Trent Spencer[1,2]

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High-risk Neuroblastoma (NB) is the most common extracranial solid tumor in pediatrics with a 5-year survival rate of <50%. Half of these patients will relapse after standard treatment, highlighting a need for new and effective therapeutics. To define immunotherapeutic targets, mice bearing NB patient-derived xenografts (PDX) were treated with chemotherapy and tumor cell surface glycoproteins were selectively separated and analyzed by LC-MS. Protein tyrosine kinase 7 (PTK7) was consistently and highly expressed both before and after chemotherapy. PTK7 is highly expressed on the cell surface of multiple NB cell lines and PDXs with low normal tissue expression. PTK7 is an inactive receptor tyrosine kinase and its expression in primary NB tumors is correlated with poor survival. We developed a lentiviral vector-based, hematopoietic codon-optimized, CD28-based chimeric antigen receptor (CAR) construct specific to PTK7. CAR surface expression and antigen specificity were determined in both Jurkat T cells and primary T cells. Further, PTK7 CAR-expressing T cells became activated in the presence of several NB cell lines that express cell surface PTK7. Similar activation was not observed when CAR T cells were co-cultured with a PTK7 negative cell line or NB cell lines with CRISPR/Cas9 knockout of PTK7, showing specific activation of the PTK7 CAR to antigen-positive cells. These initial data show promise for ongoing studies in primary T cells to assess the therapeutic potential of this CAR in NB and other PTK7 expressing tumors.

Jordan Lewis, PBEE

Host- parasite evolutionary history alters host susceptibility

Jordan Lewis[1,2]***,*** McKenna Penley[4], Hannan Sylla[1], Sebastian Ahumada[2,3], Levi T. Morran[1,2]

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[3] Department of Environmental Sciences, Emory University, Atlanta, GA
[4] ICF International, Atlanta, GA

Host populations often evolve defenses against parasites due to the significant fitness costs imposed by infection. However, adaptation to a specific parasite may alter host defense in general. In particular, the specificity of a host's defense may be influenced by its evolutionary history with parasites. Further, reciprocal change within an interaction may profoundly alter the range of host defense, as antagonistic coevolutionary interactions are predicted to favor defense against specific parasite genotypes. Here, we examined the effect of host evolutionary history on host defense range by assessing the mortality rates of *Caenorhabditis elegans* host populations exposed to an array of *Serratia marcescens* bacterial parasite strains. Importantly, each of the host populations were derived from the same genetic background but have different evolutionary histories with parasites (exposure to heat-killed, fixed genotype, or coevolving parasites). Overall, we observed an effect of host evolutionary history on mortality, however the effects differed between different parasite strains. Importantly, coevolved host populations were more susceptible to novel parasite strains compared to hosts with different evolutionary histories. This data demonstrates that host evolutionary history can have a significant impact on host defense, and that host-parasite coevolution can narrow host defense range.

Pai Liu, NS

Chronic LPS treatment induces AD pathology in C/EBPß transgenic mice

***Pai Liu*[1]**, Zhihao Wang[2], Yiyuan Xia[2], Xia Liu[2], Keqiang Ye[2]

[1]GDBBS Neuroscience program, Emory University
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Alzheimer's disease (AD) is the most common neurodegenerative disease with multifactorial pathologies. C/EBPβ, an age-dependent transcription factor that is mediated by inflammation, regulates the expression of AEP, also named delta-secretase. AEP simultaneously cleaves both APP and Tau and augments Aβ production and Tau hyperphosphorylation and aggregation, contributing to AD pathogenesis. Though numerous hypotheses are proposed, the exact molecular mechanism for AD remains elusive. Here we show that inflammation activated C/EBPβ/delta-secretase signaling pathway is a key mechanism regulating the pathogenesis. LPS chronic administration, which results in low-grade chronic inflammation, elicits AD pathogenesis in Thy1-C/EBPβ transgenic mice. However, C/EBPb +/- and Thy1-C/EBPb/AEP-/- mice exhibit attenuated AD pathology when treated with LPS. Thus, C/EBPβ/AEP pathway plays a pivotal role in AD onset and Thy1-C/EBPβ mice treated with LPS acts as a sporadic AD mouse model.

William McFadden, BCDB

Modular α-Synuclein is used to investigate thermodynamics and kinetics of liquid/liquid and liquid/solid protein phase transitions.

William McFadden[1]***,*** Christella Gordon[1], Youngsun Kim[1], Dr. David Lynn[1]

[1]BCDB Program and the Departments of Biology and Chemistry, Emory University, Atlanta, GA

The 3-dimensional fold of a protein is determined by a complex and dynamic energy landscape driven by inter- and intramolecular interactions. In some instances, proteins can form abhorrent aggregates induced by irreversible interactions that limit any conformational flexibility. Aggregation in protein misfolding disorders follows a two-step nucleation process. The first nucleation event occurs when individual proteins reversibly transition into a liquid-liquid phase separated (LLPS) body through desolvation. Protein aggregation follows the second nucleation event, a liquid-to-solid phase transition within the LLPS body. This process is exemplified by a well-studied protein, α-Synuclein (αSyn), an Intrinsically Disordered Protein (IDP) that forms fibrillar aggregates in patients with Parkinson's Disease (PD). We have identified electrostatic patterns neighboring critical glutamine residues that possibly contribute to initial long-range ordering. To test this model, we designed fragments of αSyn to report on ordering via a reversible covalent linkage. This backbone coupling reaction will allow for a statistical sampling of short- and long-range interactions, defining how specific residues and domains can drive the complex folding landscape, and mapping the energetic codes for the design of IDPs.

Michael Mortillo, GMB

A cross-comparison of cognitive ability across 8 genomic disorders

Michael Mortillo[1], Jennifer G. Mulle[1]

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Genomic disorders are diseases that result from rearrangement of the human genome. Most genomics disorders are caused by copy number variants (CNV), deletions or duplications of several hundred kilobases. Many CNV loci are associated with autism, schizophrenia, and most commonly, intellectual disability (ID). However, there is little comparison of cognitive ability measures across CNV disorders. This study aims to understand whether existing data can be leveraged for a cross-comparison of cognitive ability among multiple CNV. We conducted a systematic literature search to identify publications reporting cognitive ability measures for 8 CNV, including 3q29 deletion, 7q11.23 deletion, 15q11.2 deletion, 16p11.2 deletion and duplication, 17p11.2 deletion, and 22q11.2 deletion. We screened 503 papers; 156 of these met eligibility criteria. For each paper we extracted key data including the instrument(s) used to measure cognitive ability, and cognitive ability scores (full-scale IQ (FSIQ), performance IQ (PIQ), and verbal IQ (VIQ)). Total study subjects evaluated for cognitive ability ranged from 32 (3q29 deletion, 1 paper) to 4,732 (22q11.2 deletion, 73 papers). We found marked variation in assessment instruments used across CNV. There was also a lack of standardization in how the measures of central tendency were reported. We found that 17p11.2 deletion subjects had the lowest mean FSIQ (50.21), PIQ (54.97), and VIQ (55.27), while 16p11.2 deletion and duplication subjects had the highest FSIQ (82.13, 86.21), PIQ (85.24, 81.34), and VIQ (80.75, 85.65). Thus, despite some challenges, limited conclusions using existing published data are supported.

