BGSA 27th Symposium
November 2nd, 2022

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8:45-9:00am	**Leonard Frisbie, ISB** "Carcinoma associated mesenchymal stem cells promote ovarian cancer metastasis by increasing tumor heterogeneity through direct mitochondrial transfer"	Ballroom A
9:00-9:15am	**Tyler Fortuna, ISB** "SMN regulates GEMIN5 expression through the Tudor domain and acts as a modifier of GEMIN5 toxicity"	Ballroom A
9:15-9:30am	**Fabliha Chowdhury, MPHL** "Unraveling the therapeutic potential of a quinone-nitroalkene molecule for sickle cell anemia: Preclinical screening and development"	Ballroom A
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Nakada Lecturer

Volunteer Judge Thank You

Student Judges
Yajushi Khurana
Ahmed Emam Abdelnaby
Rithika Behera
Taylor A Gatesman
Catherine Phelps
Jordan John Peter Warunek
Jenna Marie Nosek
Ananya Mukundan
Sierra R Wilson
Julian Dana
Jessica Louise Nuwer
Olayemi G Akinyele

Faculty Judges
Chris Cunningham
Mac Hooks
Alok Joglekar
Judith Yanowitz
Nathan Lord
Amantha Thathiah
Bharat Bhushan
Creg Workman
David Boone
Deepika Vasudevan
Hun-Way Hwang
Issam Al Diri
Jami Salomon
John Alcorn
Johnathan Beckel
Jon Piganelli
Larry Kane
Laurie Silva
Marlies Meisel
Matthew Culyba
Michael J. Palladino
Michael Jurczak
Nam Vo
Sarah Ross
Srivatsun Sadagopan
Stacy Gelhaus Wendell
Thiago Bruder do Nascimento

Speaker Abstracts

Abbott, Darryl

she/her/hers
Program in Microbiology and Immunology (PMI) Year 4
Advisor: Timothy Hand

Differentiating the role of maternal IgA and infant intestinal environment on the growth of immunogenic bacteria upstream of infant T cell responses
Darryl Abbott, Ansen Burr, Kathyayini Gopalakrishna, Timothy Hand

The postnatal intestinal immune system undergoes significant development during initial bacterial colonization following loss of sterility postpartum. Due to their immature immune system, neonates are at significant risk for enteric infection and diseases associated with invasion by the microbiome, such as necrotizing enterocolitis (NEC). The developing T cell population is tightly regulated, as aberrant T cell responses during development can predispose individuals to allergy and autoimmune disorders. A key predictor of infant health is breastfeeding, which supplies antimicrobial peptides and antibodies from the maternal mucosal immune system, the most abundant of which is Immunoglobulin A (IgA). We hypothesized that maternal IgA (mIgA) in breastmilk shapes intestinal colonization by limiting the ability of immunogenic bacteria to colonize, thereby limiting infant T cell activation. Segmented filamentous bacteria (SFB), an adherent bacterium, is a strong inducer of Th17 responses and IgA in the small intestine. Surprisingly, given previous results, imaging and microbiome analysis revealed that mIgA does not control SFB. 16S sequencing of pups of IgA deficient mothers confirmed no SFB growth, but revealed an increase in Enterobacteriaceae, an immunogenic species implicated in infant health. Using bacterial lysate stimulation, imaging and antibiotic clearance we are investigating the role of Enterobacteriaceae in infant T cell activation. This work has significant parallels to human studies, which show that loss of IgA binding to Enterobacteriaceae precedes development of NEC. Taken together these results will have implications both for formula fed associated pathologies (NEC) and autoimmune disorders associated with aberrant responses to the microbiome (Crohns Disease)

Almeida Rojo, Ana

she/her/hers
Center for Neuroscience (CNUP) Year 5
Advisor: Yanhua Huang

Sleep Deprivation Engages the Hypocretin/Orexin System to Regulate Reward Seeking
Almeida Rojo AL, Heim B, Cai L, and Huang YH

In the US, about 35% of adults do not get sufficient sleep. Preclinical studies have shown that sleep deprivation (SD) alters reward processing in humans and that SD and chronic sleep restriction (CSR) alter reward seeking/responding in rodents. However, mechanisms in SD-modulated reward are not clearly understood. The hypocretin/orexin system presents a candidate mechanism in SD-modulation of reward. Orexin receptors (OX1R and OX2R) exhibit partly overlapping but distinct expression in the brain with certain sexual dimorphism. Functionally, both OXRs play a role in regulating sleep and wakefulness, promoting arousal, and regulating natural reward. Thus, we hypothesize that SD recruits the orexin system to modulate natural reward-seeking in a sex- and receptor subtype-dependent manner.

Male and female mice were trained to self-administer sucrose pellets. After obtaining a 2-4-day baseline, mice underwent SD for 6 hours (ZT0-6). Mice then received systemic administration of OX1R or OX2R antagonist prior to self-administration (SA) test. Changes in c-Fos expression in the reward circuit were quantified 90 min from entering the SA chamber. Following normal sleep, OX1R or OX2R signaling did not modulate sucrose SA in males or females. After SD in female mice, OX2R but not OX1R antagonism reduced sucrose SA. By contrast, in males, neither OX1R nor OX2R antagonism had a significant effect on sucrose SA. c-Fos quantifications are ongoing. Preliminary results suggest SD-induced, OX2R-dependent changes in the nucleus accumbens (NAc) and paraventricular nucleus of the hypothalamus (PVN).

These results suggest that in female mice, SD preferentially engages OX2R signaling to increase sucrose reward seeking. This effect may be mediated by OX2Rs in the NAc and/or PVN.

Chowdhury, Fabliha

she/her/hers
Molecular Pharmacology (MPHL) Year 5
Advisor: Dr. Adam Straub

Unraveling the therapeutic potential of a quinone-nitroalkene molecule for sickle cell anemia: Preclinical screening and Development

FA Chowdhury1, MP Miller2, KC Wood2, M Sharma2, S Yuan2, SN Taiclet2, DJ Sinchar2, ER Rochon2, FJ Schopfer1,2, BA Freeman1,2, AC Straub1,2
1Department of Pharmacology and Chemical Biology University of Pittsburgh School of Medicine; 2 Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh School of Medicine

Sickle cell anemia (SCA) is a hereditary disorder caused by the formation of hemoglobin S (HbS). HbS undergoes polymerization and generates sickled red blood cells (RBCs). Along with having reduced oxygen carrying capacity, sickled RBCs are also prone to frequent hemolysis releasing the pro-oxidant heme in the circulation. Oxidative stress created by the free heme leads to sterile inflammation, vaso-occlusion and cardiovascular complications. Although there is an existing high mortality and morbidity rates of such patients, safe and effective therapeutic options remain limited. We have developed a novel quinone-nitroalkene hybrid molecule called CP50, which has shown to activate two important proteins: 1) nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional regulator of cellular resistance to oxidants and 2) cytochrome b5 reductase 3 (CYB5R3), an anti-stress enzyme in the cardiovascular system. Our preliminary data demonstrates that CP50 provides protection against cellular stress by upregulating the ribosomal small and large protein (RPS/RPL) transcription via a CYB5R3-dependent mechanism. It also induces heme-oxygenase 1 (HO-1), an enzyme that degrades heme, via an Nrf2-dependent mechanism in primary aortic endothelial cells. In CD34+ hematopoietic stem cells, CP50 augments the expression of the F-cells, which produce fetal hemoglobin (HbF) and are resilient to sickling and hemolysis. In an animal model of SCA, we show that CP50 induces hematopoiesis in vivo and inhibits hemolysis in vitro. Together, our studies indicate that CP-50 has the potential to alleviate the multi-organ pathology associated with SCA.

Fortuna, Tyler

he/him/his
Integrative Systems Biology (ISB) Year 5
Advisor: Udai Pandey

SMN regulates GEMIN5 expression through the Tudor domain and acts as a modifier of GEMIN5 toxicity

Tyler R Fortuna, Sukhleen Kour, Anuradha Venkatakrishnan Chimata, Anixa Muiños-Bühl, Eric N Anderson, Caroline Ward, Om Chauhan, Casey O'Brian, Dhivyaa Rajasundaram, Deepa S Rajan, Brunhilde Wirth, Amit Singh, Udai Bhan Pandey

GEMIN5 is essential for core assembly of small nuclear Ribonucleoproteins (snRNPs), the building blocks of spliceosome formation. Biallelic mutations in GEMIN5 lead to a neurodevelopmental syndrome among patients presenting with developmental delay, motor dysfunction, and cerebellar atrophy by perturbing snRNP complex protein expression and assembly. Currently, molecular determinants of GEMIN5-mediated disease have yet to be explored. Here, we identified SMN as a genetic suppressor of GEMIN5-mediated neurotoxicity in vivo. We discovered that an increase in SMN expression by either SMN gene therapy replacement or the antisense oligonucleotide (ASO) Nusinsersen, significantly upregulated the expression of GEMIN5 in mammalian cells and mutant GEMIN5 derived iPSC neurons. Further, we identified a strong functional association between the expression patterns of SMN and GEMIN5 in patient Spinal Muscular Atrophy (SMA) derived motor neurons harboring loss of function mutations in the SMN gene. Interestingly, SMN binds to the C-terminus of GEMIN5 and regulates GEMIN5 expression through the Tudor domain. Lastly, we show that SMN upregulation ameliorates defective snRNP biogenesis and alternative splicing defects caused by loss of GEMIN5 in iPSC neurons and in vivo. Collectively, these studies indicate that SMN is a potent regulator of GEMIN5 expression and neuropathologies.

Frisbie, Leonard

he/him/his
Integrative Systems Biology (ISB) Year 3
Advisor: Lan Coffman

Carcinoma associated mesenchymal stem cells promote ovarian cancer metastasis by increasing tumor heterogeneity through direct mitochondrial transfer
Frisbie, LG; Pressimone, CA; Atiya, HA; Pearson, AT; Coffman, LG

Ovarian cancer is characterized by early, diffuse metastatic spread with most women presenting with widespread abdominal metastasis at the time of diagnosis. Prior work demonstrates carcinoma-associated mesenchymal stem cells (CA-MSCs) enhance ovarian cancer metastasis through a process of direct cellular interaction and formation of heterocellular CA-MSC and tumor cell complexes. Here we demonstrate CA-MSCs enhance metastasis via increasing tumor cell heterogeneity through mitochondrial donation. CA-MSCs directly interact with ovarian cancer cells forming tunneling nanotubules (TNTs) by which live mitochondria are transferred. This mitochondrial donation preferentially occurs to ovarian cancer cells with the least endogenous mitochondria (‘mito poor’ cancer cells). Mito poor cancer cells demonstrate decreased proliferation, increased sensitivity to chemotherapy and decreased OXPHOS compared to ‘mito rich’ cancer cells. CA-MSCs rescue the phenotype of these mito poor cancer cells restoring their proliferative capacity, increasing chemotherapy resistance and increasing OXPHOS. Using a knockdown of the mitochondrial motor protein, MIRO1, we demonstrate mitochondrial transfer is necessary for the CA-MSC-mediated rescue of mito poor cancer cells. Importantly, CA-MSC mitochondrial donation occurs in vivo and is associated with decreased survival in an orthotopic ovarian cancer mouse model. Quantification of tumor cell clonal heterogeneity demonstrates CA-MSCs significantly enhance heterogeneity dependent on the capacity to donate mitochondria to tumor cells. Collectively, we report CA-MSC mitochondrial transfer as a critical mediator of ovarian cancer survival, heterogeneity and metastasis representing a potentially powerful therapeutic target.

Taylor, Tiffany

she/her/hers
Program in Microbiology and Immunology (PMI) Year 5
Advisor: Sarah Gaffen

Defining the mechanistic roles of non-canonical NF-κB transcription factors in immunity to oropharyngeal candidiasis

Tiffany Taylor, Bianca Coleman, Samyuktha Arunkumar, Ipsita Dey, and Sarah Gaffen

Oropharyngeal candidiasis (OPC) is an infection of the oral mucosa caused by the overgrowth of the fungus Candida albicans. In healthy individuals, C. albicans is typically maintained as a harmless commensal organism. However, immune impairments often promote pathogenic infections. The Gaffen lab has showed that IL-17R and IL-22R signaling on specific subsets of oral epithelial cells (OECs) is essential to prevent OPC. IL-17 induces expression of anti-microbial peptides and chemokines that promote fungal clearance. IL-22, though acting on a distinct OEC subset from IL-17, promotes oral tissue damage and helps to maintain an effective IL-17 response; thus IL-17 and IL-22 act cooperatively to prevent OPC. Mechanistically, IL-17R and IL-22 activates multiple transcription factors, such as IκBζ (encoded by Nfkbiz), a non-canonical member of the NF-B family. However, the role of IκBζ in fungal immunity is unknown. Nfkbiz deficient mice exhibit tonic inflammation and enhanced apoptosis in epithelial cells of the skin and eyes. Thus, we hypothesized that IκBζ in OECs is necessary for cytokine-driven signals that mediate host defense against C. albicans. To test this, we deleted Nfkbiz in OECs using conditional knockout mice targeting the superficial (keratin 13+) or basal (keratin 14+) OEC layers. Mice were infected with C. albicans and parameters of infection were assessed. Deletion of IκBζ in both the K13+ and K14+ OECs caused increased susceptibility to OPC, consistent with a role for IκBζ in both IL-17 and IL-22 responses. Studies using cultured OECs showed that IκBζ binds directly to the promoter of vital anti-microbial peptides that mediate antifungal immunity. Hence, IκBζ is an essential driver of host defense against C. albicans, acting in an OEC-intrinsic manner.

Poster Abstracts with Poster

SESSION #1

SESSION #2

Abdelnaby, Ahmed

he/him/his
Molecular Pharmacology (MPHL) Year 2
Advisor: Mohammed Trebak

NFAT nuclear translocation is ORAI isoform-specific

Ahmed Emam Abdelnaby, J. Cory Benson, Ryan Yoast, Scott Emrich, Ping Xin, Trayambak Pathak and Mohamed Trebak

The Ca2+ release-activated channel (CRAC) is the biophysical manifestation of store-operated Ca2+ entry (SOCE) and is mediated by five molecules, STIM1/2 and ORAI1/2/3 encoded by distinct genes. ORAI1 is known to have two alternatively translated variants, long (ORAI1) and short (ORAI1β). Ca2+ influx through CRAC is crucial for a plethora of biological processes including but not limited to proliferation, and genetic and metabolic regulation. Calcium signals near the mouth of CRAC channels lead to the activation of nuclear factors of activated T-cells (NFAT) and their translocation to the nucleus to initiate multiple gene programs. Although ORAI isoforms are all activated by store depletion, they are differentially expressed with discernible structures and functions. However, the specific calcium signature associated with each of the ORAI isoforms and the NFAT isoform they activate remains poorly understood. Here we show that both ORAI1 and ORAI1β isoforms are capable of inducing significant NFAT1 activation while ORAI2 and ORAI3 do not. Moreover, ORAI1β activation resulted in more NFAT1 translocation with faster nuclear localization dynamics than ORAI1. Our results demonstrate differential specificities and sensitivities associated with each of the ORAI molecules in mediating NFAT activation.

POSTER #2

Ahmed, Ibrahim

he/him/his
Cellular and Molecular Pathology (CMP) Year 1
Advisor: Delphine Gomez

A normal physiological expression of miR-200 is needed to maintain SMC quiescence

Ibrahim Adeola Ahmed, Cristina Espinosa-Diez, Mingyuan Du, Mingjun Liu , Jianxin Wei, Delphine Gomez

The pathological mechanisms of atherosclerotic plaque formation in vessel wall involves dysregulated lipid metabolism and trafficking leading to cholesterol-filled foam cells. Numerous regulators like lipid mediators, lipoprotein transporters, reverse cholesterol transporters, and caveolin-mediated endocytosis have been identified. During atherogenesis, smooth muscle cells (SMC) undergo phenotypic switch, acquire diverse cell forms, like foam cells, based on environmental cues, and play leading roles to the condition. miRNAs are post-transcriptional regulators of blood vessel processes, and impact phenotypic switching, proliferation, migration, and inflammation of SMC. Recently, our lab discovered the role of miR-200 family in regulating SMC quiescence through the repression of Quaking, a regulator of SMC phenotypic switching in a model of carotid artery ligation. However, how miR-200 family controls SMC's cellular energy state in atherosclerosis is not clear. In this study, we hypothesize that overexpressing miR-200 transcripts will maintain SMC quiescence secondary to cholesterol loading. Oil red staining showed lipid droplet accumulation and various foam cells presence after miR-200 over-expression and cholesterol loading. Gene expression analysis showed a decrease in miR-200 family and SMC signature contractility genes, Acta2 and Myh11, compared to the vehicle after cholesterol stimulation. Over-expressing miR-200 fails to decrease cholesterol or lipid influx after cholesterol loading but instead impaired SMC contractility phenotype while macrophage marker, Cd68, was not impacted. Our current results suggest the necessity of a steady state of miR-200 expression as a maintenance of SMC quiescence in response to lipid and cholesterol metabolism/transport.

POSTER #3

Akinyele, Olayemi Grace

she/her/hers
Molecular Pharmacology (MPHL) Year 3
Adivor: Dr. Michael Jurczak

USP30 IS AN IMPORTANT REGULATOR OF HEPATIC MITOCHONDRIAL FUNCTION IN VIVO

Olayemi Grace Akinyele, Fiona Bello, Amber Vandevender, Ian Sipula, and Michael Jurczak

Mitochondria regulate cellular metabolism and homeostasis, thus, it is crucial that cells maintain a damage-free mitochondrial network. Cells employ highly evolved quality control mechanisms to remove damaged mitochondria through mitophagy. USP30, a deubiquitinase, is an important regulator of mitophagy that antagonizes PINK1/PARKIN pathway through ubiquitin removal. However, it is unknown whether USP30 regulates mitochondrial function in liver hepatocytes in vivo. Hence, we aimed to determine the effects of modulating USP30 activity on hepatic mitochondrial function.