Jordan Owyoung, GMB

Elucidating the role of the transcription factor BCL11A in GABAergic interneuron development and schizophrenia

Jordan Owyoung[1], Andrew Escayg[1]

[1]Department of Human Genetics, Emory University, Atlanta, GA

Schizophrenia (SCZ) is a complex, severe psychiatric disorder that affects ~1% of the global population. Dysfunction of GABAergic interneurons (GINs) is commonly seen in SCZ patients; however, a direct mechanism linking GIN dysfunction to SCZ has yet to be identified. B cell leukemia/lymphoma 11A gene (*BCL11A*) is an important transcription factor that has recently been found to play a role in GIN development and is differentially downregulated in patients with SCZ. Additionally, it is known to be involved in brain development and is associated with several neurological disorders. We hypothesize that *BCL11A* may play a role in GIN dysregulation and potentially in the pathogenesis of SCZ. We will test this hypothesis in two ways. First, a CRISPR/Cas9 system will be used to generate human iPSC cells lacking functional *BCL11A*, and these cells will be subsequently differentiated into GINs. RNA-sequencing will be performed to identify gene networks that are altered, playing particular attention to networks that have been implicated in SCZ. Second, we will evaluate the *in vivo* effect of reduced *BCL11A* expression in different cell types by crossing *Bcl11a* floxed mice with Nes-Cre, Gad2-Cre, and Slc17a6-Cre mice. Since *Bcl11a* is known to be involved in GIN differentiation, we expect to see behavioral deficits similar to those found in SCZ patients in mice lacking *Bcl11a* in all neurons (Nes-Cre) and GINs (Gad2-Cre).

Rebecca Parker, CB

A comparative approach to identify novel vulnerabilities in human KRAS/LKB1-mutant lung adenocarcinoma

Rebecca E. Parker[1], Junghui Koo[1], Bhakti Dwivedi[2], Adam Marcus[1], Melissa Gilbert-Ross[1]

[1]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, GA
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Lung cancer, responsible for more deaths in men and women worldwide than any other malignancy, is a highly heterogeneous disease with many subtypes; this inter- and intratumor heterogeneity makes effective treatment difficult. Lung adenocarcinoma (LUAD), which comprises 40 percent of all lung cancers, is most commonly driven by mutations in KRAS; KRAS-mutant LUAD is further divided into molecular subtypes defined by additional mutations in tumor suppressor genes such as TP53 and LKB1 (encoded by the gene *STK11*). The KRAS/LKB1-mutant subtype of LUAD is more aggressive and has reduced progression-free and overall survival compared to KRAS-mutant/LKB1-proficient disease, and is comparatively resistant to current chemotherapy, targeted therapies, and checkpoint blockade immunotherapy. To identify novel therapeutic vulnerabilities in KRAS/LKB1-mutant LUAD, we have compared differentially expressed genes in isogenic human genetically-engineered lung organoids (GELOs) to differentially expressed genes in LUAD patients from The Cancer Genome Atlas and gene expression in human cell lines from the Cancer Cell Line Encyclopedia. We will present our top gene candidates, as well as outline future plans to perform functional studies, with the ultimate goal of identifying druggable targets to improve lung cancer treatments.

Dillon Patterson, GMB

An IRF4-MYC axis regulates the proliferative response during B cell differentiation *in vivo*

Dillon G. Patterson[1,2], Christopher D. Scharer[1], Tian Mi[1], Madeline J. Price[1], Anna K. Kania[1], Sakeenah L. Hicks[1], and Jeremy M. Boss[1]

[1] Department of Microbiology and Immunology, Emory University, Atlanta, GA

Cell division is required for the initiation of B cell differentiation and ultimately entry of cells into the plasma cell (PC) lineage. Division-coupled changes in the expression of transcription factors, such as IRF4, that coordinate the PC transcriptional program also occur; however, little is known regarding how these factors coordinate the proliferative response during differentiation. To address this gap in knowledge, we employed an adoptive transfer system to assess the cell division kinetics of wildtype (WT) and IRF4-deficient ($IRF4^{-/-}$) B cells responding to lipopolysaccharide (LPS), NP-ficoll, and X31 influenza *in vivo*. Interestingly, we found that WT B cells undergo at least 8 divisions before differentiating into PCs. In contrast, $IRF4^{-/-}$ B cells divided but stalled during the proliferative response. To assess the scope of IRF4-dependent reprogramming, WT and $IRF4^{-/-}$ B cells at discrete divisions were sorted for ATAC- and RNA-seq. RNA-seq data revealed hundreds of differentially expressed genes (DEGs) when IRF4 was deleted, a subset of which included MYC target genes. Indeed, $IRF4^{-/-}$ cells failed to upregulate MYC compared to WT cells and consequently, we observed little increase in cell size, aberrant cell cycle distribution, and fewer proliferating cells following immune challenge. Overexpressing MYC in $IRF4^{-/-}$ B cells partially or fully rescued many of these defects. ATAC-seq data exposed hundreds of differentially accessible regions, the majority of which contained a known IRF4 binding motif and mapped to a corresponding DEG. Together, these data reveal a critical role for IRF4 in maintaining the proliferative response.

<u>Session 4:</u>

Aging, Human Disease and Development

Thursday, February 25th

3:00PM

Kimberly Diaz-Perez, GMB	**3:00PM**

Analysis of consanguineous families identifies recessive mutations associated with non-syndromic orofacial clefts

Kimberly K. Diaz Perez[1], Madison Bishop[1], Lina M. Moreno Uribe[2], Luz Consuelo Valencia-Ramirez[3], Claudia Restrepo[3], Eleanor Feingold[4], Mary L. Marazita[5], Elizabeth J. Leslie[1]

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Non-syndromic orofacial clefts (nsOFCs) are common and complex craniofacial birth defects caused by the incomplete fusion of facial tissues during embryonic development. Previously reported common variants only account for 25% of the genetic etiology of OFCs. We hypothesize that rare genetic variation may explain a fraction of the unattributed heritable risk. Previous studies suggest that nsOFCs do not follow a single Mendelian inheritance pattern, as autosomal dominant (AD), recessive (AR), and multifactorial inheritance modes all contribute to the etiology of nsOFCs. In this study, we focused on identifying rare homozygous recessive mutations associated with nsOFCs using whole-genome sequencing in consanguineous trios. We analyzed 22 Turkish and 27 Colombian OFC cases where the parents were 2nd, 3rd, or 4th-degree relatives as calculated by identity by descent sharing using PLINK and KING. We identified 17 protein-altering homozygous recessive mutations in OFC-associated genes in these trios. Several homozygous variants fall within AD genes, which we hypothesize are acting as hypomorphic variants, while other variants are in AR genes, acting in a loss of function manner. An example is in a family with a homozygous missense mutation (p.Glu625Lys) in *FLNB*, which causes AD Larsen syndrome and AR Spondylocarpotarsal syndrome. Larsen syndrome is caused by missense mutations, whereas stop-codon mutations cause Spondylocarpotarsal syndrome. This family supports the hypothesis that homozygous variants in dominant genes might act as hypomorphic variants, producing a non-syndromic, less severe phenotype. Overall, analyzing consanguineous families has the power to rapidly identify causal variants, and future functional studies will test these hypotheses.

Nourine Kamili, IMP	**3:15PM**

Galectin-8 regulates group B streptococcus uterine outgrowth by engaging microbial sialylated mimics of host glycans

Nourine A. Kamili[1], Connie M. Arthur[1], Anna Blenda[2], Sean R. Stowell[3]

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Group B streptococcus (GBS), the leading cause of neonatal sepsis and death, colonizes the reproductive tracts of 1 in 4 pregnant women by evading host immunity via molecular mimicry. GBS capsular sialic acid mimic host glycans to engage siglecs and inhibit immune activation. Recent studies suggest that galectins, a family of innate immune lectins, may provide protection against molecular mimicry. However, most galectins recognize non-sialylated structures, suggesting that they may not protect against sialylated molecular mimicry found in GBS. In contrast, we found that galectin-8 (Gal-8), possesses strong affinity for sialylated glycans. Using a murine model of GBS colonization along the female reproductive tract, we found that Gal-8 knockout (KO) mice carried a significantly higher GBS burden than WT mice, suggesting a direct involvement of Gal-8 in regulating GBS viability *in vivo*. Previous studies with Gal-8 identified the C-terminal domain of Gal-8 (Gal-8C) as possessing antimicrobial activity. However, examination of microbial glycan-microarrays indicated that the N-terminal domain of Gal-8 (Gal-8N) uniquely recognizes GBS-isolated glycans. Testing each domain individually with GBS, we found that while Gal-8C exclusively targeted non-sialylated microbial mimics (such as blood-group-expressing stains of *E. coli),* Gal-8N intrinsically bound and killed sialylated GBS. Together these findings suggest that while most immune factors recognize sialylation as "self-antigen" and are consequently rendered inert, Gal-8 provides innate immunity against sialylated variants of molecular mimicry. Furthermore, the unique and complementary binding preferences of each Gal-8 domain demonstrates that Gal-8 has evolved to target both sialic acid-based and non-sialic acid forms of molecular mimicry.

Erica Modeste, MSP	**3:30PM**

A proteomic network approach for elucidating racial differences in Alzheimer's disease

Erica Modeste[1], Eric Dammer[1], Measho Abreha[1], Duc M. Duong[1], James J. Lah[2], Allan I. Levey[2], Nicholas T. Seyfried[1,2]

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In Alzheimer's disease (AD), hyperphosphorylation of microtubule-associated protein tau results in its dissociation and aggregation, giving rise to insoluble neurofibrillary tangles (NFTs) in the brain. NFT formation increases with AD progression and correlates closely with cognitive decline. Traditionally, it has been understood that increasing levels of tau in cerebrospinal fluid (CSF) can be reflective of NFT formation in the brain. However, despite an equal burden of NFT in the brain, African Americans (AAs) with AD do not exhibit similar elevations in CSF tau compared to Caucasians. This poses a significant problem for the timely diagnosis and enrollment of AAs into clinical trials which frequently utilize CSF tau levels as a standard inclusion criterion. Currently, the biological differences that contribute to the lower CSF tau levels observed in AAs remains unknown. To fill this gap in knowledge, we utilized advanced bioinformatic approaches to analyze the CSF proteomes of AAs and Caucasians with AD, which revealed several proteins and pathways that are significantly different in AAs with AD. Notably, proteins known to interact with pathologic tau in AD brain were significantly decreased in the CSF of AAs with AD, suggesting that tau and its interacting partners may differ by race. Furthermore, Ca^{2+}/calmodulin-dependent protein kinase II alpha (CAMK2A) emerged as a key kinase shared between this module from CSF and the tau interactome from AD brain. Together, these findings highlight a need to understand how imbalances in tau-directed activity, such as those of kinases that phosphorylate tau, affect tau's secretion into the CSF.

Courtney Christian, BCDB	3:45PM

Maintenance of muscle myosin during aging

***Courtney Christian*[1],** Hiroshi Qadota[1], Guy M. Benian[1]

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As longevity increases, age-related diseases will become a greater public health concern. Sarcopenia is the age-related decline in muscle mass and function without any underlying disease. The molecular mechanisms responsible for this pathology remain unknown. Muscle function is dependent on having properly organized and functioning thick filaments, which are primarily composed of myosin. UNC-45 is required for the folding of the myosin head initially after translation and likely re-folds the myosin head to regain functionality after thermal or chemical stress causes unfolding. Here we show that myosin, UNC-45, and its co-chaperone HSP-90 are decreased during aging in the model organism *C. elegans*. Myosin and UNC-45 protein decline appear to be independent of steady state mRNA levels. This decrease in chaperone protein correlates to decreased assembled thick filaments in muscle cells. We also see a decrease in UNC-45 protein, but not transcript, in an *hsp-90* loss of function mutant, suggesting a role for HSP-90 in UNC-45 regulation. We also observe early onset of sarcopenia when UNC-45 is lost during adulthood. This leads us to investigate the possibility that during aging a loss of HSP-90 leads to UNC-45 degradation, which then leads to a loss of muscle mass and function. A better understanding of how myosin and its chaperone proteins are regulated and affected by aging will lead to better preventative care and treatment of sarcopenia and, possibly, the age-related decline of heart muscle function.

Jennifer Truong, BCDB **4:00PM**

Active enterohepatic cycling is not required for induction of bile flow by 24-*nor*ursodeoxycholic acid in mice

Jianing Li[1], ***Jennifer K. Truong***[1], Kimberly J. Pachura[1], Anuradha Rao[1], Sanjeev Gambeer[2], Claudia Daniela Fuchs[3], Saul J. Karpen[1], Michael Trauner[3], Paul A. Dawson[1]

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Introduction: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by progressive obliterative fibrosis of the biliary tree. 24-*nor*ursodeoxycholic acid (*nor*UDCA), is a side chain-shortened bile acid analog that improved cholestasis in PSC patients during Phase II clinical trials. The superior efficacy of *nor*UDCA is attributed to the cycling between hepatocytes and biliary epithelial cells (cholehepatic shunting). Our lab has shown that the bile acid transporters, ASBT (*SLC10A2*) and OST〈-OST® (*SLC51A*-*SLC51B*), are not required for the therapeutic actions of *nor*UDCA. The mechanisms responsible for the cholehepatic shunting of *nor*UDCA remain to be identified.

Goal: The primary aims of this study were to identify novel *nor*UDCA transporter candidates and potential signaling pathways that mediate the actions of *nor*UDCA.