Tail-vein injection of AAV8-TBG-Cre into USP30fl/fl and wildtype C57BL/6J male mice was used to generate liver-specific USP30 knockout (LKO) and control (WT) mice respectively. Both groups were fed regular chow and sacrificed at 16 weeks of age, 6 weeks after AAV treatment. Mitochondrial respiratory capacity was assessed using purified mitochondria fractions extracted from the liver and the Oroboros O2K platform.

Liver-specific deletion of USP30 in mice resulted in impaired mitochondrial respiratory capacity. There was increased state 4 or leak respiration and reduced state 3 or ADP-stimulated rates of respiration in the USP30 LKO mice compared to WT. There was no difference in uncoupled respiration or the maximum capacity of the electron transport chain between groups. The impaired respiratory function in the LKO mice on regular chow may result from excessive non-selective mitophagy in otherwise healthy mice, or alternatively suggest a mitophagy-independent effect of USP30 on mitochondrial function.

These data demonstrate that USP30 is an important regulator of mitochondrial respiratory capacity in hepatocytes in vivo, and future work will focus on understanding the mechanism behind these effects.

An, Wenxi

she/her/hers
Molecular Pharmacology (MPHL) Year 4
Advisor: Sruti Shiva

Mitofusin 1-dependent regulation of mitochondrial redox and energetic function modulates vascular smooth muscle cell phenotype and proliferation

Wenxi An, Cristina Espinosa-Diez, Chris Reyes, Andrea Braganza, Sidney Mahan, Delphine Gomez, and Sruti Shiva

Atherosclerosis is characterized by compromised vessel reactivity, intimal hyperplasia, and inflammation. In response to injury, vascular smooth muscle cells (VSMC) lose their contractile function and aberrantly proliferate, driving vascular remodeling and atherosclerosis. Mitochondrial dynamics, the fusion and fission of cellular mitochondrial networks, regulate oxidant and ATP generation to maintain mitochondrial function, and changes in dynamics have been associated with VSMC phenotype switching. However, it is unknown whether mitochondrial dynamics regulate VSMC function and vascular remodeling. Preliminary data demonstrate that silencing mitofusin 1 (MFN1), a mitochondrial fusion promoter, leads to increased cellular proliferation and decreased expression of contractile genes in rat aortic smooth muscle cells (RASMC). Further, in a murine model of carotid ligation injury, deletion of SMC-specific MFN1 exacerbates intimal hyperplasia. Thus, we hypothesize that MFN1 regulates VSMC phenotypic modulation and proliferation through the regulation of mitochondrial ATP and ROS generation, and deletion of MFN1 promotes pathologic vascular remodeling. Our results show that MFN1 deletion in RASMC increased cellular proliferation accompanied by decreased ATP production and increased cell cycle progression. Treatment with dichloroacetate, to increase mitochondrial ATP, attenuated the enhanced cell proliferation indicating that MFN1 regulates cell proliferation through the change in ATP production. We also show that MFN1 deletion decreased contractile gene expression and increased cellular ROS production. Ongoing studies are testing the role of cellular ROS generation in MFN1-dependent contractile phenotype maintenance and testing the role of MFN1 in atherosclerosis mouse model.

Antos, Dani

she/her/hers
Program in Microbiology and Immunology (PMI) Year 4
Advisor: John Alcorn

Interferon λ alters immune function during influenza, bacteria super-infection
Danielle Antos, Helen E. Rich, PhD, Saran Kupul, John F. Alcorn, PhD

Each year, influenza infections result in a significant number of mortalities, a majority of which are complicated by secondary bacterial superinfection. Primary influenza infection increases susceptibility to methicillin-resistant Staphylococcus aureus (MRSA) infection by altering the host immune response, leading to more fatalities compared to single infection. Macrophages are important in superinfection resolution as they engulf, degrade, and present bacterial antigens to immune cells, leading to lymphocyte activation. While the roles of interferon-(IFN)α/β during superinfection have been characterized, type III IFNs (IFNλ) have not been as well studied. Data shows that IFNα/β are involved in type 17 attenuation after influenza infection, which indicates that IFNλ may exhibit similar functions due to overlapping signaling pathways. Though the effects of IFNλ on epithelial cells during infection have been previously outlined, the impacts of IFNλ on immune cells are less defined. We present data supporting an inhibitory role for IFNλ in vivo by altering macrophage function and dampening type 17 immunity. Global IFNλR knockout mice had lower bacterial burden during superinfection with increased levels of IL-17 and IL-22 in the airways compared to wildtype mice. Additionally, wild type and global IFNλR knockout bone marrow chimeras and conditional IFNλR knockout mice revealed that disruption of IFNλ signaling in lung immune cells leads to reduced bacterial burden during superinfection. Together, these data provide insights into still-emerging roles for IFNλ on lung immune cells, specifically lung macrophages. IFNλ is produced in high quantities during infection and lingers longer than other interferons, making its role during superinfection onset of potential importance.

Atkins, Georgia Rae

she/her/hers
Molecular Genetics and Developmental Biology (MGDB) Year 2
Advisor: Dr. Kyle Orwig

AFF4 Mutant Mouse Model of Human Azoospermia

Georgia Rae Atkins, Meena Sukhwani PhD, Yi Sheng MD/PhD, Bronwen J. Durandt, Nijole Pollock, Jimmaline J. Hardy PhD, Alexander N. Yatsenko MD/PhD, Kyle E. Orwig PhD

An infertile patient with nonobstructive azoospermia and no family history of infertility underwent whole genome sequencing and a bioinformatics pipeline to identify candidate genes for infertility. The patient was a compound heterozygote for mutations on the AFF4 gene from paternal inheritance of p.T1107A and maternal inheritance of p.K350E. Each mutation was introduced separately into the homologous region of the mouse Aff4 gene using CRISPR/Cas9 gene editing and lines were expanded. Subsequent breeding studies were completed to determine if either mutation could cause infertility. This study focused on analysis of the Aff4T1107A mouse line. Founders were fertile when heterozygote for mutation and infertile when homozygote. Further analysis of infertile founders showed spermatogenesis active in the testes, but sperm absent in the tail of the epididymis. Unexpectedly, F2 homozygotes for the mutation were found to be fertile. Homozygous founders were analyzed for CRISPR off-target edits and large base pair deletions to determine whether that might explain the infertile phenotype in founders. However, sequencing data and alignment showed no major differences from reference genome. F2 homozygotes and heterozygotes for the mutation were found to be fertile. Immunohistochemistry experiments showed potential Aff4 expression surrounding blood vessel in mouse testicular tissue and spatially specific expression in the lower caput and the beginning corpus region of mouse epididymal tissue. We will attempt to re-derive the Aff4T1107A line using different sgRNAs to see if the infertile phenotype in founders is still observed. Also, the Aff4T1107A line will be bred with the Aff4K350E line to recapitulate the compound heterozygote genotype of the patient.

POSTER #7

Bahr, Laura

she/her/hers
Cell Biology (CBMP) Year 4
Advisor: Arjumand Ghazi

Lipid-metabolic genes that coordinate innate immunity and fertility
Laura L Bahr, Francis RG Amrit, Andre Vieira, Carissa Olsen, and Arjumand Ghazi

Fertility and immunity are both energy intensive metabolic programs. Energy allocation by an organism to one process is a trade-off at the cost of the other. Lipids are an energy-rich resource whose mobilization is integral to both reproductive success and pathogen defense. We are investigating the role of lipid-metabolic pathways in the immunity-fertility dialogue through infection of the nematode Caenorhabditis elegans, a well-established molecular-genetic model organism, by the human opportunistic pathogen Pseudomonas aeruginosa (PA14).

Previously, we demonstrated that PA14 infection dramatically reduces fertility and that TCER-1, worm homolog of a human transcription elongation and splicing factor TCERG1, protects host reproductive fitness in the presence of pathogen. Our recent data suggest that PA14 infection causes rapid depletion of stored lipids in somatic tissues, but that lipid stores in developing oocytes are protected from depletion by TCER-1. Thus, TCER-1 diverts energetic resources toward reproduction. In a preliminary RNAseq study, we identified several lipid-metabolic genes antagonistically regulated by PA14 infection and TCER-1 activity. We continue to interrogate the role of TCER-1 and its targets in balancing resource allocation between the competing demands of immunity and reproduction. These results will be discussed.

POSTER #8

Balasubramanian, Sahana

she/her/hers
Cell Biology (CBMP) Year 4
Advisor: Dr. Adam. V. Kwiatkowski

Extracellular matrix driven changes in cardiomyocyte organization and adhesion
Sahana Balasubramanian, Adam Kwiatkowski

Extracellular matrix (ECM) transitions in the heart are critical for proper development and deleterious changes in ECM composition are associated with heart disease and injury. The ECM of the embryonic and perinatal heart is dominated by fibronectin, whereas the ECM in the mature heart is primarily comprised of collagen I. The fibronectin to collagen I ECM transition is associated with an increase in matrix stiffness and the activation of programs that coordinate cardiomyocyte maturation. However, it is not clear how the ECM regulates cardiomyocyte organization to promote heart tissue maturation and function. Integrins, adhesion proteins that bind ECM, also undergo spatiotemporal changes during heart development. Integrin-fibronectin adhesions decrease after birth and are replaced by integrin-collagen I adhesions, concurrent with junctional remodeling and cardiomyocyte maturation. My preliminary work has revealed that ECM composition regulates neonatal cardiomyocyte organization. On collagen, cardiomyocytes adopt a mature, rod-shaped phenotype with myofibrils coupled to cell-cell adhesions at the longitudinal ends. In contrast, cardiomyocytes plated on fibronectin are not well organized, with basal myofibrils that largely fail to integrate at long, lateral cell-cell contacts. I hypothesize that the transition from fibronectin to collagen promotes junctional maturation and cardiomyocyte polarization. Using cell biology, advance microscopy and bioengineering techniques, I will define how ECM transitions promote myofibril organization and cardiomyocyte maturation. This study will provide mechanistic insight into how cell extrinsic cues from the ECM promote cardiomyocyte organization.

Balogun, Anu

she/her/hers
Cellular and Molecular Pathology (CMP) Year 4
Advisor: Kari Nejak-Bowen

Beta-catenin inhibition as a novel strategy for Poprhyria

Anu Balogun and Kari Nejak-Bowen

Porphyrias are metabolic disorders caused by enzymatic defects in the heme biosynthesis pathway, leading to excessive accumulation of toxic porphyrins in the liver. The patterns of accumulation of toxic porphyrins define clinical features of these diseases such as hallucinations, seizures, and liver damage. These debilitating diseases remain incurable, and there is an unmet need to develop effective therapies to treat them. The xenobiotic toxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) induces hepatic porphyria in mice by inhibiting the terminal enzyme in the heme pathway, which causes the buildup of toxic porphyrins. These porphyrin intermediates cause a milieu of cellular abnormalities including inhibition of autophagy. Evidence supports crosstalk between autophagy and Wnt signaling, an evolutionarily conserved pathway that plays an important role in liver pathophysiology. Therefore, we investigated pharmacological inhibition of Wnt signaling to determine its role in autophagy during DDC-induced injury. Prior studies have unveiled a porphyrin accumulation/deaccumulation cycle that modulates porphyrin-induced protein aggregation in external and internal organs. Our data reveals that mice lacking Wnt signaling have increased induction of autophagy over baseline that contributes to the protection from injury by upregulating the autophagic process to clear accumulated toxic porphyrins. These observations collectively offer a novel opportunity to remedy porphyria by targeting the Wnt/beta-catenin signaling pathway.

POSTER #10

Baruwal, Roja

she/her/hers
Molecular Pharmacology (MPHL) Year 2
Advisor: Dr. Lan Coffman

Evaluating EZH2 as a CA-MSC therapeutic target in ovarian cancer
Baruwal R, Coffman L

Ovarian cancer (OvCa) causes more deaths than any other gynecologic cancer. The standard treatment for ovarian cancer is surgery followed by platinum-based chemotherapy. Although initial response to chemotherapy is promising, disease recurrence is observed in more than 70% of patients followed by platinum resistance. Overcoming chemotherapy resistance is critical to improving survival in OvCa. Our published data showed that OvCa cells reprogram the surrounding mesenchymal stem cells to develop tumor supporting phenotype. These reprogrammed cells, termed carcinoma associated mesenchymal stem cells (CA-MSCs), have been shown to enhance OvCa platinum resistance, highlighting the importance of stromal mediated chemoresistance in OvCa. Our data indicates that CA-MSCs can revert to normal MSCs, losing their tumor promoting properties. Given that the CA-MSC phenotype is reversible and negatively correlates with patient survival, inhibiting CA-MSC formation is a therapeutic strategy to overcome chemoresistance in OvCa. We found that MSC reprogramming into CA-MSC is mediated by epigenetic regulator, Enhancer of Zeste Homolog 2 (EZH2). We hypothesize that EZH2 is a major driver of CA-MSC reprogramming and disrupting the induction of CA-MSC EZH2 will improve OvCa response to chemotherapy. Our data shows that pharmacological inhibition of EZH2 along with EZH1, a closely related gene to EZH2, reverts CA-MSC phenotype back to MSC phenotype. Additionally, EZH1/2 inhibitor, Valemetostat, changes cisplatin sensitivity in OvCa cells in presence of CA-MSC by disrupting the tumor associated stroma. Our data contributes to improving OvCa treatment through disruption of the TME by targeting CA-MSC EZH2 induction, a fundamental step in CA-MSC epigenetic reprogramming.

Beecher, Maria

she/her/hers
Cellular and Molecular Pathology (CMP) Year 4
Advisor: Dr. Peter Lucas

MALT1 inhibition to improve sensitivity to chemotherapy in triple-negative breast cancer

Maria Beecher1,2, Dong Hu2 Linda Klei3, Linda M. McAllister-Lucas3,4 and Peter C. Lucas2,3,4,5
1.) Cellular and Molecular Pathology Program, University of Pittsburgh School of Medicine; 2) Department of Pathology, University of Pittsburgh School of Medicine; 3) Department of Pediatrics, University of Pittsburgh School of Medicine; 4) UPMC Hillman Cancer Center; 5) NSABP Foundation

Background: Breast cancer is the most commonly diagnosed malignancy in American women. The triple-negative breast cancer (TNBC) subtype has among the worst prognosis due to high rates of recurrence and metastasis. TNBC lacks targetable receptor proteins. Therefore, treatment relies upon chemotherapy, which can become ineffective upon resistance. One potential driver of TNBC treatment resistance is MALT1, the effector protein of the CARMA-BCL10-MALT1 signaling complex, which activates NF-kB in multiple cancer cell types, including TNBC. Notably, breast cancer cells demonstrate increased sensitivity to doxorubicin when MALT1 is depleted. We hypothesize that MALT1 is a pharmaceutically targetable driver of TNBC treatment resistance. Mechanistically, we hypothesize that MALT1 promotes chemotherapy resistance by enhancing DNA repair and suppressing immunogenic cell death.

Results/Conclusions: We analyzed RNAseq and proteomic data from TCGA and CPTAC and found that MALT1 is highly expressed in basal breast cancer, a subtype largely composed of TNBC. MALT1 expression is associated with reduced treatment response and survival in basal breast cancer. We utilized GDSC and CTRP databases to identify TNBC cell lines demonstrating greatest resistance to doxorubicin (MDA-MB-231, BT20, HCC1143) and showed that MALT1 protease is activated by doxorubicin treatment in these cells. Using CellTiter-Glo and Incucyte Caspase-3/7 assays, we find that MALT1 blockade, via siRNA-knockdown or MALT1 protease inhibitor treatment, results in increased apoptosis and decreased cell viability in response to doxorubicin. These studies suggest that targeting MALT1 enhances TNBC sensitivity to doxorubicin. Through these analyses, we hope to inform new approaches for improving treatment response in TNBC.

POSTER #12

Behera, Rithika

she/her/hers
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Robert Lafyatis

Transcriptional regulation of SSc dermal myofibroblasts by FOSL2 and FOXP1
Rithika Behera, Tracy Tabib, Mengqi Huang, Christina Morse, Robert Lafyatis

Systemic Sclerosis(SSc) is characterized by fibrosis, vasculopathy, and immune dysregulation. Skin fibrosis is the hallmark of SSc and is driven by the contractile action of myofibroblasts. The number of myofibroblasts in the skin correlates with the modified Rodnan skin score, the most widely used clinical measure of skin severity.Using single cell RNA sequencing, we have identified different dermal fibroblast populations and shown that SSc dermal myofibroblasts arise in two steps from SFRP2hi/DPP4 expressing progenitor population. Bioinformatic analyses of the SSc dermal fibroblast transcriptome implicated the role of transcription factors FOSL2 and FOXP1 in the first and second step of SSc myofibroblast differentiation respectively. Our aims are to understand the transcriptional regulation of FOSL2 and FOXP1 in dermal myofibroblast activity and SSc pathogenesis.

Methods: We used si-RNA to knockdown the RNA expression of FOSL2 and FOXP1 in primary dermal fibroblasts from SSc patients. The perturbed transcriptome, signaling pathways, and epigenetic changes were characterized using bulk RNA sequencing, Western blotting, and ATAC sequencing.

Results: We found that knocking down FOSL2 and FOXP1 RNA using si-RNA led to a reduction in fibrotic genes and biomarkers for SSc disease progression such as: COL1A1, THBS1, PRSS23, THY1. Activity modules of the perturbed transcriptome had a high expression in the SFRP2hi/DPP4 expressing progenitor population.

Conclusion: Our study provides a novel understanding of the transcriptional and epigenetic regulation of SSc dermal myofibroblasts by FOSL2 and FOXP1 and provides evidence of their role in the pathogenesis of SSc.We have identified target genes which are regulated by FOSL2 and FOXP1 and responsible for driving fibrosis in dermal fibroblasts.