Results: RNA-Seq analysis revealed that *nor*UDCA treatment altered the expression of a subset of liver transporter genes, with the Organic anion transporter Oatp1a4 most highly induced. However, *nor*UDCA-stimulation of bile flow and output were similar in WT and Oatp1a/1b KO mice lacking Oatp1a4. Many of the genes induced by *nor*UDCA are targets for the nuclear receptor PXR, but *nor*UDCA failed to activate PXR, FXR or VDR in cell-based assays.

Conclusions: *nor*UDCA does not behave like an endogenous bile acid in its ability to activate the classical bile acid nuclear receptors and its actions do not require the major bile acid transporters. These results support further investigation of the potential of a combination of *nor*UDCA and therapeutic modulators of bile acid transport and bile acid-activated nuclear receptor function.

Session 5:

Receptors and Signaling

Friday,

February 26th

10:00AM

Trisha Lala, NS	**10:00AM**

Detection of phosphatidylserine externalization by the synaptic receptor BAI1

Trisha Lala, Randy A. Hall

Department of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, GA

Brain-specific angiogenesis inhibitor 1 (BAI1) is an orphan adhesion G protein-coupled receptor (GPCR) found at synapses. The extracellular N-terminus of BAI1 is known to recognize phosphatidylserine (PS), a lipid that is usually concealed in the inner leaflet of the plasma membrane. However, it is unknown whether PS has any effect on BAI1 signaling activity. PS can be externalized under certain conditions, and its return to the inner leaflet is mediated by phospholipid flippases. We co-expressed BAI1 in HEK-293T cells with a brain-enriched flippase, ATP11B, and found that co-expression with ATP11B significantly reduced the G protein-dependent signaling that full-length BAI1 (BAI1-FL) engages in. These findings suggest that BAI1 detects PS in a manner that activates the signaling of this receptor. Interestingly, we found that ATP11B co-expression did not alter the signaling of a version of BAI1 lacking the extracellular N-terminus (BAI1ΔNT), suggesting that engagement of the N-terminus is necessary for the effect of PS on receptor signaling. Moreover, ATP11B specifically decreased the expression of BAI1-FL but not BAI1ΔNT. This is the first demonstration that PS can regulate the G protein-dependent signaling activity of the synaptic receptor BAI1, thereby shedding light on the detection of PS in the central nervous system.

Chad Camp, MSP	**10:15AM**

Loss of *Grin2a* Promotes Parvalbumin Interneuron Dysfunction and Synaptic Mistargeting in the Developing Hippocampus

Chad R. Camp[1], Lindsey Shapiro[2], Nima Shariatzadeh[1], Andrew Escayg[2], and Stephen F. Traynelis[1]

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De novo mutations in the *GRIN2A* gene, which encodes the GluN2A subunit of the N-methyl-D-aspartate receptor, are linked to several forms of epileptic encephalopathy (EE). The prognosis for EE patients is poor, as our current pharmacological options mainly dampen symptoms, without rectifying aberrant neuronal circuitry. 56% of epilepsy-related *GRIN2A* mutations are loss-of-function (LoF), displaying diminished receptor function and/or surface expression. These results are intriguing, as one might hypothesize that the loss of excitatory synaptic subunit would decrease excitability, rather than promote epileptiform activity. To understand this paradox, we have used *Grin2a* +/- and -/- mice as a model for LoF *GRIN2A* variants. Our data indicate that *Grin2a* -/- mice show an altered threshold for 6-Hz induced seizures compared to age-matched wildtype mice. Moreover, using both electrophysiological and immunohistochemical techniques, we show that the interneuron network in the *Grin2a* -/- mice are dysfunctional, providing a potential explanation for their altered seizure threshold. More specifically, we show that parvalbumin-positive interneurons are immature, missing intrinsic cues for interneuron apoptosis during developing, and are miswired, with synaptic outputs directed at the proximal dendrites of pyramidal cells rather than the soma and perisomatic regions. The ramifications of these abnormalities are still being explored, but it is possible that synaptic mistargeting itself could promote epileptiform activity. Viral strategies aimed at reintroduction of the *Grin2a* gene are also being explored and could represent a viable gene therapy option for EE patients suffering from *GRIN2A* LoF mutations.

Carolina Montanez, MSP	**10:30AM**

Human RGS14 and NHERF1 regulate PTH1R-G protein signaling events linked to phosphate uptake in kidney

Carolina Montañez-Miranda[1], Nicholas H. Harbin[1], and John R. Hepler[1]

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Regulator of G Protein Signaling 14 (RGS14) is a multifunctional scaffolding protein that integrates G protein, MAPK, and Ca^{++}/CaM signaling pathways. Multiple GWAS studies have implicated RGS14 with Chronic Kidney Disease and disordered phosphate metabolism. How RGS14 impacts kidney function and phosphate homeostasis remains unexplored. Phosphate homeostasis is regulated by the kidney sodium/phosphate exchanger [NPT2A:NHERF1] complex, which mediates phosphate uptake in renal proximal tubule cells. Parathyroid hormone (PTH) activation of the parathyroid hormone receptor 1 (PTH1R) blocks phosphate uptake. Our recent studies (Freidman et al-2019) show that RGS14 blocks PTH-sensitive phosphate uptake in renal cells. How RGS14 regulates PTHR1 signaling is unknown. Previous studies show that PTHR1 increases intracellular cAMP and calcium. Here we show in HEK and Opossum Kidney (OK) cells that PTHR1 stimulates intracellular cAMP and calcium, and directly couples to Gs but, surprisingly, not Gq. We find that RGS14 and NHERF1 block PTH-stimulated calcium, but not cAMP. We also show that human RGS14 binds to NHERF1, suggesting that RGS14 and NHERF1 regulate PTHR1-G signaling. Ongoing studies focus on understanding how RGS14 impacts PTH1R-G binding to NHERF1 to affect PTH1R downstream signaling and phosphate metabolism in the kidney.

Stephanie Foster, NS	10:45AM

Behavioral and molecular assessment of the role of noradrenergic galanin during opioid withdrawal

Stephanie L. Foster[1], Saumya L. Karne[1], Ewa Galaj[2], Sergi Ferré[3], David Weinshenker[1]

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Opioid withdrawal symptoms are associated with hyperactivity in several brain regions, including the brain's major noradrenergic nucleus, the locus coeruleus (LC). The neuropeptide galanin and one of its receptor subtypes, GalR1, are dynamically regulated by opioids, and enhanced galanin signaling is reported to suppress opioid withdrawal symptoms. Given that the LC expresses both galanin and GalR1, the LC is thought to be a critical site of action for galanin to suppress withdrawal symptoms. We therefore sought to 1) characterize galanin and GalR1 expression specifically in the LC at baseline and after opioid withdrawal, and to 2) test whether noradrenergic-derived galanin modulates withdrawal behaviors. We first used RNAscope to characterize galanin and GalR1 mRNA expression in the LC at baseline, after chronic morphine, or after precipitated withdrawal. We then compared withdrawal behaviors in genetically-altered mouse lines that either lacked or overexpressed noradrenergic galanin to their wild-type littermates. Surprisingly, we found that 80% of GalR1 mRNA puncta were attributable to cells adjacent to, rather than inside, the LC. We also found that baseline LC GalR1 mRNA expression is low, restricted to a small subset of LC neurons, and is not altered by opioid exposure or withdrawal. In contrast, baseline LC galanin expression was high, and was further increased by withdrawal. Neither overexpression nor depletion of noradrenergic galanin significantly altered withdrawal behaviors compared to wild-type mice. These collective findings suggest that noradrenergic galanin does not critically modulate withdrawal behaviors, which may be explained by the lack of GalR1 expression inside the LC.