POSTER #13

Belford, Anna

she/her/hers
Molecular Biology & Structural Biology (MBSB) Year 3
Advisor: James Conway

Insights into early stage capsid assembly of the T=9 D3 bacteriophage

Anna K. Belford , Alexis Huet , Josh Maurer, Robert L. Duda, & James F. Conway

The Pseudomonas aeruginosa D3 bacteriophage assembles an icosahedral capsid with T=9 geometry and shares ~50% sequence similarity of its major capsid protein (MCP) with that of the T=7 HK97 capsid. As with the HK97 phage, D3 has a scaffolding domain attached to the MCP rather than a separately-encoded scaffolding protein. This scaffolding domain is larger than that of HK97, and which is also predicted to be organized mostly as a coiled-coil. In order to better understand the relationship between the MCP and its scaffold domain, particularly its role in capsid assembly and size regulation, we are working to resolve the structures of the D3 proheads and mature capsid at high resolution. Our preliminary results from cryo-EM studies show that the scaffold domains in the prohead I is divided in two regions: a rigidly bound C-terminal part that goes along the MCP P-domain and folds back into a helix that extends towards the inside of the capsid, forming a flexible coiled-coil with the neighboring scaffolds. Their organizations diverge based on their location beneath either the penton or the hexons. By comparing the structures of prohead I, prohead II, and the fully mature capsid of the virion, we are able to reach a greater understanding of the multiple roles that the MCP plays in regulating the various structural states of the capsid during the viral life cycle.

POSTER #14

Braden, Dennis

he/him/his
Molecular Pharmacology (MPHL) Year 4
Advisor: Carola Neumann

Electrophilic nitroalkenes block DSB end resection and sensitize High-grade serous ovarian cancer (HGSOC) to PARP inhibitors.

Dennis Braden (1,2), Alparslan Asan (1,2), Bruce Freeman (1), Carola Neumann (1,2)

High-grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy, largely due to a lack of effective therapies. The approval of Poly-ADP ribose polymerase (PARP) inhibitors to exploit the fact that half of HGSOCs are deficient in the DNA double strand break (DSB) repair pathway homologous recombination (HR) has met with some clinical success. However, the utility of PARP inhibitors has been limited by the prevalence of mutations that restore HR and rescue genome stability. Previously, we demonstrated that the nitroalkene 10-nitro oleic acid (OA-NO2) blocks the growth of breast cancer, at least in part, through inhibition of the HR recombinase RAD51. Here, we demonstrate that OA-NO2 also has anti-cancer activity in HGSOC cell lines and sensitizes HR-proficient HGSOC to treatment with PARP inhibitors. Mechanistically, OA-NO2 is broadly able to inhibit homology-dependent DSB repair through the prevention of DSB end resection independently of RAD51. Using an unbiased mass spectrometry-based target screen coupled with click chemistry we were able to identify and confirm the putative mediator of this effect as the ATP-dependent DNA helicase Q4 (RECQL4). RECQL4 is an emerging cancer therapeutic target without any available inhibitors, and its overexpression has been linked to poor therapeutic outcomes across a variety of cancers, including ovarian. Interestingly, we demonstrate that OA-NO2 inhibits RECQL4 activity in a biochemical helicase assay. Ongoing experiments aim to further characterize the functional impact of OA-NO2 adduction on RECQL4 helicase activity and to determine whether RECQL4 inhibition is necessary for the PARPi inhibitor sensitizing effects of OA-NO2 in HGSOC.

POSTER #15

Brynes, Adam

he/him/his
Program in Microbiology and Immunology (PMI) Year 4
Advisor: John V. Williams

Human Metapneumovirus Small Hydrophobic Protein Disrupts Multiple Immune Signaling Pathways

Adam Brynes, Yu Zhang, John V. Williams

Human metapneumovirus (HMPV) is a leading cause of respiratory infections in children and the elderly. Like all pathogens, HMPV must evade immune defenses to replicate successfully, however, the viral proteins that accomplish this are poorly characterized. One example is HMPV's small hydrophobic (SH) protein. HMPV SH has been shown in vitro to inhibit signaling through the TNF receptor (TNFR1) and both type I and type II interferon receptors (IFNAR and IFNGR). Additionally, our research shows that SH is required for viral inhibition of IL-6 signaling. Despite acting on these important signaling pathways, the mechanism by which SH inhibits them is not known. Research performed by our laboratory indicates that HMPV SH inhibits type I and type II interferon signaling upstream of STAT1. More recent experiments suggest that this may be accomplished by an SH-mediated decrease in levels of cellular JAKs, a possible mechanism by which IL-6 signaling may also be inhibited. We present evidence that HMPV SH is required to inhibit IL-6 signaling during infection and further investigate SH's mechanism of action as well as the domains required for its activity. A better understanding of HMPV's interactions with immune signaling pathways could pave the way for future treatments.

Butterfield, Hannah

she/her/hers
Cellular and Molecular Pathology (CMP) Year 2
Advisor: Peter Lucas, Linda McAllister-Lucas

Dual role for the CBM signalosome in H3K27M Diffuse Midline Glioma (DMG) tumor progression

Hannah Butterfield (1,2,3), Juliana Azambuja (3,4), Andrea Cruz (1,3), Gabi Debom (3), Matthew Halbert (1,3), Taylor Gatesman (1,3), Sameer Agnihotri (5), Peter C. Lucas (3,6,7,8) and Linda M. McAllister-Lucas (3,8)
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Background: H3K27M-mutant diffuse midline glioma (DMG) is a devastating pediatric brain tumor. Surgical resection is difficult due to the location of these tumors within the brainstem. Despite decades of clinical trials, effective treatments have not been identified and DMG is uniformly fatal with a median survival of 9-12 months.

MALT1 is the downstream effector molecule of the CARD/BCL10/MALT1 (CBM) signalosome, a protein signaling complex that induces NF-B transcriptional activity. CBM activity within tumor cells drives cell survival in multiple neoplasms and was recently implicated in glioblastoma (GBM). Our lab found that in an orthotopic syngeneic GBM mouse model, deficient MALT1 protease activity within the host tumor microenvironment (TME) results in M1 tumor-associated microglia/macrophage (TAM) polarization, decreased tumor growth and increased survival. While the DMG TME is lymphocyte-depleted, TAMs are present in DMG tumors. These observations led us to question how MALT1 protease influences tumor cells and TAMs in DMG.

Methods/Results: Single cell RNAseq data revealed that the CBM signalosome member CARD9 is expressed in the microglial infiltrate of H3K27M DMG, suggesting that the CBM signalosome may play a role in the DMG TME. In addition to a role for MALT1 in TAMs, we find that DMG cancer cells demonstrate constitutive MALT1 activity and that MALT1 protease inhibition selectively reduces DMG cancer cell survival.

Conclusions/Future Directions: Our preliminary analyses suggest that MALT1 could play a role in both DMG tumor cells and TAMs to promote tumor progression and that MALT1 protease inhibition could offer therapeutic benefit. We are developing a syngeneic mouse model to evaluate the effect of inhibiting MALT1 protease activity in DMG.

POSTER #17

Casey, Allison

she/her/hers
Molecular Genetics and Developmental Biology (MGDB) Year 2
Advisors: Adrian Lee // Steffi Oesterreich

AP2B - a biomarker for Invasive Lobular Breast Cancer?

Allison N. Casey*,1,2,3, Osama Shah*,1,2,4, Dorothy Carter4 Jennifer M. Atkinson1,5, Jagmohan Hooda1, Steffi Oesterreich1,2,4,5,7 Adrian V. Lee1,2,3,4,5,6

Breast cancer is the most common cancer among American women, with an estimated 250,000 new cases of invasive breast cancer diagnosed per year. Of all breast cancers diagnoses, 80% are estrogen receptor positive (ER+) and are broadly distinguished into two main histological subtypes – No special type (NST) and Invasive Lobular Carcinoma (ILC. Despite distinct clinical, pathological, and molecular characteristics distinguishing the subtypes, ILC and NST are treated the same clinically. Diagnosis of ILC is largely based on histomorphology. Given increasing evidence for altered management of patients with ILC vs NST and opening of clinical trials with entry criteria based on histology, it is important to improve diagnosis. We set out to identify differentially methylated genes using TCGA and METABRIC. This analysis identified AP-2β, member of the AP-2 transcription factor family, as the top hypomethylated gene in ILC compared to NST. AP-2β has previously been implicated in the regulation of cell proliferation and differentiation during development. We therefore hypothesize that AP-2 is a specific and sensitive biomarker for ILC that marks the lobular lineage of breast tumors, and that its overexpression mediates survival of E-cadherin null cells. Using patient derived organoids from primary disease of the breast, we have shown an enrichment of AP-2β in ILC. Moreover, we aim to assess if E-cadherin loss-induced apoptosis of normal breast cells can be rescued with AP-2 overexpression, and if this is caused by activation of a FOXA1/GATA3/ER complex. Successful completion of this project may lead to the identification of a new biomarker and potential drug target in ILC, but will also contribute to improved understanding of ILC development and biology.

Chakraborty, Trirupa

she/her/hers
Integrative Systems Biology (ISB) Year 2
Advisor: Jishnu Das

Characterizing the impact of genomic variation in open chromatin regions in human B cells

Trirupa Chakraborty1, Swapnil Keshari1, Jingyu Fan1, Nicholas Pease1, Daniel McGrail3, Nidhi Sahni2, Harinder Singh1 and Jishnu Das

A fundamental question in gene regulation is how thousands of active enhancers coordinate cell-type-specific patterns of gene expression. The study of open chromatin regions (OCRs) - regions of accessible chromatin provides critical insights into this question. We compiled a comprehensive list of OCRs in GM12878 – a human B-lymphocyte cell line. Next, we analyzed the distribution of GWAS variants associated with 5 autoimmune diseases involving B cells in the context of these OCRs. We identified hypervariable OCRs (hvOCRs) based on the enrichment of these variants and compared them to other not enriched OCRs. We found that variants within these hvOCRs have a greater functional impact than variants in other OCRs. CADD scores, which capture different kinds of evolutionary constraints, indicate that variants in hvOCRs have more deleterious fitness consequences, at an organismal level than those in other OCRs. Additionally, using a recent deep learning approach, DeepSEA Sei we found that variants within hvOCRs were significantly more likely to have molecular phenotypes than those in other OCRs. hvOCRs were found to be enriched within higher-order transcriptional clusters of promoters, and enhancers called “hubs” defined using structural and functional genomic datasets from GM12878, underscoring functional consequences of variation within these hvOCRS. Further, while some of these hvOCRs were unique to each disease, others were shared across multiple autoimmune diseases, suggesting that variation within these hvOCRs may have broader impacts on B cell function. Overall, our analyses suggest that variation within hvOCRs have important phenotypes and warrants further investigation.

Chapman, Caitlyn

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Molecular Pharmacology (MPHL) Year 4
Advisor: Dr. Tija Jacob

Mechanisms of diazepam-induced GABAA receptor plasticity underlying benzodiazepine tolerance

Caitlyn Chapman1; Joshua M. Lorenz-Guertin1; Sabyasachi Das1; Nadya Povyesheva2; Tija C. Jacob1

Benzodiazepines (BZDs) are anxiolytic and anticonvulsant drugs that act as positive allosteric modulators of inhibitory heteropentameric GABA type A receptors (GABAARs). Use of these key clinical drugs is limited by the development of tolerance, the underlying molecular mechanisms of which are unknown. We hypothesize that adaptive mechanisms during sustained BZD treatment generate a hyperexcitable state that impairs BZD sensitivity of canonical synaptic αβγ2 GABAARs via phospho-dependent mechanisms, while additional mechanisms promote the assembly and synaptic accumulation of BZD-insensitive GABAARs. BZDs specifically bind at the interface of α1-3 or α5 subunits with the γ2 subunit, while other subunit combinations are BZD-insensitive. Membrane fractionation results from 7-day DZP vs vehicle treated mice show increased synaptic BZD-insensitive receptors and reduced extrasynaptic GABAARs without loss of γ2-GABAARs. Similarly, γ2-GABAAR co-immunoprecipitation mass spectrometry (co-IP/MS) in cortical neurons support enhanced formation of BZD-insensitive receptors with 7d DZP. We are further investigating this by in vitro FRET and in vivo co-IP/MS. Supporting a role for phospho-dependent mechanisms in tolerance, our in vivo MS analysis shows an overall upregulation of kinase expression with 7-day DZP. Ongoing biochemical and live imaging experiments are dissecting how both kinase activity and γ2 phosphorylation are involved in reducing DZP sensitivity in tolerance. This is facilitated by γ2 subunit phospho-mutant constructs containing a pH-sensitive GFP and fluorogen-activating peptide (γ2pHFAP). Uncovering DZP-induced GABAAR neuroadaptations defining tolerance will provide a foundation for approaches promoting the development of prescription drugs with safer long-term use.

POSTER #20

Chatrizeh, Mona

she/her/hers
Cellular and Molecular Pathology (CMP) Year 2
Advisor: Michael J. Morowitz

Plant based enteral nutrition is superior to artificial nutrition in recovering antibiotic induced immune suppression

Mona Chatrizeh, Jianmin Tian, Brian Firek, Matthew Rogers, Dennis Simon, Michael Morowitz

Immune suppression and bone marrow dysfunction are ubiquitous among critically ill patients. Short term, this places an already vulnerable population at additional risk of life-threatening infections. Long term, immune suppression can persist in the form of chronic critical illness which significantly worsens functional outcomes. Many studies have attempted to rescue immune function early in the care of critically ill patients but have generally failed. This may be in part due to lack of consideration about the integral role of the gut microbiome in regulating hematopoiesis and immune function. Recent murine studies have illustrated antibiotic induced dysbiosis impairs hematopoiesis and suppresses bone marrow function. Clinically, our group completed some of the first genomic studies illustrating microbiota derangements in critically ill patients, likely because of liberal use of antibiotics. In addition to antibiotics, most critically ill patients rely on enteral nutrition which shapes their microbiome. Previously we have shown artificial enteral nutrition (AEN), the default and most commonly used formula for patients requiring enteral nutrition promotes dysbiosis. In contrast, high fiber plant based enteral nutrition (PBEN) is well tolerated, promotes the growth of healthy commensal gut anaerobes, and improves outcomes in murine models. We demonstrate PBEN randomized mice exhibit improved immune recovery with higher lymphocyte and white blood cell counts following antibiotic induced bone marrow suppression. We also provide evidence that critically ill patients randomized to PBEN have higher lymphocyte counts than those that received CEN. Together, these data suggest nutrition can be a clinically relavent strategy to boost immune function in critically ill patients.

Cheng, Winnie (Yu-Wei)

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Cellular and Molecular Pathology (CMP) Year 2
Advisor: Toren Finkel

The Role of Lysosomes in Maintaining Cellular Senescence
Yu-Wei Cheng, Toren Finkel

Cellular senescence is one of the hallmarks of aging and contributes to a plethora of aging-related diseases including atherosclerosis, cardiomyopathy, and coronary heart disease. Senescence is defined as a state where the cells undergo growth arrest due to permanently exiting the cell cycle. As senescent cells accumulate in aged tissues, the capacity for tissue regeneration decreases which leads to tissue dysfunction and deterioration. Furthermore, the senescence-associated secretory phenotype (SASP) and prolonged secretion of pro-inflammatory cytokines and chemokines also result in chronic inflammation. Since cellular senescence is pro-aging and can be deleterious, identifying how senescent cells can be removed is therefore of great therapeutic interest. Although the causes of senescence have been extensively studied, how the senescence state is maintained remains elusive. One of the characteristics of cellular senescence is the expansion of the lysosome compartment. Given the role of TFEB and TFE3 in lysosome biogenesis, we propose that TFEB and TFE3 contribute to the expansion of the lysosome compartment during senescence, and depletion of TFEB and TFE3 in senescent cells will lead to an insufficient pool of functional lysosomes which ultimately lead to cell death and senescent cell clearance. Recently, our lab has developed an unbiased proteomic approach to identify proteins that are recruited to the lysosomal surface called Lyso-TurboID. This novel technique targets TurboID, a biotin ligase, to the lysosomal surface which enables biotinylation of lysosomal surface proteins that can then be purified. Thus, in addition to examining the role of TFEB and TFE3 during senescence, using this technique, we will also identify additional players involved in the process.

POSTER #22

Cole, Aidan

he/him/his
Molecular Pharmacology (MPHL) Year 2
Advisor: Dr. Katherine Aird

Metabolic reprogramming of cancer via the paracrine effects of senescence
Aidan R. Cole and Katherine M. Aird

Cellular senescence is a stable cell cycle arrest induced by various stressors, including multiple cancer therapies. Senescent cells may be tumor promoting as they produce and secrete a senescence associated secretory phenotype (SASP) comprised of pro-inflammatory and pro-tumorigenic proteins and metabolites. The SASP causes metabolic reprogramming and have been shown to stimulate to cancer progression and chemoresistance in paracrine manner. How SASP-initiated metabolic changes affect deleterious cancer phenotypes is currently under-explored. We use ovarian cancer as a model system because standard-of-care platinum -based and/or poly(ADP-ribose) polymerase inhibitor therapies induce senescence, and 90% of ovarian cancer deaths result from chemoresistance. Whether therapy-induced senescence promotes ovarian cancer chemoresistance via metabolic reprogramming is unclear. TCGA ovarian cancer patient data shows a highly significant positive correlation between the well-known SASP factor interleukin-6 (IL6) and nicotinamide N-methyltransferase (NNMT). NNMT is responsible for methylating nicotinamide, preventing its salvage to NAD+, a critical metabolite for many processes important in cancer. In vitro induction of senescence using multiple approaches increases NNMT expression in ovarian cancer cells. Culturing proliferating cancer cells in conditioned media from senescent cells induced NNMT expression, suggesting that increased NNMT is a paracrine effect of the SASP. Future experiments will seek to determine the mechanism underlying the paracrine-mediated up-regulation of NNMT by the SASP and its associated cellular reprogramming. These studies will provide insights into this process and may identify therapeutic targets to prevent or delay chemoresistance in ovarian cancer.