Emily Legan, BCDB	**11:00AM**

Conformational dynamics and mechanoregulation govern the hemostatic response of von Willebrand factor

Emily R. Legan[1], Wenpeng Cao[2], Nicholas A. Arce[1], Yingchun Wang[1], Pete Lollar[1], X. Frank Zhang[2], Renhao Li[1]

[1]Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA
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Von Willebrand factor (VWF) is a large, concatemeric plasma glycoprotein that initiates blood clotting by binding and arresting platelets at the site of vascular injury. Dysregulation of VWF levels or activity can lead to adverse bleeding or clotting events. An example of altered VWF activity occurs in type 2B von Willebrand disease, a genetic disorder defined by enhanced association of the VWF A1 domain to its cognate platelet receptor glycoprotein (GP)Ibα, leading to variable platelet levels and bleeding severity in patients depending on the causative mutation. The molecular basis underlying heightened VWF activity and platelet count heterogeneity is not well understood. Previous studies have posited that the flanking residues of the VWF A1 domain functions as an inhibitory regulatory mechanism, but whether these residues dampen A1 activity by adopting inherent structural elements or by steric hindrance remains contested. Here, we have characterized the conformational dynamics and mechanical properties of mammalian-derived A1 fragments bearing various type 2B mutations to further investigate the mechanisms of A1 autoinhibition. Hydrogen-deuterium exchange studies show faster exchange kinetics in the secondary GPIbα binding site in type 2B mutants, consistent with their enhanced binding to GPIbα. Further, single molecule optical tweezer experiments reveal that type 2B mutations reduce the unfolding force of the flanking regions compared to wildtype. Combined, these data suggest that the flanking residues of the A1 domain adopt a metastable structure that inhibits aberrant binding of VWF to platelets.

Flash Talks

Friday, February 26th

1:00-2:30PM

Samantha Reed, NS

The potential of astrocyte-derived extracellular vesicles as a treatment for mesial temporal lobe epilepsy

Samantha Reed[1], Lindsey Sharpiro[1], Jennifer Wong[1], Ma Rong[2], Guoku Hu[2], Andrew Escayg[1]

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[2] Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE

Temporal lobe epilepsy (TLE) is the most common form of treatment-resistant epilepsy in adults and mesial temporal lobe epilepsy (MTLE) is the most common type of TLE. MTLE is characterized by cellular changes in the brain including neuroinflammation and neuronal death which are believed to contribute to MTLE symptoms. Thus, one potential therapeutic strategy may be to target these early pathological changes. Extracellular vesicles (EVs) have gained attention for their anti-inflammatory and neuroprotective properties. EVs are small vesicles released by cells into the extracellular space where they play a role in intercellular communication. They can contain a variety of cargos, including neuroprotective and anti-inflammatory RNAs and proteins. Astrocyte-derived EVs (astro-EVs) have been shown to reduce neuroinflammation in models of stroke and traumatic brain injury but have not yet been tested in models of epilepsy. In this study, I examined the anti-inflammatory effect of astro-EVs in the pilocarpine mouse model of MTLE. I administered astro-EVs for five days following pilocarpine treatment and measured levels of inflammatory gene expression in the brain using qRT-PCR. Astro-EV treatment attenuated expression of microglial and macrophage markers such as CD11b and CD68. Overall, this suggests that astro-EVs may be able to reduce pathological changes in a mouse model of MTLE and future research should evaluate the ability of astro-EVs to reduce other features of MTLE, such as the development of seizures.

Emilio Rodriguez, MMG

Regulation of *ispD* in the *Neisseria meningitidis* urethral clade US_NmUC

Emilio Rodriguez[1], Yih-ling Tzeng[1], David Stephens[1]

[1]Department of Medicine, Division of Infectious Diseases, Emory University, Atlanta, GA

Neisseria meningitidis (Nm) is a leading cause of bacterial meningitis and sepsis; death can occur within hours of symptoms appearing, and globally over a million cases of meningococcal disease have caused 135,000 deaths annually. *N. meningitidis* normally colonizes the nasopharynx, but a novel meningococcal clade, US_NmUC, has been identified to be responsible for recent meningococcal urethritis outbreaks. Whole genome sequencing of > 200 US_NmUC isolates revealed that an Nm ancestor underwent multiple homologous recombination events with *Neisseria gonorrhoeae* (Ng), one of which integrated a 3.3 kb segment of gonococcal DNA into the US_NmUC genomes. Preliminary data showed that one of the gonococcal genes from the 3.3 kb segment, *ispD*, is involved in the terpenoid synthesis pathway and has a 50-fold higher expression in US_NmUC isolates compared to non-US_NmUC meningococci. To determine if the increased expression of *ispD* is the result of newly created promoters, LacZ transcriptional and translational reporters with different 5' regions of the operon were generated to measure promoter activities by a β-galactosidase assay. Two different regions originating from the gonococcal genome displayed significantly higher promoter activities, suggesting the possibility of multiple promoters mediating *ispD* expression in US_NmUC isolates. IspD has been shown to be essential in several bacteria including *E. coli, Salmonella enterica,* and *Providencia stuartii*. Viable *ispD* deletion mutants were created in US_NmUC, non-clade Nm, and Ng, demonstrating that *ispD* was not essential in *Neisseria*. The biological consequence of integrating the gonococcal *ispD* into US_NmUC remains under investigation.

James Rose, GMB

Unique abilities of human memory T cell subsets are associated with both specialized gene expression modules and epigenetic priming

James R. Rose[1], Andrew R. Rahmberg[1], Christopher D. Scharer[1], Jeremy M. Boss[1]

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA

Memory T cells are a diverse set of cells that respond to immunologic challenge more quickly and with greater efficacy than their naïve counterparts. Their potential in the resting and stimulated state has not been explored for each of the subsets. To define their capabilities, RNA-seq and ATAC-seq data assessing the transcriptome and chromatin accessibility potential of both resting and activated human memory T cells was analyzed. Clustering gene expression modules identified a core set of genes highly expressed in both resting central memory and effector memory cells (but not in naïve or terminally differentiated effector cells). Memory subsets also expressed unique gene modules enabling specialized functions such as cell cytotoxicity, migration, or self-renewal. Subset differences in gene expression were reflected in differentiated chromatin accessibility and enhanced variation around binding motifs for key T cell differentiation transcription factors such as Tbet, EOMES, and LEF1. Upon activation memory T cells showed substantial rapid upregulation of genes including cytokines which were absent in activated naïve T cells. These differences in gene expression from naïve T cells were often preceded by areas of open chromatin in a resting memory state, suggesting mechanisms of epigenetic priming for rapid recall or augmentation of effector T cell function in memory. In summary, memory T cell subsets are distinct in both resting gene expression functionalities and differentiated epigenetic landscape—both of which enable unique, rapid, and effective response to antigen.