POSTER #23

Colussi, Nicole

she/her/hers
Molecular Pharmacology (MPHL) Year 3
Advisor: Francisco Schopfer

Membrane lipids as substrates for fatty acid nitration and signaling activity
Nicole C. Colussi, Sonia R. Salvatore, Fei Chang, Francisco J. Schopfer

Nitrated fatty acids (NO2-FAs) are endogenous electrophilic molecules that form as a consequence of metabolic and inflammatory processes. Fatty acids in tissues are mostly esterified into complex lipids with very low levels in the free acid form, making triglycerides and phospholipids the primary targets for nitration. Esterified NO2-FAs in cell membranes can be released by phospholipases to exert their signaling functions by post-translationally modifying target proteins through Michael addition reactions. Though most cellular and in vivo studies have focused on nitration of the free acid form, the evaluation of lipid nitration in membranes has been hindered by its chemical and analytical complexity. We first established the nitration of conjugated linoleic acid (CLA) present in phospholipids during macrophage activation with LPS. Nitrated products were characterized by LC-MS/MS and compared to those with direct NO2-CLA incorporation into cell membranes. We plan to apply these analytical approaches to cellular and isolated heart models of cardiac ischemia injury, for CLA is nitrated during this oxidative event and plasma NO2-CLA levels correlate with survival. To define the site of NO2-CLA formation and protein targets, we designed and synthesized alkynyl-CLA for bio-orthogonal labeling and click chemistry. This minimally modified lipid tracer will allow the detection of protein targets and facilitate the visualization of nitroalkylated proteins. The localization of protein targets in cells and cardiac tissue will be established using clearing assisted tissue click chemistry-based imaging. Finally, the proteomic and localization data will be integrated with the protective effects of NO2-CLA to elucidate the mechanism of action of this endogenous signaling lipid.

Connelly, Jaclyn

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Molecular Pharmacology (MPHL) Year 4
Qiming Jane Wang

Protein kinase D2 confers neuroprotection by promoting AKT and CREB activation in ischemic stroke

Jaclyn A. Connelly1, Xuejing Zhang1, Yapeng Chao1, Yejie Shi2, Tija C. Jacob1, Q. Jane Wang1*
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The protein kinase D family plays a major role in stress-induced adaptive responses and is a component of the diacyglycerol signaling network long-implicated in ischemia/reperfusion (I/R)-induced tissue injuries, including those caused by ischemic stroke. However, the role of PKD2 in ischemic stroke has not been investigated. This study aims to determine if PKD2 activity contributes to neuronal survival in the context of ischemia/reperfusion (I/R) brain injury. We found that PKD2 was upregulated and activated in response to in vivo or in vitro I/R injury in mouse brain tissues and primary neurons, implying a potential role in neuronal survival. Indeed, kinase-dead PKD2 knock-in (KI) mice exhibited larger infarction volumes and worsened neurological scores, indicative of increased brain injury, when subjected to 1 h transient middle cerebral arterial occlusion (tMCAO) followed by 24 h reperfusion to simulate ischemic stroke, further confirming a neuroprotective role of PKD2 in I/R injury. In addition, mouse primary neurons deficient in PKD2 activity also showed increased cell death as compared to the wild-type neurons when subjected to I/R. We have further identified AKT and CREB as two main signaling nodes through which PKD2 regulates neuronal survival during the acute phase of I/R injury. Our findings support a neuroprotective role of PKD2 in ischemic stroke, highlighting the therapeutic implication of targeting PKD2 for neuroprotection against ischemic injury.

POSTER #25

Conway, Grace

she/her/hers
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Flordeliza Villanueva, MD

Understanding the Mechanisms of Ultrasound-Targeted Microbubble Cavitation-Mediated Blood Brain Barrier Opening

Grace E. Conway, Anurag N. Paranjape, Xucai Chen, Flordeliza S. Villanueva

Introduction: Stem cell therapies are exciting new treatments for neuronal regeneration after ischemic stroke, but they have yet to become the standard of care. One challenge for these therapies is penetrating the blood brain barrier (BBB). Ultrasound-targeted microbubble cavitation (UTMC) using contrast agents (microbubbles, MB) and ultrasound (US) applied to the brain is being explored to transiently open the BBB. While UTMC-mediated BBB opening is a promising delivery strategy, its underlying mechanisms are poorly understood. We hypothesize that tight junctions (TJ) play a role in mediating BBB permeability after UTMC.

Methods: We utilized transwells with murine brain endothelial cells (EC) and astrocytes on opposite sides of a support membrane. US (1 MHz, 10 μs duration, 10 ms pulse interval) at 250 kPa was applied for 20 sec to MB in contact with ECs. Permeability was assessed using dextran. Immunofluorescence with staining for CD-31 (EC) and ZO-1 (TJ) was performed on transwells fixed 15 and 60 min after UTMC. Z-stacks of the transwells were acquired via confocal microscopy to measure paracellular gap area and mean voxel intensity of ZO-1. Data was normalized per EC. Unpaired 2-tailed t-test with correction for multiple t-tests were performed.

Results: UTMC increases dextran flux across the BBB ($p<0.05$). As shown in Figure 1, compared to no UTMC, there is an increase in paracellular gap area per cell ($p<0.05$). From 15 to 60 min after UTMC, there is a decrease in gap area ($p=0.056$) and an increase in the mean voxel intensity of ZO-1 per EC ($p<0.05$).

Conclusions: UTMC increase BBB permeability through paracellular gaps. Between 15 and 60 min after UTMC, there is closure of some paracellular gaps and a re-establishment of endothelial TJ in this model of the BBB.

Cruz-Lorenzo, Emily

she/her/hers
Program in Microbiology and Immunology (PMI) Year 2
Advisor: Tera Levin

Characterizing the antibacterial TirA pathway in the social amoeba Dictyostelium discoideum

Emily Cruz-Lorenzo, Edward Culbertson, Tera Levin

Amoeba such as Dictyostelium discoideum (Dd.) are bacterial predators that can consume pathogens like Legionella pneumophila (Lp.), the causative agent of Legionnaire's Disease, which leads to deadly outbreaks of pneumonia. As prolific bacterial phagocytes, Dd. have evolved multiple pathways to prevent and combat intracellular bacterial infections, many of which are conserved in mammalian cells. Through repeated amoeba predation, Lp. have evolved to evade these immune strategies to infect cells including human alveolar macrophages. As such, Dd. immunity serves as a strong selective pressure driving the emergence of pathogens equipped with virulence factors that drive infection of mammalian cells. However, much of Dd. antibacterial immunity is poorly understood. Previous studies identified a gene known as tirA that is important for Dd. antibacterial responses such as production of bactericidal reactive oxygen species, extracellular DNA traps, and bacterial predation. While it is known that TirA is important for antibacterial immunity, the mechanism by which it enables these responses remains undefined. TirA contains a Toll/interleukin-1 receptor (TIR) signaling domain. TIR domains are widely distributed throughout the tree of life including Toll -Like Receptors and are important for pathogen recognition and initiation of innate immune responses. Preliminary phylogenetic analysis of TirA's TIR domain suggests it functions enzymatically to cleave NAD+, generating intermediate signaling molecules that promote death of infected cells to restrict intracellular bacterial replication. I will uncover the TirA pathway using our Lp.-Dd. system to broaden our knowledge of Dd. antibacterial immunity and understand how this pathway contributes to Lp. virulence.

POSTER #27

Cruz, Andrea

she/her/hers
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Sameer Agnihotri

The Role of Tumor Microenvironment Derived Growth Factors in Pediatric Brain Tumors

Andrea Cruz, Abigail Locke, Katherine Halligan, Lauren Sanders, Allison Cheney, Ann-Catherine Jean Stanton, Robert F. Koncar, Alberto Broniscer, Olena Morozova, Thomas Pearce, Daniel Marker, Clayton Wiley, Stephen C. Mack, Mariella Filbin, Ian F. Pollack, Baoli Hu, and Sameer Agnihotri

High-grade gliomas (HGGs) are the most common fatal intrinsic brain tumors in pediatric patients. H3K27-altered diffuse midline gliomas (H3K27-DMGs), a subgroup of HGGs, are especially aggressive with a 5-year survival rate of <2% following diagnosis. These patients are in dire need of effective therapies. A common feature of H3K27-DMGs is infiltration of microglia, macrophages, other myeloid cells, collectively referred to as GAMs, and a small population of T-cells. The contribution of non-tumor cells in the tumor microenvironment (TME) can both promote and or inhibit tumor growth, thus representing an opportunity in the pursuit of novel therapeutics. In preliminary studies, we have determined that H3K27-DMG cells stimulate microglial cell secretion of heparin-binding EGF-like growth factor (HBEGF). HBEGF expression is elevated in many human cancers and known to activate the EGFR signaling pathway, which is linked to tumor cell proliferation and growth. I hypothesize that microglial-derived HBEGF activates EGFR via paracrine signaling in H3K27-DMGs. The specific aims of this research are to: (1) validate preliminary findings demonstrating that non-tumor cells in the TME produce HBEGF to promote tumor progression, and (2) investigate the functional consequence(s) of HBEGF-induced EGFR activation in H3K27-DMGs. In co-culture studies, we show that microglial cells promote H3K27-DMG cell proliferation and that HBEGF blockade attenuates glioma cell proliferation. This research uncovers an HBEGF-EGFR axis between GAMs and H3K27-DMGs and highlights an underappreciated role of EGFR signaling in pediatric HGGs.

Dalton, Michael

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Molecular Biology & Structural Biology (MBSB) Year 3
Advisor: Jonathan Coleman

Intrinsic Fiducials for Small Membrane Protein Structure Determination
Michael P. Dalton, Jonathan A. Coleman

Solute Carrier Proteins (SLC) are small membrane transport proteins that play a critical role in many physiological processes. Despite their critical importance, our understanding of how these proteins function is limited due to the difficulty of structure determination. This is particularly true for major facilitator superfamily (MFS) proteins where there are often limited protein features to drive computational alignment of particles. While tools like cryoEM have greatly enhanced the ability to determine membrane protein structures, fiducial markers are needed to help promote particle alignment. Fragment antigen-binding antibodies (Fab) or nanobodies (Nb) are the most commonly used examples, but generating these is not a trivial task. Often this is the largest barrier for membrane protein structure determination, taking a significant amount of time and resources. In the end, the resulting fiducial may be too flexible to enable high resolution structure determination, requiring more iterations of fiducial development and screening. A feature intrinsic to the protein sequence would eliminate this barrier as it would require no additional generation time, and can be purified concurrently with the transporter of interest. Here we investigate the use of an intrinsic fiducial consisting of GFP and anti-GFP Nb labeled at the each of the protein's termini to solve a novel structure of an MFS transporter. We have generated three fully functional constructs, each with varying termini lengths, and demonstrated successive improvements in fiducial rigidity and overall particle resolution in cryoEM reconstructions. We believe this method will enable us to solve a previously unknown structure and also prove to be an accessible means for membrane protein structure determination.

Dresden, Brooke

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Program in Microbiology and Immunology (PMI) Year 2
Advisor: John Alcorn

Staphylococcus aureus cell wall-anchored protein SasD is required for virulence during pulmonary infection

Brooke P. Dresden2,3, Jennifer A. Grousd1,2, Anthony R. Richardson3, Jennifer M. Bomberger3, Vaughn S. Cooper3 and John F. Alcorn1,2
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Staphylococcus aureus is an opportunistic pathogen capable of causing severe infections such as pneumonia and sepsis. Methicillin-resistant S. aureus (MRSA) is more virulent than methicillin-sensitive S. aureus, and as such MRSA has become the dominant cause of staphylococcal pneumonia. This rise of antibiotic-resistant infections highlights the importance of finding and understanding new potential drug targets. Currently, there is very little research on the in vivo role of bacterial adherence factors in respiratory infections. S. aureus cell-wall anchored proteins (CWAPs) are important in bacterial adhesion and immune evasion. We identified an uncharacterized CWAP, surface protein D (SasD), as a novel virulence factor during staphylococcal pneumonia. A mutant lacking SasD (sasD A50.1) was created in the MRSA strain JE2 via transduction. In mice infected with sasD A50.1, bacterial burden, levels of inflammatory cytokines, and mortality were all decreased compared to wildtype JE2. Mice infected with the sasD A50.1 also had reduced immune cell infiltrate in the bronchoalveolar lavage compared to mice infected with wildtype JE2. Cloning of a plasmid expressing SasD into sasD A50.1 led to a partial restoration of bacterial burden and immune infiltrate in mice. Additionally, macrophages exposed to sasD A50.1 in vitro showed increased viability and decreased levels of inflammatory cytokines compared to wildtype S. aureus, implying SasD may impact macrophage cell death pathways. In the future, we aim to understand the mechanisms behind these phenotypes. These studies help characterize SasD as a potential target during infection to reduce inflammation and infection severity during pneumonia caused by S. aureus.

POSTER #30

Duray, Alexis

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Program in Microbiology and Immunology (PMI) Year 4
Advisor: John Alcorn

Deletion of Nrf2 during Influenza-Staphylococcus aureus super-infection leads to increased bacterial burden

Alexis Duray, Kevin J. McHugh and John F. Alcorn

Secondary bacterial infection can lead to worsened disease outcomes during viral infection. Millions of people every year are infected with influenza and in severe cases will go on to acquire secondary bacterial pneumonia. As such, alleviating or preventing secondary bacterial infections is an area of intensive research. Here, we show that loss of nuclear factor erythroid 2-related factor 2 (Nrf2) in mice during influenza infection worsens secondary Staphylococcus aureus (SA) infection. While Nrf2 has been intensively studied in the context of cancer and viral infection due to its role in promoting the expression of antioxidant response genes, its role in bacterial infection, as well as viral-bacterial super-infection remains understudied. In mice, global knockout of Nrf2 results in increased SA burden in the lung during influenza super-infection. This is also accompanied by increases in lung immune cell infiltrate and the increase of IL-17A, indicating a role for altered T lymphocyte function. Global Nrf2 knockout mice also have decreased expression of the macrophage scavenger receptor MARCO, indicating increased SA burden could be a result of macrophage dysfunction. Future experiments will include targeted knockouts of the gene, including in epithelial cells, macrophages and T cells to determine the impact each population has on the global phenotype, as well as functional assays to determine changes in effector functions.

Ermine, Kaylee

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Molecular Pharmacology (MPHL) Year 4
Advisor: Dr. Lin Zhang

Targeting Defective Necroptosis in Colorectal Cancer to Overcome Therapeutic Resistance

Ermine, K (1, 2), Chen, D (1), Yu, J (2, 3), and Zhang, L (1, 2)
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Colorectal cancer (CRC) is a leading cause of cancer related deaths and is typically treated with chemotherapeutic drug 5-fluorouracil (5-FU), but resistance often occurs. It is well-established that 5-FU induces apoptosis in cancer cells, but apoptosis inhibition does not block the anticancer effect of 5-FU, suggesting a role of non-apoptotic cell death. Necroptosis is a regulated form of necrosis controlled by Receptor-Interacting Protein kinase 1 (RIP1), RIP3, and Mixed Lineage Kinase domain-Like (MLKL), and its role in anticancer therapy is unclear. TCGA RNAseq data show that RIP3 is downregulated in primary CRC tumors, which is correlated with worse outcomes. RIP3 downregulation also occurs in CRC cells and is associated with decreased 5-FU sensitivity. Therefore, we hypothesize that necroptosis plays a critical role in anticancer therapy and that restoring defective necroptosis may enhance therapeutic sensitivity. Our studies show that RIP3-expressing CRC cells undergo both apoptosis and necroptosis in response to 5-FU, which is abrogated in RIP3 knock-out cells. Additionally, stable restoration of RIP3 in RIP3-silenced (RIP3-) CRC cells appears to enhance 5-FU response when apoptosis is inhibited. Furthermore, the requirement of RIP3 for necroptosis induction in RIP3- cells was bypassed by using natural compound OSW-1. We found that combining OSW-1 with 5-FU results in a synergistic effect, along with both necroptosis and apoptosis induction. Together, our results suggest that necroptosis plays an important role in 5-FU-induced cell death and inducing necroptosis could enhance 5-FU sensitivity, overcoming resistance. These results provide novel insight into the role of necroptosis in anticancer therapy, which could lead to improved and personalized treatments.

POSTER #32

Farrell, Corinne

she/her/hers
Cell Biology (CBMP) Year 5
Advisor: Michael Butterworth

Sexual dimorphic regulation of microRNAs alters sodium transport in the kidney distal nephron

Farrell, C., Ozbaki Yagan, N., Liu, X, Bodnar, A.J., Ho, J. and Butterworth, M.B

Hypertension affects more than one billion people worldwide. A key regulator of blood pressure is aldosterone. Aldosterone increases sodium transport in the kidney distal nephron. Premenopausal women are less likely to develop hypertension than age-matched men, due to estrogen signaling. We previously demonstrated that aldosterone alters the expression of miRs in collecting duct epithelial cells to modulate the Na+ transport response to aldosterone. However, the sex-specific regulation of miRs and role of estrogen to alter miR expression has not been explored. This study investigates how estrogen alters aldosterone signaling by regulating the expression of miRs in the CCD. Cells were incubated with estrogen for time and dose responses to determine the impact on aldosterone stimulation and Na+ transport. RT-qPCR quantified miR and mRNA expression and western blot quantified protein expression after aldosterone and estrogen stimulation. In vivo regulation of miRs by aldosterone was confirmed using isolated CCD cells from mice placed on low Na+ diets. A sex-specific upregulation of the miR-17~92 cluster was observed in female mice placed on a low-Na+ diet to stimulate aldosterone release. MiR19 was also upregulated in mCCD cells stimulated with estrogen. E2 pretreatment blunted aldosterone stimulation, by targeting SGK1 mRNA. Luciferase assays demonstrated that miRs-19a&b bind to the 3’-UTR of SGK1. Overexpression of miR-19 in mCCD cells using miR mimics inhibited aldosterone stimulation of Na+ transport. The miR-17~92 cluster is regulated by aldosterone and estrogen. Mir-19 targets SGK1 and may account for estrogen’s inhibition of aldosterone signaling. This miR cluster may be responsible for the sex-specific differences in aldosterone signaling.