Sarah Samaranayake, CB

Efficacy of a tumor membrane vesicle (TMV)-based vaccination approach with alum adjuvant in a HER2+ murine breast cancer model

Sarah Samaranayake[1]**,** Luis Munoz[1], Lenore Monterroza[1], Rohini Neha Guin[1], Ramireddy Bommireddy[1], Periasamy Selvaraj[1]

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Breast cancer is predicted to be the most common cancer diagnosis for U.S. women in 2021 with the second highest estimated mortality rate. Breast cancer often develops due to overexpression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2). For HER2+ breast cancer patients, HER2-targeted therapies offer promising survival outcomes by blocking HER2 oncogenic signaling. When anti-HER2 therapies fail to prevent disease recurrence and metastases, novel treatments are needed to address resistance rooted in tumor heterogeneity. Our lab approaches intra-tumoral and inter-patient heterogeneity with tumor membrane vesicle (TMV) vaccines, a unique antigen delivery system prepared from tumor tissue. TMVs can be modified with a novel protein transfer method to incorporate glycolipid-anchored immunostimulatory molecules (GPI-ISMs) to enhance anti-cancer immune responses upon uptake by antigen presenting cells. TMV vaccines have demonstrated efficacy in other murine models, but this is the first investigation using TUBO, a HER2+ murine model established from a spontaneous mammary carcinoma in a BALB/c mouse expressing rat HER2. Our interest in alternative TMV adjuvants led us to alum, which is among the most common vaccine adjuvants due to its safety profile. Alum creates a depot effect at the injection site for adsorbed antigen, in addition to other mechanisms of immune cell activation still under investigation. An *in vivo* therapeutic vaccination study using combinations of TMVs, alum, and GPI-IL-12 did not affect tumor growth. TUBO may require combinations with other GPI-ISMs, while alum should be investigated in other models to further explore its activity with TMVs.

Juliet Santiago, NS

Exosomes: A novel contributor of microglia-mediated neuroinflammation and neurodegeneration

***Juliet Santiago*[1]**, Sruti Rayaprolu[1], Hailian Xiao[1], Nicholas T Seyfried[1], Srikant Rangaraju[1]

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Alzheimer’s disease (AD) is the most common neurodegenerative disorder defined by continuous pathological protein aggregation (amyloid-beta and tau) and deterioration of cognitive function. A key pathological component of AD is neuroinflammation which is mediated by immune cells of the brain called microglia. Although microglia-mediated neuroinflammation has emerged as a causal disease mechanism, there are still critical gaps in our understanding of what microglial protein changes occur in AD and how microglia can perpetuate AD pathology. Exosome release has emerged as a mechanism of microglia-mediated neuroinflammation and neurodegeneration. Given the role of exosomes in the transfer of macromolecules between cells to facilitate intercellular communication, it is possible that microglia-derived exosomes transfer pathogenic cargo which could perpetuate AD. However, the role of microglia-derived exosomes in neuroinflammation and AD pathology remains poorly investigated. To this end, our goal is to define microglial proteomic changes in AD and define the molecular composition and state-specific effects of microglia-derived exosomes in AD pathology. We hypothesize that the protein profiles of microglia and microglia-derived exosomes impact AD pathology. Here, we present a study to 1) determine the effect of microglia state on exosome composition and exosome-mediated responses *in-vitro* and 2) define microglial and microglia-derived exosomal proteomic changes associated with AD pathology *in-vivo*. Preliminary findings suggest successful exosome isolation from microglial cell lines, confirmed by expression of the exosomal marker, CD9. Furthermore, we can label the microglia proteome *in-vivo* using a biotinylation approach and are developing approaches to enrich microglia-derived exosomes for downstream mass spectrometry studies.

Jessica Shartouny, IMP

Viral determinants of reliance on multiple infection in influenza

Jessica Shartouny[1], Chung-Young Lee[1], and Anice Lowen[1]

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Influenza A viruses (IAV) infect diverse species with many factors determining whether a virus can infect a particular host. When species barriers are overcome in a naïve population, IAV has the potential to cause widespread outbreaks. Previous work from our lab has demonstrated that IAVs rely on more than one virus particle infecting the same cell for effective production of progeny viruses in a host- and strain-dependent manner. This reliance on multiple infection is seen strongly in influenza A/guinea fowl/Hong Kong/WF10/99 (H9N2) virus (WF10) when infecting mammalian cells while not as strongly in avian cells. Influenza A/mallard/Minnesota/199106/99 (H3N8) virus (MaMN99), however, did not show the same high level of reliance. Subsequent investigation of singular genome segments mapped the multiple infection phenotype to the polymerase segment PA. We hypothesize that poor adaptation to the host increases reliance on multiple infection. This could, in turn, augment reassortment of gene segments between two infecting viruses and accelerate host adaptation. Using a multiple infection model based on tagged homologous viruses, we are able to examine the genetic factors that contribute to the need for multiple infection. Our data suggest that IAV strains more or less closely related to WF10 show a corresponding multiple infection phenotype that is linked to their PA segment. Viruses engineered with mutations in functional regions of PA and the alternative gene product PA-X are likewise being examined to pinpoint which areas are involved in facilitating cooperation between multiply-infecting viruses.

Debresha Shelton, GMB

The effects of light-induced retinal damage (LIRD) in C57BL6/J mouse is mediated by RPE-65 activity

Debresha Shelton [1], Wenfei Wu[1], Salma Ferdous[1], Vivian Summers[1], Isabelle Gefke[1], Micah Chrenek[1], Preston Girardot[1], Jeffery Boatright[1], John M. Nickerson[1]

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PURPOSE: Controversy exists in the field regarding the extent of heterogeneity in microglial responses in a damage dependent context in the eye. We investigated the hypothesis that mutations in genes involved in the visual cycle, namely *rpe65*, can be used to study changes in damage kinetics and immune cell recruitment by modulating the extent of phototoxicity.

Methods: Mice with wildtype Rpe65(Leucine at aa450) and the low activity variant (Leu450Met) to study how changes in Rpe65 affects damage kinetics after light-induced retinal damage (LIRD). Mice were aged to P60+. The L450 and L450M animals were exposed to LIRD at 50,000 lux for 5 hours at ZT12-ZT17. Animals were grouped into day 3, 5, 7, and 10 post LIRD. Ocular morphology was measured via *in vivo* imaging.

Animals were sacrificed at *in vivo* data collection and ocular tissue was collected for retinal pigment epithelium (RPE) flat mounts and stained for IBA1 (microglia).

Results: Preliminary data suggest that L450 mice exhibit earlier morphological changes in fundus images, ocular structures, and an increased presence of autofluorescent dots (putative microglia) compared to animals expressing the L450M variant, as early as three days post LIRD. Additionally, data show a significant difference in IBA+ cell deposition in the RPE of L450 and L450M mice at days 3, 5, and 7 post LIRD (p-value:0.05), with the greatest disparity at day 7 (p-value: 0.001).