Forman-Rubinsky, Rachel

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Molecular Genetics and Developmental Biology (MGDB) Year 5
Advisor: Michael Tsang

Uncovering the role of cited4a in zebrafish heart regeneration
Rachel Forman-Rubinsky, Daniel A. Zuppo, Michael Tsang

Myocardial infarction leaves the heart permanently damaged and more susceptible to future cardiac failure. This is because almost all mammalian adult cardiomyocytes (CMs) are in a post-mitotic, non-proliferative state and therefore fail to replace the damaged tissue. In contrast, Zebrafish can fully regenerate their hearts following injury. Through RNA-sequencing of adult zebrafish hearts during regeneration we identified genes that become highly expressed at the onset of CM proliferation at 3 days post-amputation(dpa) compared to uninjured hearts. As expected, the upregulated transcripts included many cell-cycle genes involved in CM proliferation. We also identified cited4a, a transcriptional co-activator classified by its interaction with the CBP/p300 complex. Surprisingly cited4a does not appear to be involved in activating CM proliferation, as its expression was highest in non-proliferating CMs following injury. To understand the role of cited4a during heart regeneration we created a cited4apt38a mutant carrying a frameshift mutation caused by a 95bp deletion. cited4a mutants have more proliferating CMs at 7dpa and less fibrosis remaining at 20dpa compared to WT. Additionally, the area of CM dedifferentiation marker embCMHC expression was significantly expanded in cited4a mutants. These observations demonstrate that loss of cited4a leads to accelerated heart regeneration. Through interactions with the CBP/p300 complex or other transcription factors, cited4a may prevent activation of transcriptional programs required for dedifferentiation and subsequent proliferation in a subset of CMs during heart regeneration. This response to injury could be important for limiting CM dedifferentiation to maintain heart function during regeneration and limiting cardiac growth.

POSTER #34

Garcia, Geyon

he/him/his
Molecular Genetics and Developmental Biology (MGDB) Year 2
Advisor: Lan Coffman

Uncovering the mechanism by which high-risk mesenchymal stem cells orchestrate high grade serous ovarian cancer initiation

Geyon L. Garcia (1), Huda Atiya, PhD (2), Taylor Orellana, MD (3), Lan Coffman, MD/PhD (4)

High grade serous ovarian carcinoma (HGSOC) is the 5th most deadly cancer in women. Despite the high mortality of HGSOC, little is known of how this HGSOC initiates. Recent evidence from our group and others have demonstrated that mesenchymal stem cells (MSCs) play an important role in HGSOC initiation. MSCs are key progenitor cells in the fallopian tube stroma that support the growth of local epithelial cells. Our group has shown that carcinoma associated mesenchymal stem cells (CA-MSCs) develop from normal MSCs via epigenetic changes brought about by the developing cancer. CA-MSCs subsequently contribute to cancer progression and metastasis. We recently uncovered a potential precursor to CA-MSCs termed high risk MSCs (hrMSCs) that are present in healthy human fallopian tube stroma as well as the stroma of HGSOC and other precursor lesions. To our surprise hrMSCs promote HGSOC development from healthy, non-cancerous, primary fallopian tube epithelial cells (FTE). The mechanism by which this occurs remains unknown. Interestingly, we've discovered that hrMSCs cause DNA damage in the surrounding epithelia as evidenced by gH2AX foci and western blot. gH2AX foci co-localized with 53BP1 foci; a protein that localizes and binds to DNA double strand breaks (DSB). Therefore, I hypothesize that hrMSCs elicit DNA DSBs that serve as a selective pressure on epithelial cells thereby promoting malignant transformation.

Gatesman, Taylor

she/her/hers
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Sameer Agnihotri

Inhibiting insulin signaling reverses resistance to PI3K-mTOR inhibitors in aggressive pediatric high-grade gliomas

Taylor A. Gatesman, Katharine E. Halligan, Matthew E. Halbert, Ann-Catherine J. Stanton, Andrea F. Cruz, Brian J. Golbourn, Ian F. Pollack, Stephen C. Mack, Sameer Agnihotri

Pediatric high-grade gliomas (pHGGs) are among the most lethal brain tumors with a 5-year survival rate of only 20%. MYCN pHGGs represents one subgroup with an unmet need for therapeutics. MYCN belongs to the family of MYC transcription factors that regulate numerous cancer hallmarks. While no direct inhibitors of MYCN are in clinical trial, current strategies focus on targeting the MYCN regulators or downstream effectors. Lack of pre-clinical models contribute to limited therapeutic efficacy. To address these knowledge gaps, we developed a novel mouse model of MYCN pHGG using the FLEx-Cre switch system, whereby neural stem cells are selectively delivered with MYCN cDNA and shRNA targeting the tumor suppressor genes p53 and Pten. We identified that this model harbors hyper-activation of the PI3K/AKT/mTOR signaling pathway. We demonstrate that dual PI3K-mTOR blood brain barrier penetrant inhibitors are effective in reducing pHGG growth and MYCN protein levels. We also developed a novel drug-resistant model of MYCN pHGG as a mechanistic tool to identify relevant resistance mechanisms to re-acquisition of the PI3K-AKT-mTOR pathway. We identified the insulin growth factor signaling pathway as our top mechanism of resistance. We hypothesized that MYCN is a critical driver of pHGG and can be effectively targeted via dual inhibition of the PI3K-mTOR and IGF/Insulin pathways. We tested next generation inhibitors of IGF and PI3K-mTOR pathways and performed genetic and pharmacological assays in our MYCN pHGG gliomas. We also investigated this mechanism in human MYCN pHGG cells. Dual inhibition in our MYCN pHGG model and human MYCN cells were synergistic, leading to significant decreases in cell growth and MYCN signaling.

POSTER #36

Gilmer, Gabrielle

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Cellular and Molecular Pathology (CMP) Year 2
Advisor: Fabrisia Ambrosio

Systematic review and meta-analysis of menopausal knee osteoarthritis animal models and mathematical modeling of estrogen treatment

Gabrielle Gilmer, Allison Bean, Hirotaka Iijima, Natalie Jackson, Christopher Evans, Rebecca Thurston, Fabrisia Ambrosio

Post-menopausal women are disproportionately affected by osteoarthritis (OA), yet most animal models of OA are in male mice. Here, our purpose was to (1) summarize the state-of-the-science aimed at understanding menopausal effects on OA in animal models and (2) investigate how dosage and timing of initiation of estrogen treatment affects cartilage degeneration. A systematic review identified articles studying menopausal effects on cartilage in animal models. Meta-analyses were performed on overlapping cartilage metrics in conjunction with rigor and reproducibility analyses. Mathematical modeling was used to evaluate the relationship between cartilage degeneration and the timing of initiation or dosage of estrogen treatment. Thirty-eight manuscripts were included. The most used animal models were the knees of ovariectomized young rodents. Most studies did not adequately report inclusion criteria, animal monitoring, protocol registration, and data accessibility. Cartilage histological scoring, cartilage thickness, type II collagen expression, and CTX-II were significantly worse in post-menopausal animals compared to non-menopausal animals. Estrogen and SERM treatment modulated markers of cartilage degeneration, suggesting a chondroprotective role. Moreover, cartilage health was improved with earlier initiation of estrogen treatment and higher doses. To improve translatability, animal models that include aging and perimenopause should be incorporated into studies of menopause and OA. Our review highlights the significant need for increased attention to rigor and reproducibility and mechanistic studies into non-estrogen mediated pathways of OA. Lastly, timing of treatment initiation and dosage may be important factors modulating therapeutic effects of estrogen on cartilage.

Gonzalez-Ferrer, Shekina

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Program in Microbiology and Immunology (PMI) Year 4
Advisor: Janet Lee

STAT1 regulates excessive neutrophilic inflammation and limits lung Injury in a model of pneumonia-induced sepsis

S. Gonzalez-Ferrer, H. Peñaloza, A. McCollum, R. van der Geest, N. Kohli, M. Tabary, Z. Xiong, T. L. Suber, W. Bain, D. Van Tyne, A. Ray, P. Ray, J. Le

Acute respiratory distress syndrome (ARDS) is often characterized by immune dysregulation with excessive neutrophilic inflammation. Studies have shown an interferon signature in BALF and peripheral blood of ARDS patients, but the relationship between excessive neutrophilic inflammation and this interferon signature, if any, remains unclear. Using Stat1-/- mice to study the impact of loss of interferon signaling on pneumonia-induced sepsis, we hypothesized that interferon signaling regulates neutrophil response and limits lung injury in a model of pneumonia-induced sepsis. C57BL/6 (WT) and Stat1-/- (KO) mice were intratracheally inoculated with a Klebsiella pneumoniae clinical respiratory isolate. By bulk-RNA seq analysis, KO mice exhibited an exaggerated neutrophil transcriptional signature in the lungs but showed no significant differences in lung bacterial burden at 24 h post-infection when compared to WT mice. By 48h, KO mice exhibited significantly higher levels of the pro-inflammatory cytokines and chemokines in the lung, which was accompanied by increased lung bacterial burden and extrapulmonary dissemination to the kidney and liver, whereas WT mice showed minimal to no KP burden at extrapulmonary sites. By 72h, KO mice displayed increased BAL neutrophil counts, free NE activity, total protein content, and IgM when compared to WT mice. H&E-stained lung sections of KO mice also showed more inflammation at 72h compared to WT mice. In an experimental model of bacterial pneumonia-induced sepsis, loss of interferon signaling led to an early neutrophil-dominant transcriptional response in the lung that preceded widespread bacterial dissemination and development of lung injury.

Goossen, Christian

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Molecular Pharmacology (MPHL) Year 2
Advisor: Dr. Patrick Pagano

Endothelial NOX1-Mediated AMPKα1 Propagates Hypoxia-Induced Pulmonary Hypertension via the Cip/Kip family of CDK inhibitors

Christian J. Goossen and Patrick J. Pagano

Pulmonary arterial hypertension (PAH) is a rare, devastating disease with the initial pathognomonic instigator being a combination of genetic and environmental, injurious factors including hypoxemia and reactive oxygen species (ROS) signaling. We postulated that NADPH oxidase 1 (NOX1) and attendant ROS mediate EC proliferation and hemodynamic changes occurring in PAH. Findings from our lab have shown induction of NOX1 expression by approximately 1.6 ± 0.10-fold ($n=7-9$, $p<0.01$) in human pulmonary artery endothelial cells (HPAECs) under hypoxic conditions. Furthermore, the laboratory's preliminary findings implicate AMPK isoform alpha-1 (AMPKα1) as a potential lynchpin in the NOX1 pro-proliferative pathway, showing an induction of activated (phosphorylated) AMPKα1 by 2.03 ± 0.22-fold ($n=4$, $p<0.001$), partially reverted when treated with a small interfering RNA inhibiting NOX1 expression (siNOX1) in HPAECs under hypoxic conditions. Using the bioinformatic tools we discovered that phosphorylated/activated AMPKα1 is associated with activated cyclin-dependent kinase inhibitor 1B (p27kip1), cyclin-dependent kinase inhibitor 1 (p21cip1), and cyclin dependent kinase inhibitor 1C (p57kip2) - all members of the Cip/Kip family of CDK inhibitors. The labs preliminary findings implicate this, showing a 1.62 ± 0.11 -fold decrease ($n=5-6$, $p<0.001$) of p21cip1 in HPAECs under hypoxia, partially reverted with the treatment peptidic inhibitor selective for NOX1 (NoxA1ds) developed in the laboratory. In conclusion, based on preliminary data, we postulate that NOX1 mediates oxidative activation of AMPKα1, which, in turn, is expected to phosphorylate multiple Cip/Kip proteins and lead to their disinhibited binding from cyclin/CDK and cell cycle progression - EC hyperproliferation in PAH.

Griswold, Kira

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Program in Microbiology and Immunology (PMI) Year 4
Advisor: Dr. Terence Dermody

Transneuronal dissemination of mammalian orthoreovirus

Kira Griswold,1,2 Pavithra Aravamudhan,2,3 Megan Smith,4 Alan Watson,4 William DePas,3 Terence Dermody1,2,3
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Mammalian orthoreovirus is a nonenveloped virus that spreads along neural routes to infect the central nervous system (CNS). Reovirus transmission within peripheral neurons is predominately retrograde. However, it is not known how reovirus is transmitted between neurons in the brain. We hypothesize that reovirus transits bidirectionally in the CNS. Using whole-brain 3D imaging of reovirus infection, we observed that reovirus targets neuronal subtypes that may be synaptically connected and represent a neural circuit. Neural circuits are networks of neurons connected by axonal projections across brain regions to execute functions. To investigate mechanisms of reovirus neurotransmission, we used cultures of primary rat dorsal root ganglion (DRG) neurons. Accumulation of reovirus progeny in DRG neuron culture supernatants does not coincide with release of lactase dehydrogenase, indicating that reovirus release occurs in the absence of cell membrane disruption. To assess transneuronal dissemination, we cultured DRG neurons in microfluidic devices, in which neuron cell bodies and axons are present within fluidically isolated chambers. Reovirus transport in DRG neurons cultured without neurons in the opposing chamber was predominately retrograde. However, the presence of neuron-to-neuron contacts in microfluidic chambers appeared to allow reovirus to transport bidirectionally. Moreover, reovirus infectivity is enhanced when synapsed DRG neurons are treated with tetrodotoxin, a neurotoxin that inhibits neural firing and strengthens synaptic connections. Together, these data suggest that reovirus spread within the brain occurs between synaptically connected neurons, allowing reovirus to use neural circuits as highways for viral dissemination.

Hahn, Gabriella

she/they
Molecular Genetics and Developmental Biology (MGDB) Year 3
Advisor: Judy Yanowitz

Characterization of chd-7 and ztf-6 in dauer formation

Gabriella M. Hahn, Martín Jofré, Dane K. Hoffman, Luciana Godoy, Dr. Daniel Hochbaum, Dr. Judith Yanowitz

In Caenorhabditis elegans, Insulin/Insulin-like signaling (IIS/DAF-2) and TGF-β (DAF-7) signaling converge to regulate expression of DAF-9 to either promote development in favorable conditions or promote dauer in unfavorable conditions. Dauer is a specialized life stage that lengthens lifespan and protects the worm until conditions are favorable to continue reproductive development. CHD-7 (chromodomain helicase domain protein) and ZTF-6 (zinc finger putative transcription factor) are both transcriptional targets of the switch protein DAF-12 that ultimately makes the decision to dauer or not. We have recently shown that CHD-7 acts downstream in the TGF-β pathway to regulate dauer formation. chd-7 mutant worms do not undergo proper dauer morphogenesis and arrest as “partial” dauers with abnormalities in the specialized dauer cuticle and overgrowth of the germ line. The mutants also proceed into normal development faster than control worms but exhibit a drastic loss in fertility post-recovery. We showed that expression of DAF-9 is upregulated in chd-7 mutant dauers, consistent with a role of CHD-7 as a transcriptional repressor and suggesting it may work directly with the TGF-β SMADs to repress target genes. While we have a more complete picture of CHD-7’s role in dauer, we are in the early steps of investigating ZTF-6. I will show preliminary data of how loss of ztf-6 affects dauer formation and morphology as well as post-dauer phenotypes such as morphological defects and fertility. We are also interested in potential interactions between CHD-7 and ZTF-6 to regulate development. chd-7 and ztf-6 mutants share phenotypes, suggesting they may interact directly and/or share transcriptional targets.

POSTER #41

Halbert, Matthew

he/him/his
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Wendy Mars

MAT2A loss compromises methionine metabolism and represents a vulnerability in histone mutant gliomas

Matthew E. Halbert, Brian J. Golbourn, Katharine Halligan, Ann-Catherine Jean Stanton, Abigail L. Locke, Stephanie M. Casillo, Michelle Wassell, Taylor A. Gatesman, Andrea F. Cruz, Stephen C. Mack, Sameer Agnihotri

H3K27-mutant diffuse midline gliomas (DMGs) are inoperable and resistant to chemo/radiotherapies. Median survival ranges from 8-11 months, with 2% of patients surviving beyond 5 years. H3K27M mutations lead to global epigenetic and transcriptional reprogramming driven by the global loss H3K27 trimethylation. Loss of H3K27me3 is an initiating event in gliomagenesis. This disease lacks appropriate models to predict disease biology and response to treatment. Therefore, we developed a novel syngeneic H3K27M mouse model. An unbiased integrated systems biology approach identified that H3K27M relied on the amino acid methionine and the enzyme Methionine Adenosyltransferase 2A (MAT2A). MAT2A is a central regulator of one-carbon metabolism by converting methionine to S-adenosylmethionine, the universal methyl-donor. In complementary genetic approaches, we applied these findings to patient-derived cell lines with the H3K27M mutation. We hypothesize that MAT2A abrogation, genetic/pharmacological, would alter DMG viability by disrupting the methylome. We provide a novel mechanism whereby H3K27M cells are sensitive to MAT2A loss, because Adenosylmethionine Decarboxylase 1 overexpression disrupts MAT2A regulation. This results in H3K27M cells having lower MAT2A protein levels, conferring a sensitivity by inhibiting residual MAT2A. Genetic/pharmacological aberrations to MAT2A resulted in reduced proliferation. In vivo syngeneic and patient-derived xenograft models with both inducible MAT2A knockdown or methionine restricted diets showed extended survival. These results suggest novel interactions between methionine metabolism and the epigenome of H3K27M gliomas and provide evidence that MAT2A, presents exploitable therapeutic vulnerabilities in histone mutant gliomas.