Conclusion: Our data suggest that changes in Rpe65 activity can change both the extent of damage and the kinetics of cells involved in damage resolution.

Sarah Strassler, BCDB

Substrate recognition by the tRNA methyltransferase Trm10.

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The highly conserved methyltransferase Trm10 (TRMT10A in humans) modifies a specific subset of tRNAs on their 9th nucleotide. However, of the 26 tRNAs in yeast with guanosine at position 9, only 14 are substrates for Trm10 and no common sequences or other posttranscriptional modifications have been identified among these substrates. This finding suggests some other tRNA feature(s) must allow Trm10 to accurately distinguish between tRNA substrates and non-substrates. This project will test the hypothesis that tRNA substrate recognition by Trm10 is dependent on specific structural features and conformational dynamics in both Trm10 and tRNA. Using the sensitive RNA structure probing method selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE), tRNA conformational changes upon Trm10 binding were identified that may allow Trm10 to gain access to the tRNA core region. In addition, preliminary protein dynamic studies using hydrogen-deuterium exchange mass spectrometry (HDX-MS) have revealed a potential role for specific protein conformational changes in tRNA methylation. Further SHAPE and HDX-MS studies with Trm10 variants and substrate/ non-substrate tRNAs will be used to fully define the role of structural plasticity in tRNA and Trm10 during correct substrate recognition. Additionally, x-ray crystallography will be used to determine the first structure of a full-length Trm10 protein bound to a tRNA substrate, revealing a structural snapshot of the complex and the molecular interactions essential for substrate recognition. Collectively, these studies will reveal a novel mechanism of protein-RNA recognition and how tRNA modifying enzymes discriminate between structurally similar tRNA species for accurate substrate selection.

Brooke Talbot, PBEE

Genomic clusters of methicillin-resistant *Staphylococcus aureus* (MRSA) causing bloodstream infections (BSIs) in hospitalized adults, 2018-19

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Staphylococcus aureus is a bacterial species that can cause infections resulting in sepsis or death and be difficult to treat with antibiotics, such as methicillin-resistant *S. aureus* (MRSA). Bloodstream infections (BSI) caused by MRSA are common in hospitals, but the molecular epidemiology between BSI cases is not well understood. Using genomic and clinical data, we investigated 105 MRSA isolates from adults presenting with BSI at two Philadelphia hospitals between July 2018-June 2019. Raw genomic sequences were analyzed using the Staphopia workflow to assess data quality and describe the MLST, SCCmec type, and antibiotic resistance and virulence genes. To create phylogenetic comparisons between isolates of the same clonal cluster (CC), sequences were aligned against an appropriate reference strain using Parsnp, and Gubbins was used to remove recombinant regions and create maximum-likelihood trees using RAxML. Trees were visualized using the R package ggTree. Single-nucleotide polymorphism (SNP) distance matrices were created using Disty McMatrixface to identify isolates that were closely related (<35 SNPs). In total, 36.2% (n=38) of isolates belonged to CC5, 49.5% (n=52) belonged to CC8, and 14.3% (n=15) belonged to eight other CCs. We identified six clusters of two or more patients with closely related BSI infections (two CC8 clusters and four CC5 clusters). Three clusters had subjects with overlapping hospital stays, while three clusters did not have temporally overlapping hospital stays, suggesting transmission via a hospital reservoir. Wider implementation of whole-genome sequencing may efficiently detect MRSA hospital outbreaks and lead to better infection control of persistent MRSA strains.

Julia Tanquary, BCDB

Molecular targets and functions of bacterial toxins

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Antibiotic resistance is a serious threat to human health and it is clear that antibiotic persistence contributes to the resurgence of bacterial infections. Within a population of bacteria, persistent bacteria are genetically identical to those susceptible to antibiotics, yet exhibit different phenotypic responses upon antibiotic exposure. Typically, persistent bacteria halt cellular processes conducive to growth to enter a dormant state, thereby evading antibiotics and thus cell death. Although the cellular mechanisms that induce dormancy are unknown, bacterial toxin-antitoxin complexes have been implicated in persistence as they are activated under stress to temporarily halt growth. For example, *Escherichia coli* DinJ-YafQ, where DinJ is the antitoxin and YafQ is the cognate toxin, is activated by oxidative stress to inhibit protein synthesis and pause growth. To date, the roles of many bacterial toxin-antitoxin complexes in persistence have been investigated through overexpression models that result in cell death and therefore do not recapitulate the endogenous, protective role of the toxin. To overcome this experimental limitation, I genetically engineered an *E. coli* strain that contains an inducible degron, a TEV protease recognition sequence, in chromosomally-encoded DinJ. When TEV protease expression is induced, the DinJ antitoxin is proteolyzed, releasing the bound YafQ toxin. Upon proteolysis of DinJ, a reduction in cell viability, but not cell death, is observed, which is consistent with the protective nature of toxins. This novel strain provides a unique tool to more accurately investigate the role of chromosomally-encoded toxins in persistence in their native context.

Jesse Tehrani, GMB

Cadmium accumulation in the placenta associates with aberrant microRNA expression: results from a small RNA-Seq analysis

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As the master regulator of early development, the placenta is responsible for initiating changes in maternal physiology to sustain pregnancy as well as promoting fetal growth and development. MicroRNAs (miRNAs) are epigenetic post-transcriptional regulators of gene expression which participate in critical processes during early development, including embryogenesis, implantation and placentation. Cadmium is an environmental toxicant with no known biological role in humans. Human exposure primarily occurs through consumption of contaminated food or through the use of tobacco products. Gestational cadmium exposure has been associated with adverse health outcomes in newborns, but the molecular mechanisms by which these are initiated remain unclear. In this study, 281 mother-infant pairs from the New Hampshire Birth Cohort Study (NHBCS) were selected for trace element profiling and small RNA transcriptomic analysis. We identified four **d**ifferentially **e**xpressed placental **miR**NAs (DEmiRs) were significantly associated with cadmium concentrations in placenta (FDR <0.1): miR-509-3p, miR-10b-5p, miR-10b-3p, and miR-193b-5p. Two of these DEmiRs (miR-509-3p and miR-193b-5p), maintained direction of effect in an independent analysis within the Rhode Island Child Health Study (RICHS)(n=115) but were not significantly associated with placental cadmium concentrations. Bioinformatic miRNA target prediction was used to identify potential mRNAs targeted by these DEmiRs, revealing gene targets participating in various cell signaling pathways critical for placentation, angiogenesis and response to hypoxia. These results indicate that accumulation of cadmium within the placenta may disrupt fetoplacental gene expression via changes in miRNAs. Future work aims to identify associations between early life health outcomes and differential expression of cadmium-sensitive fetoplacental miRNAs.