POSTER #42

Hall, Robert

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Molecular Pharmacology (MPHL) Year 2
Advisor: Adam Straub

Discerning the mechanisms of CYB5R1 regulation of ferroptosis in endothelial cells
Robert Hall

The cytochrome b5 reductase (CYB5R 1-5) family of enzymes are known to regulate redox balance in the cardiovascular system. We recently conducted a large-scale genomic screen comparing differences in gene profiles from all five CYB5R enzymes that were knocked down in primary human aortic endothelial cells (HAECs). Pathway analysis revealed that loss of CYB5R1 increased genes pertinent to ferroptosis. Interestingly, one publication demonstrated that CYB5R1 mediates ferroptosis in HeLa cells. Ferroptosis is an iron-dependent form of cell death triggered by intracellular iron accumulation and elevated hydrogen peroxide (H2O2) levels, which together lead to lipid peroxidation and cell death. Importantly, glutathione peroxidase 4 (GPX4) is a key enzyme that protects against ferroptosis by quenching lipid peroxides. Emerging evidence supports the idea that ferroptosis contributes to numerous cardiovascular diseases. However, the mechanisms of CYB5R1 regulation of ferroptosis in endothelial cells have not been elucidated. We have demonstrated that CYB5R1-deficient HAECs exhibit heightened non-mitochondrial oxygen consumption, H2O2 generation, and lipid peroxide levels. Leveraging this information, we tested whether loss of CYB5R1 modulates ferroptosis in HAECs. We show that CYB5R1 is localized to the outer mitochondrial membrane (OMM) and the loss of CYB5R1 exacerbates ferroptosis when triggered by GPX4 inhibition. These effects are completely reversed with ferrostatin-1, an inhibitor of lipid peroxidation. Mechanistically, it is known that CYB5R enzymes can reduce coenzyme Q, a potent antioxidant. Together, we hypothesize that mitochondria bound CYB5R1 protects endothelial cells from ferroptosis by mitigating membrane lipid peroxidation through reducing OMM coenzyme Q pool.

POSTER #43

Harancher, Mitch

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Molecular Genetics and Developmental Biology (MGDB) Year 4
Advisor: Kyle Orwig

A Germline Gene Editing Approach to Address Iatrogenic Infertility and Disease Transmittance Using Sickle Cell Disease Models

Mitchell R. Harancher1,2, Kien T.D. Tran1,2, Yi Sheng2, Amanda M. Colvin Zielen2, and Kyle E. Orwig1,2

Sickle cell disease (SCD) is a crippling disease that significantly shortens lifespan, decreases quality of life, and has a fiscal burden of $2.98 billion per year in the U.S. Individuals with SCD have a high risk for infections, severe pain crises, organ pathogenesis, anemia, and psychological stress, all of which worsen disease progression. Disease symptoms can be managed with medical treatments such as blood transfusions and hydroxyurea or even cured with hematopoietic stem cell transplantation. However, these treatments can cause infertility. Reports indicate that SCD patients place great value in maintaining their fertility.To combat infertility, the fertility preservation program in Pittsburgh is actively cryopreserving ovarian tissues and testicular tissues for young sickle cell patients to safeguard their future fertility (Funded by: P50HD096723). Prepubertal boys have spermatogonial stem cells in their testes that can potentially be transplanted to restore spermatogenesis in healthy survivors of SCD. This creates an opportunity for targeted gene correction of SCD in spermatogonial stem cells (SSCs), ex vivo, prior to transplantation. We acquired and established a breeding colony of the Towne’s mouse model of SCD disease and confirmed the SCD phenotype in blood and tissues. We validated sgRNAs to target CRISPR/Cas9 gene editing to the human beta-globin locus. In parallel, we are testing the potential of Towne’s mouse SSCs to be maintained in long-term culture, which is necessary for gene editing as well as subcloning and testing of gene edited lines. In the future, gene edited Town’s mouse SSCs will be transplanted into busulfan-treated Towne’s mouse recipients to restore spermatogenesis and produce progeny with a corrected beta-globin locus.

POSTER #44

Harkness, Trey

he/him/his
Molecular Pharmacology (MPHL) Year 2
Advisor: Robert J. Binder, PhD

Dissecting the roles of CD91 in Heat Shock Protein-mediated cancer immunosurveillance
Harkness, Trey and Binder, Robert J., PhD

Cancer immunosurveillance is dependent on the heat shock protein (HSP) receptor, CD91, which is expressed on professional antigen-presenting cells (APCs). While CD91-facilitated cross-presentation of HSP-chaperoned peptides has been well studied, CD91-mediated signaling is not completely defined. The binding of CD91 by immunogenic HSPs leads to production of unique inflammatory cytokine profiles by APCs. This requires the phosphorylation of tyrosine residues on intracellular domains of CD91 and activation of NF-B. However, adaptor proteins associated with CD91 and molecules involved in signal transmission have not been characterized and their role in cancer immunosurveillance remains untested. Our general strategy to identify adaptor proteins involves crosslinking and co-immunoprecipitation of CD91 and its associated signaling complex following stimulation of CD91 with the immunogenic HSPs gp96, calreticulin, and hsp70. Preliminary studies with gp96 have identified Shc as an adaptor protein and AXL receptor tyrosine kinase and tyrosine-protein kinase Fgr as membrane associated kinases. The impact on their inhibition on cytokine release by APCs is being tested. We have also developed a novel mouse expressing mutant, non-signaling CD91, and its impact on cancer immunosurveillance will be determined. Investigation of these complexes will aid in understanding the signaling pathway that activates production of cytokine profiles within APCs. This project will allow us understand the pathways to priming immune responses responsible for cancer immunosurveillance and also to develop better therapeutic strategies for cancer and autoimmune diseases.

Hillebrand, Gideon

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Program in Microbiology and Immunology (PMI) Year 2
Advisor: Tom Hooven

A CRISPR/Cas-based gene knockdown approach identifies group B Streptococcus surface-associated proteins that dampen macrophage inflammatory signaling

Kathyayini P. Gopalakrishna, Gideon H. Hillebrand, Venkata H. Bhavana, Jordan L. Elder, Lisa M. Rogers, David M. Aronoff, Thomas A. Hooven

Group B Streptococcus (GBS) can lead to severe maternal infections, preterm delivery, or stillbirth. Macrophages are important cellular components of innate immunity and play key roles in pregnancy homeostasis by clearing pathogens from the placenta and maintaining a cytokine milieu conducive to pregnancy retention. Little is known about how specific GBS surface-associated proteins interact with macrophages and how those interactions might affect intrauterine or neonatal inflammation. We developed a CRISPR/Cas-based GBS gene expression knockdown library and applied it to a macrophage co-incubation model to assess the effect of conserved GBS surface-targeted proteins on macrophage cytokine expression. Our results demonstrate the potential of CRISPRi as a method for rapid generation and screening of GBS targeted gene expression knockdown strains. Our macrophage co-incubation data indicate that multiple previously uncharacterized GBS surface-associated and secreted proteins suppress pro-inflammatory responses in cultured THP1 macrophages. Three candidate genes have significant dampening effects on both TNFα and IL-1β pathways. We hypothesize that these newly identified surface proteins “cloak” GBS from innate immune responses, potentiating persistent colonization and host invasion. Ongoing work to validate, further characterize, and evaluate these candidate proteins with primary human placental macrophages is underway.

POSTER #46

Hinchie, Angela

she/her/hers
Molecular Pharmacology (MPHL) Year 5
Advisor: Jonathan Alder

A variant telomere template influences the mechanism of telomere addition in humans

Angela Hinchie BS, Samantha Sanford PhD, Rachel Sutton MS, Anishka Parikh, Agustin Gil Silva BS, John F McDyer MD, Patricia Opresko PhD, Jonathan Alder PhD

The sequence of telomere repeats, TTAGGG, has been exceptionally well conserved in a vast number of species including all vertebrates. This sequence is essential for normal telomere function, both to efficiently bind sequence specific Shelterin components and recruit telomerase. Changing this sequence by expressing telomerase with a variant template leads to a DNA damage response and inhibits cell proliferation. Despite this, we identified an idiopathic pulmonary fibrosis patient heterozygous for a variant in the telomerase template, expected to add a variant sequence to the telomere. Overexpression of the variant sequence in cell culture led to a minor DNA damage response and decreased proliferation. We analyzed the telomere content of two family members with the template variant and found a variant telomere content of 2.4 and 9.1% in parent and child respectively compared to 0.6% in controls. The template variant profoundly inhibited telomerase processivity, which could be partially rescued with a compensatory mutation. In both biochemical and cell culture experiments there is no evidence that the variant telomere addition leads to a dominant negative effect. Despite the clear decrease in telomerase function, the original patient was found to have telomere length within the normal range. The relative tolerance of this sequence in heterozygous individuals suggests that telomeres may tolerate more degeneracy than previously thought. Telomere dysfunction caused by sequence changes in addition to and/or independent from length may be sufficient to cause disease in humans.

POSTER #47

Hong, Lisa

she/they
Molecular Pharmacology (MPHL) Year 4
Advisor: Carola Neumann

Targeting Autophagy in PARPi-resistant Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is the most lethal breast cancer subtype and lacks targeted therapy. Poly-ADP ribose polymerase inhibitors (PARPi) are available as adjuvant therapy for patients expressing germline mutBRCA1/2; however, the inevitable resistance underlies the need for a better understanding of PARPi resistance mechanisms like macroautophagy, hereafter autophagy. Autophagy is a homeostatic response to various stimuli like DNA damage. In response to PARPi treatment, autophagy promotes drug resistance and tumor survival in ovarian cancer, but the role in PARPi resistance in TNBC is poorly understood. Targeting autophagy is an emerging field for anti-cancer therapy. Our lab has previously demonstrated that nitrated fatty acids (NFA), which are endogenous signaling molecules involved in processes like anti-inflammatory pathways, selectively target TNBC over benign breast epithelium through post-translational modification of protein cysteine residues. Preliminary data finds that a specific NFA, OA-NO2, functions as an autophagy inhibitor, binding to autophagy-related proteins p62 and ATG7. We hypothesize that PARPi-induced DNA damage initiates drug resistance through autophagy that can be overcome by OA-NO2 targeting critical cysteine residues of essential autophagy proteins. This study will advance the understanding of autophagy-conferred PARPi resistance and overcome PARPi resistance through autophagy inhibition with OA-NO2. To better model TNBC patients receiving treatment, our study has generated PARPi-resistance in three different TNBC cell lines against three PARPi through continuous drug exposure. Preliminary data reveals cross-resistance between PARPi and future studies will examine the synergism of combination treatment between PARPi and OA-NO2.

Huckestein, Brydie

she/her/hers
Program in Microbiology and Immunology (PMI) Year 5
Advisor: John Alcorn

Severe influenza infection causes long-term inflammation, lung damage, and metabolic changes 21 days post-infection

Brydie Huckestein, John F. Alcorn

Severe influenza infection causes widespread damage to the lung epithelium. This damage can lead to long-term complications like alveolitis and reduced lung function. Our laboratory and others have shown that influenza infected mice have persistent lung damage, inflammation, and epithelial metaplasia for up to 60 days post-infection (dpi). To understand the cause of these long-term complications, 6-week-old C57BL/6 mice were infected with the mouse adapted H1N1 strain A/PR8/1934 and sacrificed 21 dpi. Flow cytometry performed on the lung showed that mice have higher levels of neutrophils, exudate macrophages (exM), memory T cells, and effector T cells compared to mock infected controls. This coincides with increased levels of IL-6, IL-12p40, G-CSF, and other pro-inflammatory cytokines that likely contribute to persistent inflammation. Lung OXPHOS and ROS levels were also higher in the lung 21 dpi, encouraging us to look at mTORC1 activation by flow cytometry. mTORC1 activation was increased in neutrophils, interstitial macrophages, and exM 21 dpi. We hypothesized that treating mice with an mTORC1 inhibitor during the repair phase following viral clearance could reduce inflammation and subsequently improve alveolar regeneration. Mice that were treated with rapamycin 14-20 dpi did not have significantly different levels of cytokines or expression of alveolar type II genes 21 dpi. However, the lungs of rapamycin-treated mice had fewer neutrophils and exM seen by flow cytometry. These results suggest that, while rapamycin treatment does not significantly impact lung damage by 21 dpi, it does alter the immune landscape. A longer-term study is needed to determine whether the changes we saw in immune cell populations influence lung repair.

POSTER #49

Ingram Zachary

he/him/his
Program in Microbiology and Immunology (PMI) Year 3
Advisor: Zandrea Ambrose

Characterizing Sequential Host Factor Binding to HIV-1 Capsid using a Unique Mutant
Zachary Ingram, Chris Kline, and Zandrea Ambrose

Following entry into the cell, the HIV-1 capsid protects the viral RNA genome while directly influencing cell trafficking, reverse transcription, and nuclear import via direct interactions with host proteins. How spatiotemporal interactions of capsid with host proteins affects HIV-1 replication is not understood. We have shown that the host cytoplasmic cleavage and polyadenylation specificity factor 6 (CPSF6) binding to capsid increases its trafficking speed and is competitively blocked by host cyclophilin A (CypA). As CPSF6 and CypA are not co-localized in target cells, we hypothesize that the localization of CPSF6 and CypA allow differential binding to HIV-1 that regulates capsid trafficking and nuclear import. To test this mechanism, we are characterizing the HIV-1AC-1 mutant, which has increased CypA binding affinity that leads to defective replication. CypA binding to HIV-1 capsid can be inhibited by a drug, which rescues HIV-1AC-1 replication and suggests CypA may block required host factor interactions. To assess whether CPSF6 can bind HIV-1AC-1 capsid, we infected cells expressing cytoplasmic CPSF6, which forms visible higher order complexes upon capsid binding. CPSF6 complexes readily form when CypA is inhibited from binding HIV-1AC-1 capsid but fail to form when CypA is bound, suggesting that increased CypA binding can block CPSF6 from binding the HIV-1AC-1 capsid. Surprisingly, mutations that disrupt CPSF6 binding restore HIV-1AC-1 infection to near wildtype levels regardless of CypA binding. Therefore, the loss of capsid binding to CPSF6 and other host factors may contribute to the CypA-dependent restriction of HIV-1AC-1. Future studies of HIV-1AC-1 will characterize early replication events of HIV-1AC-1 in relation to CPSF6 and CypA binding to capsid.

POSTER #50

Johnson, Aaron

he/him/his
Molecular Pharmacology (MPHL) Year 5
Advisor: Sruti Shiva

Myoglobin Decreases Mitochondrial Fatty Acid Oxidation in Breast Cancer Cells Independent of Fatty Acid Binding

Aaron Johnson, Courtney Sparacino-Watkins, Bob Zhang, Eric Goetzman, and Sruti Shiva

Breast cancer affects 1 in 8 women, and nearly 40% of breast tumors aberrantly express the small heme protein myoglobin (Mb). Several studies suggest that Mb expression in breast tumors is associated with better patient outcomes, yet the molecular mechanism by which Mb slows cell growth is unclear. Mb regulates mitochondrial function by delivering O2 in muscle tissue and by binding fatty acids (FAs) via lysine residue K46 to promote fatty acid oxidation (FAO) in the heart. However, Mb-dependent FA binding and its role in FAO and cell proliferation have not been explored in cancer. We hypothesize that Mb downregulates FAO by FA binding, which decreases proliferation. We utilized MDA-MB-468 cells expressing endogenous Mb, which is knocked down using siRNA, and MDA-MB-231 cells, which stably express GFP or GFP-tagged Mb. Cell growth was assayed by crystal violet staining. FAO was analyzed by Seahorse XF Analysis and 14C-palmitate oxidation. Mb K46X mutants were generated by site-directed mutagenesis. FA binding was tested by cellular thermal shift assays and UV-Vis spectroscopy. Mb expression decreases proliferation and oxygen consumption rates (OCR) compared to cells without Mb. Using etomoxir to inhibit FAO, Mb-expressing cells show a decreased FA-dependent OCR compared to cells without Mb. To determine if Mb's FA binding underlies these effects, we generated Mb K46X mutant cells to disrupt FA binding, yet no changes in proliferation or OCR were observed, suggesting that Mb modulates FAO via a mechanism distinct from FA binding. We are currently exploring if Mb expression regulates FAO enzymes, such as CPT1. These data reveal a novel function of Mb as a regulator of FAO and cancer cell growth and suggest that altering Mb and/or FAO may offer new therapeutic avenues.

POSTER #51

Julian, Dana

she/her/hers
Cellular and Molecular Pathology (CMP) Year 2
Advisor: Julia Kofler, MD

Quantitative Digital Image Analysis to Investigate White Matter Rarefaction in Alzheimer's Disease

Dana R. Julian1, Ying Ding2, Hansruedi Mathys3, Karl Herrup3, Thomas Pearce1, Julia K. Kofler1

Alzheimer's Disease (AD) is a relentless progressive neurodegenerative disease leading to severe cognitive decline and affects approximately 26.6 million people worldwide. Myelin loss precedes cognitive symptoms in AD by as much as 20 years and is canonically considered a result of ischemic injury and axonal loss. Recent evidence shows direct oligodendrocyte involvement in this multifactorial process. The goal of this study was to develop a quantitative digital image analysis pipeline to analyze myelin quantity, oligodendrocyte density, and vascular markers in postmortem human AD tissue by immunohistochemistry (IHC) and in situ hybridization (ISH). We stained tissue with antibodies targeting myelin (MAG, MBP, PLP), oligodendrocyte lineage cells (Olig2, SOX10), and vimentin. We developed image analysis algorithms using machine learning algorithms in the QuPath software for automated and high-throughput data collection. These data were processed through algorithms identifying perivascular zones and subcortical vs. deep white matter to distinguish areas with different vulnerabilities to chronic ischemic injury, a proponent of white matter rarefaction. This methodology allows us to further interrogate oligodendrocyte populations specifically vulnerable to ischemic injury. Understanding these subpopulations is critical to developing therapeutics for early intervention in white matter loss in AD patients.

Kamlapurkar Shriya

she/her/hers
Molecular Pharmacology (MPHL) Year 2
Advisor: Dr. Nadine Hempel

Elucidating the role of the extracellular antioxidant enzyme, Glutathione Peroxidase 3, in pro-tumorigenic inflammatory signaling in ovarian cancer

Shriya Kamlapurkar, Caroline Chang, Rébécca Phaëton, Nadine Hempel

Ovarian cancer (OVCA) is the most lethal gynecologic malignancy in the United States and is often detected at an advanced stage. OVCA progression involves transcoelomic metastasis which refers to the dissemination of cells into the peritoneal ascites where they experience redox stress and a shift in nutrient availability. Thus, malignant cells must adapt to survive in anchorage-independent conditions within the ascites, which includes upregulation of antioxidant defense mechanisms. While we have previously demonstrated that mitochondrial antioxidants play an important role in OVCA, it is unknown if metastatic OVCA cells alter their extracellular antioxidant enzyme status to cope with stress in this unique tumor environment. Assessment of global changes in antioxidant enzyme expression in high-grade serous OVCA specimens from the Cancer Genome Atlas revealed that patient specimens could be segregated into two clusters based primarily on high or low expression of GPx3, and that high GPX3 expression correlates with poor patient survival. GPx3 catalyzes the reduction of hydrogen peroxide (H2O2) to water and oxygen, using glutathione as the electron donor. shRNA mediated knock-down (KD) revealed that GPx3-mediated scavenging of extracellular H2O2 is crucial for OVCAR3 cell survival in metastatic cell culture assays. Moreover, GPx3 KD in ID8 tumor cells decreased omental tumor burden in a syngeneic OVCA mouse model. RNA-Seq data following GPx3 KD in OVCAR3 cells revealed a marked reduction in expression of inflammatory response genes and genes involved in epithelial-to-mesenchymal transition. Our current studies are focused on the molecular mechanisms behind GPx3's role in pro-tumorigenic inflammatory signaling to explore therapeutic strategies to target GPx3-high tumors.

Kaufman, Keith

Center for Neuroscience (CNUP) Year 3
Advisor: Ross Williamson

Diverse Influences of Pupil-linked Arousal on Auditory Processing in Cortex
Keith J Kaufman, Rebecca F Krall, Megan P Arnold, Tomas S Omedas, Ross S Williamson

Stimulus-independent nervous activity associated with arousal state, as indexed by pupil diameter, varies continuously and influences membrane potentials, cortical state, and sensory processing. Previous studies have shown that the response strength, reliability, and tuning properties of layer (L) 2/3 neurons in auditory cortex (ACtx) are modulated by arousal. The processing of acoustic information recruits a diverse set of cortical excitatory neurons. These cells can be categorized as intratelencephalic (IT), extratelencephalic (ET), or corticothalamic (CT), distinct in terms of their anatomy, morphology, and synaptic properties. These differences will likely lead to state-dependent changes in sensory coding.

We combined two-photon imaging and pupillometry in awake mice to research how arousal regulates the response properties of L2/3 IT, L5 IT, ET, and CT neurons in ACtx. We found that response amplitude scaled monotonically with arousal in ET and CT neurons, but not in IT neurons. With the exception of L5 IT neurons, the neural activity of all cell types became less correlated when animals were more alert. Changes in neural response distributions as a function of arousal also differed between the projection cell types. We tested whether this arousal-dependent modulation degraded sound encoding by employing a statistical neural decoding analysis to predict stimulus identity at each state using the different neural populations. This analysis revealed an inverse-U function whereby intermediate states had the highest decoding accuracy, suggesting stimulus representations are less faithfully encoded at low and high arousal levels. Together, these findings provide a detailed description of arousal-dependent changes in the sensory coding of specific cortical projections.

Keeney, Matt

he/him/his
Molecular Pharmacology (MPHL) Year 5
Advisor: Tim Greenamyre

Redox regulation of LRRK2 kinase activity in Parkinson's disease pathogenesis

Matthew T. Keeney1,2,3*, Eric K. Hoffman1,2, Roberto Di Maio1,2, Teresa G. Hastings1,2, and J. Timothy Greenamyre1,2
Pittsburgh Institute for Neurodegenerative Diseases1, Department of Neurology2, Molecular Pharmacology Graduate Program3 – University of Pittsburgh School of Medicine, Pittsburgh, PA

The most common form of familial Parkinson's disease (PD) results from missense mutations in leucine rich-repeat kinase 2 (LRRK2). These mutations result in a toxic gain of function: elevated kinase activity. Accumulating evidence indicates that wild-type LRRK2 kinase activity is also enhanced in idiopathic PD (iPD). However, the mechanism behind wild-type kinase activation, independent of an activating mutation, remains uncertain. It has been hypothesized that LRRK2 is a redox sensitive protein, but there has been no definitive evidence. Thus, oxidative stress, an important contributor to PD pathogenesis, may exert its effects, in part, by modulating LRRK2 kinase activity. Our data demonstrates that H2O2 rapidly elicits LRRK2 activation, and this effect can be prevented by the thiol antioxidant, N-acetyl cysteine. Furthermore, oxidation of the neurotransmitter dopamine and impaired vesicular trafficking induced kinase activation occurs in a redox dependent manner. Thus, suggesting that LRRK2 is regulated by reactive oxygen species. In the kinase activation loop, there are two vicinal cysteine residues, Cys2024 and Cys2025, which might act as a redox switch that turns kinase-inactive LRRK2 to kinase-active LRRK2. We now report that LRRK2-C2024A, LRRK2-C2025A, and LRRK2-C2024A/C2025A cell lines prevent H2O2-, rotenone-, and impaired vesicular trafficking-induced LRRK2 activation. Thus, our data suggest that (a) LRRK2 is a redox sensitive kinase and (b) both Cys2024 and Cys2025 are crucial residues that regulate LRRK2 kinase activity. These findings may help explain the mechanism that drives oxidative stress induced LRRK2 activation in iPD.

POSTER #55

Kemp, Felicia

she/her/hers
Program in Microbiology and Immunology (PMI) Year 3
Advisor: Craig Byersdorfer

Metabolic profiles of chronically stimulated murine T cells

Felicia Kemp, Carina Sclafani, Manda Ramsey, Craig Byersdorfer, M.D., PhD.

Cellular metabolism is a key determinant in T lymphocyte fate and function. Upon activation, T cells rapidly undergo metabolic reprogramming to rely predominantly on glycolysis to proliferate and produce cytokines. However, previous work in our lab demonstrated that during graft versus host disease (GVHD), murine T cells also upregulate fatty acid oxidation (FAO). This observation was only seen in allogeneic mismatched animals and not seen following immunization. Recent work in human T lymphocytes found chronic stimulation in vitro made cells more reliant upon FAO than T cells maintained under control conditions. We hypothesized that chronically stimulated murine T cells would similarly upregulate FAO in vitro using the same mechanisms as GVHD-causing T cells. While chronically stimulated T cells adopted an effector memory phenotype, with PD-1 being upregulated, they did not express exhaustion markers LAG3 or TIM3. Chronically stimulated T cells accumulated more neutral lipids (Bodipy-493) and transported less glucose (2-NBDG) than control treated T cells on Days 15 and 21 suggesting a shift away from glycolysis. This trend was supported by reduced ECAR levels in chronically stimulated T cells at Day 21. Interestingly, long-term control T cells demonstrated higher FAO compared to chronically stimulated T cells on Day 15. Together, these data indicate chronically stimulated T cells transport and store fats but are not reliant upon lipid metabolism or glycolysis and likely utilize alternative metabolic pathways like glutaminolysis. Ongoing studies will examine alternative forms of chronic stimulation such as histocompatibility mismatch and their impact on T cell metabolic adaptations.

POSTER #56

Khurana, Yajushi

she/her/hers
Computational Biology (Comp Bio) Year 2
Advisor: Dr. Jianhua Xing

LIVETracker: an inclusive and extensible tool for live single-cell imaging trajectory extraction and analyses

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With recent advances in long-term time-lapse microscopy, an active research direction emerges on studying how individual cells respond to stimuli through collecting long single-cell trajectory datasets and quantifying dynamical cell behaviors and morphology features. Single-cell behaviors and morphological analysis provide crucial information to phenotype cells and understand cell/developmental biology, for which each cell must be detected and tracked over spatial and temporal scales. We present LIVETracker, a deep-learning segmentation-based live-cell imaging analysis package that provides users with customizable tools to segment cells, track cells, and compute cell features at the single-cell level. To improve segmentation and tracking quality, LIVETracker includes a correction convolution neural network (C-CNN) module to correct over-segmentation and under-segmentation cases, and a space-time memory network to incorporate temporal information. With current trends of applying large pre-trained models to downstream tasks of various domains, LIVETracker provides the community with segmentation models pre-trained on published datasets and live single-cell and fixed-cell datasets acquired in our lab. Along with preset manual features and autoencoder-based features at a single-cell level, we provide an extensible module for users to implement customized single-cell feature extractor functions. We highlight the power of LIVETracker with image analyses on TGF-β induced epithelial to mesenchymal transition (EMT), a key cell phenotypic transition process whose dynamics can be reconstructed through live single-cell imaging studies.

Krutsenko, Yekaterina

she/her/hers
Cellular and Molecular Pathology (CMP) Year 4
Advisor: Dr. Paul Monga

Dual loss of β-catenin and γ-catenin from cholangiocytes causes intrahepatic cholestatic injury in mice

Yekaterina Krutsenko, Minakshi Poddar, Sucha Singh, Satdarshan P Monga

β-catenin as a key effector of the canonical Wnt signaling, as well as a structural component of adherens junctions (AJs), is indispensable for normal liver development, homeostasis, and regeneration. Previous studies have shown that, in hepatocytes and other epithelial cells, γ-catenin, a highly homologous desmosomal protein, is capable of compensating for the functions of β-catenin at AJs. In the present study we inducibly and specifically delete both β- and γ-catenins from biliary epithelium, to avoid the compensatory effects of γ-catenin, and study the biological implications of dual elimination. We utilized Opn-iCreERT2+/-; Ctnnb1-fl/fl; Jup-fl/fl mice (DKO), in which both β-catenin (Ctnnb1 gene) and γ-catenin (Jup) are acutely deleted from biliary epithelium by the Tamoxifen-inducible, cholangiocyte-specific osteopontin-driven expression of Cre-recombinase. DKO mice showed increased morbidity and mortality as shown by Kaplan-Meier survival analysis. The mice were grossly yellow and lethargic suggesting severe liver injury among the survivors. Indeed, DKO animals showed elevated serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, hyperbilirubinemia, and high cholesterol. Histological examination revealed multiple biliary infarcts, inflammation, stellate cell activation, and portal fibrosis in DKO livers. While there was the presence of CK19-positive bile ducts, these structures showed decreased and sometimes absent lumen in the DKOs. The observed phenotype is histologically reminiscent of human cholangiopathies, and a detailed characterization of the phenotype may lead to the discovery of novel cellular and molecular mechanisms contributing to disease pathogenesis.

POSTER #58

Kubota, Nanami

she/her/hers
Program in Microbiology and Immunology (PMI) Year 3
Advisor: Vaughn Cooper

Prisoner's dilemma: Prophage can enable their bacterial hosts to exploit cooperative bacteria and drive the population to lower fitness

Nanami Kubota, Michelle R. Scribner, and Vaughn S. Cooper

Evolutionary game theory, and specifically the game known as Prisoner's Dilemma, can help explain how cheaters are able to invade populations despite being less reproductively fit than cooperators. Most strains of Pseudomonas aeruginosa contain Pf bacteriophages integrated in their genomes, and these filamentous phages can propagate without lysing their host. Pf phage infection is typically not costly and some benefits to P. aeruginosa have been described, including increased antimicrobial resistance. However, cell death may result when multiple Pf phages attack a cell or when Pf replication is uncontrolled. We observed extremely high levels of Pf5 phage replication when P. aeruginosa PA14 was experimentally evolved in media simulating nutrients from the cystic fibrosis airway. This Pf5 spread was caused by a mutated repressor gene (pf5r) within the prophage, which imposes significant costs to the host bacterium by reducing both growth rate and total yield. Despite their lower absolute fitness relative to their ancestor strain, these bacteria containing hyperactive prophage could outcompete the ancestor in direct competition. These pf5r mutants are cheaters as releasing phage in the environment selfishly increases their own fitness at the cost of other competitors. It is unsurprising that viruses act selfishly, but more remarkable is that these mutant phage shift competitions between bacterial genotypes to prisoner's dilemma, in which all genotypes evolve lower fitness as the pf5r mutation sweeps across the population. This study demonstrates that relationships between bacteria and prophage are unstable and can quickly remodel evolving microbial communities, including potentially during chronic infections caused by P. aeruginosa.

POSTER #59

Lackner, Emily

she/her/hers
Cell Biology (CBMP) Year 1
Advisor: Dr. Ora Weisz

Acute and reversible modulation of kidney proximal tubule endocytic capacity by fluid shear stress

Emily Lackner1, Kate E. Shipman1, Kimberly R. Long1, Isabella A. Cowan1, and Ora A. Weisz1,2
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The proximal tubule (PT) of the kidney is comprised of cells that are uniquely specialized for efficient uptake of proteins that escape the glomerular filtration barrier. These proteins bind to the multiligand receptors megalin and cubilin and are internalized via clathrin-dependent endocytosis into small apical early endosomes that rapidly mature into larger apical vacuoles. Dissociated ligands are delivered from vacuoles to lysosomes for degradation, while receptors are recycled via dense apical tubules to the cell surface. We have optimized a proximal tubule cell model cultured under orbital shear stress that recapitulates the essential morphologic and functional features of the PT in vivo, including high megalin and cubilin expression and robust apical endocytic capacity. Using this model, we found that acute reduction in orbital speed trigger rapid changes in the endocytosis of albumin, as well as an increase in surface megalin and cubilin at the apical membrane. We hypothesize that in vivo, this modulation of endocytic capacity enables PT cells to preserve uptake efficiency in response to changes in glomerular filtration rate. We use biochemical and imaging approaches combined with mathematical modeling to identify the trafficking step(s) modulated by fluid shear stress. These data will be used to adapt our recently developed kinetic model of megalin trafficking in PT cells to determine effects of shear stress changes on individual trafficking rates. This integrative approach will allow us to identify small changes in trafficking that would be difficult to detect through one of these methods alone. By identifying the impacted steps, we can then determine the signaling pathways that are impacted by fluid shear stress.

POSTER #60

Lamerand, Sydney

she/her/hers
Center for Neuroscience (CNUP) Year 4
Advisor: Bradley Taylor

Spinal S1PR1 Agonism Reduces Neuropathic Pain in Multiple Sclerosis
S.R. Lamerand, K.L. Nguyen, B.K. Taylor

Sphingosine-1-phosphate receptors (S1PRs) are an emerging target for the treatment of persistent pain. Emerging literature reports that the S1PR agonist fingolimod, a disease modifying agent for multiple sclerosis (MS), reduces pain-like behaviors in models of inflammatory and neuropathic pain. We previously reported that systemic administration of fingolimod reduced behavioral signs of mechanical allodynia (hypersensitivity to plantar application of von Frey filaments) in an experimental autoimmune encephalomyelitis (EAE) model of MS associated neuropathic pain (MSNP). This reduction was mimicked by S1PR1 agonists and blocked by S1PR1 antagonists. These results indicate an S1PR1-dependent mechanism, but whether this occurs in the peripheral tissues, spinal cord, or brain remains unclear. Based on our preliminary data suggesting that intrathecal (i.t.) injection of fingolimod or S1PR1 or S1PR1/5 agonists decrease MSNP we tested the hypothesis that fingolimod behaves as an S1PR1 agonist in the dorsal horn of the spinal cord to reverse MSNP. After induction of EAE with MOG33-55, both male and female C57BL/6 mice develop mechanical and cold hypersensitivity within 14 days. Next, mice received an intrathecal injection (i.t. 5ul) of either fingolimod, the S1PR1 agonist SEW2871, the S1PR1 antagonist NIBR0213, fingolimod+NIBR0213, or vehicle. This was followed by repeated assessment of mechanical sensitivity. We report that fingolimod and SEW2871, but neither NIBR0213, vehicle nor FTY720+NIBR0213 attenuated mechanical, but not cold, hypersensitivity. We conclude that the spinal cord engages S1PR1 agonism to reduce mechanical allodynia in EAE. These results point to spinal S1PR1 as a target for future pharmacotherapy of MSNP.

Lau, Louis

he/him/his
Program in Microbiology and Immunology (PMI) Year 5
Advisor: Harinder Singh

Non-canonical unfolded protein response transcription factor CREB3L2 regulates antibody secretion in murine B cells.