Germán G. Vargas-Cuebas, MMG

Clostridioides difficile glycine Stickland metabolism and its modulation in response to the host peptide LL-37

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Clostridioides difficile (*C. difficile)* is the leading cause of antibiotic-associated diarrhea. This human pathogen is able to ferment amino acids to generate energy through Stickland reactions, in which a donor amino acid is oxidized while an acceptor amino acid is reduced. The glycine Stickland pathway is conserved among *C. difficile* strains, and represents one of the preferred acceptor amino acids in Stickland reactions. We have observed changes in *C. difficile* glycine Stickland transcription in respond to the host peptide, LL-37. Even though it has been shown that Stickland metabolism is important for *C. difficile* growth *in vitro*, how this metabolism impacts pathogenesis and how it is regulated remains largely uncharacterized. I hypothesize that *C. difficile* colonization and pathogenesis is increased by glycine Stickland metabolism, and that this metabolic pathway is modulated in response to LL-37. To test this hypothesis, we generated a ∆*grdAB* mutant and analyzed its phenotypes in minimal media. Our preliminary data show that the amino acid glycine enhances *C. difficile* growth through the glycine Stickland pathway. Using transcriptional reporter assays we showed that expression of the *grd* operon is increased by LL-37. Interestingly, the ability to catabolize glycine impacts the total amount of toxin produced in minimal media. Future studies will aim to determine how LL-37 regulates the *grd* operon, and how this metabolic pathway impacts virulence in *C. difficile.*

Katherine Westover, GMB

Genome-wide dysregulation of R-loops in ATM neurological pathogenesis

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R-loops are three-stranded RNA:DNA structures formed when an RNA strand anneals to one strand of a duplex DNA, leaving the other strand free. R-loops have been shown to play important roles in key biological processes such as transcriptional regulation and DNA damage repair (DDR). One critical player in DDR is the ATM serine/threonine kinase. This kinase phosphorylates key proteins involved in the process and when mutated causes a severe neurodegenerative disorder known as Ataxia telangiectasia (AT). The precise mechanism underlying ATM-mediated R-loop regulation and its implication in neurodegeneration remains largely unclear. This project first focuses on understanding how R-loops are formed and processed under normal DNA damage response at genome-wide scale as well as how ATM loss-of-function leads to global R-loop alteration in both ATM depleted neuronal cell culture and AT-patient derived Purkinje neurons. In light of recent findings that ATM phosphorylates an RNA m6A methyltransferase METTL3 to modulate R-loop formation via decorating m6A on R-loop and our preliminary data that a DNA damage agent Zeocin causes ectopic accumulation of global R-loop formation, we will mechanistically define the interplay of ATM-METTL3 in response to DNA damage through base-pair modification in regulating R-loop formation and how disruption of this process precisely leads to neurodegeneration at the cellular and molecular level. Our data will shed light on the previous under-appreciated physiological and pathogenic roles of R-loop in the mammalian central nervous system and could provide strong molecular foundation in therapeutic development.

Keenan Wiggins, GMB

Gating schemes to best analyze class switched memory B cell formation.

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B lymphocytes are a type of cells that belong to the immune system with a one role to make antibodies to invading pathogens like viruses. One class of these cells is memory cells that remember the same pathogen for faster antibody production in future infections. Recently, there has been a growing interest in memory B cells. Following activation of the adaptive immune response by foreign antigens, memory B cells are formed or reactivated. There are many questions that remain on how memory B cell differentiate and what cues help them stay or differentiate in other cells in the B cell lineage. Our lab has shown previously that epigenetic complexes are involved in the reprogramming of B lymphocytes. Having a better understanding of the epigenetic landscape in memory B cells can provide insight into development of better therapies to defend ourselves from infections. We show that mice infected with the flu (PR8) develop IgG class switched memory B cells that are antigen specific as early as 10 days post infection. We use different gating schemes to determine how best to define the different memory B cell sub-populations. Development of this system will help our future studies looking into what cues and factors help regulate the differentiation into and out from memory B cells.

Shyra Wilde, MMG

Mechanism of interleukin-6-mediated restriction of group A *Streptococcus*

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Group A *Streptococcus* (GAS) is a human-restricted pathogen that causes mild infections such as pharyngitis (“strep throat”), invasive and life-threatening infections like Streptococcal Toxic Shock Syndrome, and post-infection sequelae like rheumatic heart disease. Immunosuppressed patients are at higher risk for invasive GAS infections, and recent data demonstrate that interleukin-6 (IL-6)-inhibiting biologics, used to manage autoimmune disorders, are associated with invasive GAS infections in humans and increased GAS survival within phagocytes *in vitro*. The mechanism for IL-6-mediated killing of GAS remains unclear, but recent data show that reactive oxygen species (ROS) are necessary for limiting intracellular GAS. GAS is catalase-negative, yet hypervirulent strains are resistant to IL-6-mediated immune restriction and can still withstand oxidative burst *in vitro*. Through genetic screens and analysis of clinical isolates, we have found that the hyaluronic acid capsule of GAS, which acts as a physical barrier against opsonization, also serves as a redox sink to absorb free radicals produced during oxidative burst. This allows GAS to survive in an intracellular niche within phagocytes, but naturally acapsulated GAS and strains with defined mutations in the capsule biosynthetic operon are significantly more susceptible to oxidative stress *in vitro*. Intracellular growth of naturally acapsulated GAS strains is rescued when ROS scavengers are present. Together, these data demonstrate a conditional requirement for IL-6 and ROS in the immune restriction of GAS, which is bypassed by some strains through production of capsule.

Paul Zakutansky, BCDB

The RNA binding protein QKI regulates the lncRNA *GOMAFU* during human neural cell development.

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Alternative splicing, the inclusion or exclusion of specific exons in mRNAs, is a key component in controlling normal neuronal development but affected in neuropsychiatric diseases such as schizophrenia (SCZ). Recent discoveries indicate that long non-coding RNAs (lncRNAs), play sophisticated roles in gene regulation including alternative splicing. lncRNAs are poorly conserved in general and highly expressed in the human brain. Abnormalities in lncRNA expression are implicated in neurodegenerative and neuropsychiatric diseases. A particular human lncRNA of interest is *GOMAFU*, which is abundantly expressed in human iPSC-derived neural progenitor cells (hNPCs) and brain neurons but negligible in glia. *GOMAFU* is restricted in the nuclei and regulates alternative splicing of SCZ risk factor gene transcripts. Abnormal expression of *GOMAFU* is found in SCZ patient neurons. However, molecular mechanisms that govern *GOMAFU* expression in human neurons still remain elusive. Recent studies suggest *GOMAFU* may form a functional pathway with an RNA-binding protein (RBP) called Quaking I (QKI), another SCZ risk factor known to regulate alternative splicing in neural progenitor cells (NPCs) and neuron-glia lineage development. We found multiple quaking response elements in human *GOMAFU* enriched in the 3' exon. Furthermore, during human neuron development, the decline of QKI isoform expression conversely correlates with increased *GOMAFU* expression. Upon CRISPR-Cas9 elimination of the nuclear QKI-5 isoform, we observed significant up-regulation of *GOMAFU,* suggesting QKI-5 suppresses *GOMAFU* expression. Our studies provide evidence for QKI in regulating lncRNA expression and identified the QKI-*GOMAFU* SCZ risk factor pathway during human neuronal development.