Louis Lau, Godhev Manakkat Vijay, Harinder Singh

Activated B cell differentiation into antibody-secreting cells (ASCs) involves ER & Golgi biogenesis and remodeling that enable increased antibody synthesis and secretion. These processes are mediated by the unfolded protein response (UPR). Canonical UPR pathways utilize the TF effectors XBP1, ATF4, and ATF6, which are activated upon ER or Golgi stress. XBP1 has been shown to be a major regulator of antibody secretion in ASCs and plasma cells (PCs), whereas ATF6 primarily affects antibody glycosylation patterns. Using scRNA-seq to analyze the generation of ASCs and plasma cells, we discovered that the Creb3l2 gene, encoding a non-canonical UPR TF, is specifically expressed in ASCs and PCs. CREB3L2 has been shown to regulate transcription of genes encoding secretory components and remodeling of the ER and Golgi in secretory cells of the pancreas and pituitary gland. To analyze the function of CREB3L2 in ASCs, I utilized CRISPR/Cas9-RNP editing in the murine CH12 B lymphoma CH12 to generate out-of-frame deletions in exon 2 of the Creb3l2 gene (Creb3l2-/-). Loss of CREB3L2 resulted in severely impaired IgM secretion in the steady-state as well as under LPS stimulation of CH12 cells, without impairing expression of μs or μm heavy chains. Importantly, XBP1 expression was not substantially impacted by the loss of CREB3L2 suggesting that it regulates antibody secretion in a manner independent of XBP1. RNA-seq analysis of Creb3l2-/- CH12 cells reveals putative CREB3L2 positively regulated target genes including genes involved in plasma cell survival. Thus, CREB3L2 regulates high levels of antibody secretion in ASCs, independent of XBP1 or ATF6 UPR pathways and the plasma cell differentiation program.

POSTER #62

Lehrich, Brandon

he/him/his
Cellular and Molecular Pathology (CMP) Year 1
Advisor: Satdarshan P. Monga, MD

β-Catenin Activation Promotes B-cell Exclusion in the Hepatocellular Carcinoma Microenvironment

Brandon M. Lehrich, Evan R. Delgado, Junyan Tao, Silvia Liu, Aatur D. Singhi, Satdarshan P. Monga

Background: Current immunotherapies for hepatocellular carcinoma (HCC) are focused on T-cell specific immune checkpoint inhibitors (ICIs). Work in other cancer models have linked ICI response to B-cell signaling in the tumor microenvironment. Our group has demonstrated that β-catenin-mutated HCCs are resistant to ICIs. Here, we investigated if β-catenin-mutation in HCCs may play a role in B-cell exclusion and impact subsequent response to ICIs.
Methods: Public HCC datasets were assessed for mutations in the β-catenin gene, CTNNB1, and B-cell related gene signatures. Our clinically relevant mouse HCC models of T41A-CTNNB1/G31A-NFE2L2, S45Y-CTNNB1/hMET, and MYC/hMet treated with and without anti-PD-1 therapy, were assessed for the presence or absence of B-cells by immunohistochemistry (IHC). Next, the influence of β-catenin suppression on B-cells was explored using an antisense technology.
Results: Overall, 26% of HCC cases in The Cancer Genome Atlas (TCGA) showed CTNNB1 mutations. Ingenuity pathway analysis of TCGA RNA-sequencing data demonstrated that multiple pathways in B-cells were significantly altered in CTNNB1 mutated vs non-mutated cases. In our murine HCC models, we noticed decreases in CD20+ cells on IHC in both β-catenin-driven models compared to MYC/hMET model. Additionally, we noted no differences in CD20+ cells following anti-PD-1 treatment in both β-catenin models. Moreover, using antisense technology to suppress β-catenin in HCC models increased CD20+ immune cell infiltration in the tumor microenvironment and simultaneously significantly reduced tumor burden.
Conclusion: Future studies aim to elucidate the mechanism of B-cell signaling in ameliorating tumor burden following β-Catenin inhibition with anti-PD-1 therapy in CTNNB1-mutated HCC.

POSTER #63

Leon-Colon, Emmanuel

he/him/his
Program in Microbiology and Immunology (PMI) Year 2
Advisor: Tim Hand

Intestinal Memory T cells Express a Transcriptome Associated With Tissue Residence and Enteric Nerve Interaction

Emmanuel León Colón, B.S., Amrita Bhattacharjee, Ph.D., Kristen Smith-Edwards, Ph.D., and Timothy W. Hand, Ph.D.

Following primary bacterial infection, CD4+ memory T cells arise from the effector population that consequently migrate to barrier sites, such as the small intestinal lamina propria (siLP), where tissue residence is established, conferring quicker and efficient responses upon rechallenge. Depletion of these cells after bacterial reinfection impairs pathogen clearance, suggesting a protective role in intestinal immunity. However, the mechanisms by which CD4+ tissue resident memory (TRM) T cells protect within the siLP are not well defined. Analysis of vaccine-specific CD4+ T cells during effector and memory stages after oral immunization with the double mutant heat-labile toxin (dmLT), an inactivated form of the LT, revealed a transcriptome associated with tissue residency as well production of the neuropeptide CGRP-a that is differentially expressed in the gut compared to the spleen. We also observed CGRP-a-producing CD4+ T cells in the proximity of enteric neurons during the effector response. It is understood that intestinal motility is beneficial for pathogen mechanical clearance and, interestingly, pharmacological neutralization of CGRP-a results in severe constipation. We therefore hypothesize that these enteric CD4+ TRMs produce CGRP-a to communicate with enteric neurons resulting in increased motility to propel bacteria from the intestine. Our current objective is to validate CGRP-a expression with a reporter mouse and test its role in enteric infection by depleting it from CD4+ T cells. This will allow us to elucidate the crosstalk between the adaptive immune system and enteric neurons that contribute to intestinal immunity.

Little, Jack

he/him/his
Cellular and Molecular Pathology (CMP) Year 1
Advisor: Peter Lucas and Linda McAllister-Lucas

MALT1 coordinates the expression of an immunosuppressive secretome in GPCR+ triple-negative breast cancer

Jack Little, Linda McAllister-Lucas, Peter Lucas

Breast cancer is the most common cancer in women and has the second highest mortality rate behind lung cancer. Classically, breast malignancy is classified by expression of estrogen receptor (ER), progesterone receptor (PR), and/or HER2. Triple-negative breast cancer (TNBC) represents the case where tumors lack expression of any of the most common markers (ER-/PR-/HER2-). This subclass of breast cancer represents approximately 15% of breast cancer with over 200,000 new cases diagnosed each year. Due to lack of targetable molecular markers, treatment options rely on non-specific, systemic chemotherapy and radiation with poor response rates overall. There is a growing need to identify targetable markers in TNBC to develop specific, personalized therapies. We and others have recently uncovered the tumorigenic role of MALT1 in TNBC. MALT1 has long been implicated in the pathogenesis of lymphoma, but its role in solid tumors is less defined. This intracellular protein is established to form a signaling complex (CBM complex) downstream of a variety of receptors, including G protein-coupled receptors (GPCRs). In a significant subset of TNBC tumors, GPCRS such as PAR1, AGTR1, and LPARs are upregulated and CBM complex activity is increased. Each of these receptors play many physiologic roles in normal tissue but are incompletely understood in solid tumor pathogenesis. Our lab has shown that overexpression of these GPCRs in experimental models results in increased tumor growth and an immunosuppressive tumor immune microenvironment (TIME). However, the mechanism to how GPCR-MALT1 signaling drives an immunosuppressive TIME is poorly understood. Here, we report on efforts towards defining the role of GPCR-MALT1 signaling in driving the production of an immunosuppressive secretome.

POSTER #65

Liu, Zhaojin

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Molecular Pharmacology (MPHL) Year 2
Advisor: Lin Zhang

Targeting Aurora Kinase A for Potentiating Colorectal Cancer to KRASg12c Targeted Therapy

Zhaojin Liu, Ning Wei, Kaylee Ermine, Jian Yu, Lin Zhang

The oncogenic mutation of KRAS is found in approximately 90% of pancreatic cancer, 45% of colorectal cancer (CRC), and 35% of non-small cell lung cancer (NSCLC) in the United States. G12C is one of the three dominant mutants of KRAS in cancer, leading to unregulated proliferation, and resistance to cell death. KRASg12c remained undruggable until the recent development of the first potent small-molecule allosteric inhibitor that covalently binds to KRASg12c. In 2021, KRASg12c inhibitor sotorasib (AMG510) was approved, and adagrasib (MRTX849) has gained solid progress in the monotherapy of KRASg12c NSCLC. However, both KRASg12c inhibitors show virtually no efficacy when used as monotherapy in CRC, which makes it necessary to discover potential combination treatments to overcome this resistance. Apoptosis plays a key role in cancer therapy, which relies on the activation of multiple caspases, promoted, or inhibited respectively by proapoptotic or antiapoptotic Bcl-2 family proteins. Our group previously reported that inhibition of aurora kinase A (AURKA) could sensitize the KRAS-mutant CRC to anti-EGFR treatment by inducing apoptosis. We found that in SW837 and SW1463, two CRC cell lines with endogenous KRASg12c, and in KRASg12c mutant knock-in SW48, neither MRTX849 nor AMG510 could substantially induce cell death. The addition of alisertib, an AURKA inhibitor in clinical trials, could lead to decreased cell viability, enhanced apoptosis, and Mcl-1 downregulation in these cell lines. We hypothesize that the inhibition of aurora kinase A could lead to sensitization to anti-KRASg12c treatment in KRASg12c mutant CRC cells through the downregulation of Mcl-1. This project would provide preclinical insight to support a novel combination therapy strategy of KRASg12c CRC.

POSTER #66

Luis Mena Hernandez

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Program in Microbiology and Immunology (PMI) Year 2
Advisor: Harinder Singh

Dissecting the functions of BCR signaling in positive selection of GC B cells using SABRs

Luis Mena Hernandez, Nicholas Pease, Alok Joglekar and Harinder Singh

High-affinity memory B cells (MBCs) and plasma cells (PCs) are generated through germinal center (GC) responses that involve clonal expansion, somatic hypermutation, and positive selection. Insightful experiments bypassing the requirement for B cell receptor (BCR) signaling suggest that T follicular helper (Tfh) cells are sufficient in promoting positive selection of GC B cells by tuning cell division times for each B cell clone. However, alternate evidence implicates a role for BCR signaling in GC B cell positive selection. To address this controversy, we are engineering novel signaling and antigen presenting bifunctional receptors (SABRs) and hypothesize that BCR signaling contributes to positive selection. These chimeric bifunctional receptors encode cognate or control peptides and are fused to BCR signaling domains. The novel SABRs will enable precise initiation of BCR signaling during cognate B-T cell interactions. The consequences of BCR signaling on GC B cell clonal expansion and positive selection will be tested using competitive transfer of SABR engineered B cells into immunized recipient mice. This platform will allow us to dissect the contributions of BCR and Tfh cell signals in GC B cell positive selection. The proposed work will advance our fundamental understanding of B cell affinity maturation and accelerate the development of more potent and durable vaccines.

POSTER #67

Lynskey, Michelle

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Molecular Pharmacology (MPHL) Year 5
Advisor: Roddy O'Sullivan

Understanding the effect of HIRA-mediated chromatin assembly at ALT telomeres
Michelle Lynskey and Roderick O’Sullivan

The Alternative Lengthening of Telomeres (ALT) pathway is a pathogenic mechanism of telomere extension that maintains the proliferation of several of the most aggressive cancers. Recurring abnormalities detected in ALT tumors include mutations that inactivate the ATRX-DAXX chromatin remodeling complex which coordinates histone H3.3 deposition at telomeres. Loss of this complex causes telomeric instability, chromatin decompaction, and elevates the levels of the telomeric repeat-containing long noncoding RNA, TERRA. Recently we showed that another H3.3 deposition complex named HIRA mobilizes to ALT telomeres to compensate for ATRX-DAXX inactivation by maintaining chromatin at telomeres. However, rather than mitigating the effects of ATRX-DAXX loss, HIRA appears to be essential for maintaining telomere extension by ALT thereby ensuring the survival of ATRX-DAXX-deficient ALT cancer cells. How HIRA achieves this remains unclear.

We uncovered a non-canonical role for HIRA in the regulation of TERRA association with telomeric chromatin preventing transcription-replication (T-R) collisions that would disrupt efficient ALT telomere maintenance. In this study, we aim to characterize how HIRA affects the multifaceted roles of TERRA and if this relationship is crucial for ALT activity. Using separation of function HIRA mutants that allow us to distinguish the deposition of new histone H3.3 via UBN1/2 from the recycling of old histone H3.3 via ASF1a, we delineate the mechanism by which HIRA regulates TERRA, R-loop homeostasis, and potential T-R collisions at ALT telomeres.

POSTER #68

Mannes, Philip

he/him/his
Cellular and Molecular Pathology (CMP) Year 4
Advisor: Sina Tavakoli

In vivo molecular imaging of chemokine-like receptor 1 (CMKLR1) to monitor ongoing inflammation in a preclinical bleomycin-induced lung injury model Philip Z. Mannes; Clayton Barnes; Qin Zhu; Kathryn Day; Joseph Latoche; Jessie Nedrow; Carolyn Anderson; Janet S. Lee; Sina Tavakoli

INTRODUCTION: There are currently few clinical tools to monitor ongoing lung inflammation and stratify patient care using a personalized medicine approach. A promising target is chemokine-like receptor 1 (CMKLR1), a GPCR expressed by various leukocytes that is implicated in various lung diseases. We evaluated a novel CMKLR1-targeted positron emission tomography (PET) radiotracer in a preclinical model of bleomycin-induced lung injury and characterize CMKLR1 expression over the course of bleomycin-induced lung injury.
METHODS: PET/CT imaging with 64Cu-NODAGA-CG34 was conducted in a bleomycin-induced murine model of lung injury, and the radiotracer uptake in the lungs was quantified by percent injected dose per gram (%ID/g). Following imaging studies, radiotracer uptake in individual organs (%ID/g) was measured with ex vivo gamma counting. Cellular patterns of CMKLR1 expression throughout the course of bleomycin-induced lung injury was determined by flow cytometry quantification of 6CF-CG34.
RESULTS: In vivo uptake of 64Cu-NODAGA-CG34 both globally and focally was highest at 1 week and 2 weeks following bleomycin treatment and decreased nearly to baseline by 4 weeks. Quantification of 64Cu-NODAGA-CG34 uptake in the lungs by ex vivo gamma counting strongly correlated with in vivo uptake obtained by PET. Further, we found that the increased CMKLR1 expression in the lungs of mice at 1- and 2-weeks post-bleomycin was mostly driven by monocyte-derived macrophages.
CONCLUSION: In vivo molecular imaging of CMKLR1 with 64Cu-NODAGA-CG34 measures ongoing inflammation in the context of a preclinical fibrotic lung injury model. PET imaging of CMKLR1 provides a potential strategy to non-invasively quantitate, spatially localize and monitor the dynamics of lung injury.

POSTER #69

Martin, Matt

he/him/his
Molecular Biology & Structural Biology (MBSB) Year 2
Advisor: Jonathan Coleman

Expression and Purification of Synaptic Vesicle Protein 2A-Synaptotagmin 1 Complex
Matt Martin, Anshumali Mittal and Jonathan Coleman

Synaptic vesicle protein 2A (SV2A) is a transmembrane glycoprotein that is ubiquitously expressed in the human brain. Along with its other isoforms SV2B and SV2C, SV2A has similarities with members of the major facilitator superfamily of transport proteins, but no endogenous substrates for any of the three SV2 isoforms have been found to date. Case studies of humans with mutant SV2A alleles report intractable epilepsy, developmental delays, and other neurological abnormalities. SV2A has also been identified as the binding site for the antiepileptic drug levetiracetam. These observations suggest that SV2A plays an important role in neurotransmission, but its exact function is still poorly understood. The calcium sensing protein synaptotagmin 1 (Syt1) forms a complex with SV2A. This interaction is inhibited by Ca2+. Given Syt1's role in synaptic vesicle fusion, one proposed function of SV2A is sequestration of Syt1 until an action potential occurs, raising Ca2+ levels in the presynaptic neuron and allowing Syt1 to unbind SV2A and initiate vesicle fusion. Structural and biochemical analysis of SV2A, both alone and in complex with Syt1, will give insight into its role in neurotransmission. Using fluorescence size exclusion chromatography as a screening tool, we successfully developed a purification method to obtain SV2A-Syt1 complex in quantities sufficient for cryo-EM. In addition to this, we have mapped the epitope which a pan-SV2 antibody binds to, and developed a tool to pulldown all 3 SV2 isoforms from brain tissues using immobilized fAb. Pulled down protein can be eluted using a peptide corresponding to the epitope sequence, and eluted protein can be analyzed by mass spectrometry to identify SV2 binding partners and putative small molecule ligands.

POSTER #70

Martucci, Nicole

she/her/hers
Cellular and Molecular Pathology (CMP) Year 6+
Advisor: George Michalopoulos

Inhibition of PIK3CD Suppresses Hepatocyte Proliferation by More than 50% in the Regenerating Liver after Partial Hepatectomy

Nicole J. Martucci,John W. Stoops,William C. Bowen,Wendy M. Mars,George K. Michalopoulos

The extracellular matrix is important for survival, differentiation, and normal functioning of cells within the liver; integrins are key signaling receptors in this process. Previous data from our lab has shown that with hepatocyte specific knockout of integrin linked kinase, there is hepatocyte proliferation, increased matrix deposition, and unorganized biliary cell/ductal proliferation; this led us to investigate the signaling pathways downstream of ILK that might be responsible for the observed phenotype. Through this, we uncovered a possible central role of PI3K delta (PIK3CD), a protein thought to be immune specific and absent in hepatocytes. The objective of this study was to determine the role PIK3CD has in the liver. From literature searches and our initial data collection, our hypothesis was that the inhibition of PIK3CD would suppress hepatocyte proliferation.

WT mice that were treated with the PIK3CD inhibitor, Cal-101, exhibited a significant decrease in hepatocyte proliferation on days 1, 2, 4, and 7 after PHx, compared to vehicle control, through quantification of nuclear Ki67 staining. By day 7, proliferation subsided in both the control and Cal-101 mice and liver to body weight ratios were similar. Additionally, western blot analyses revealed differences in p-AKT, MET, EGFR, and NFkB after PHx. In vitro culture data also supported a role for PIK3CD in hepatocyte proliferation as a significant decrease was observed in primary hepatocytes treated with Cal-101 compared to control over a 6-day culture period.

This data shows a previously unknown essential role for PIK3CD in controlling hepatocyte proliferation in the regenerating liver.

McCollum, Alexis

she/her/hers
Program in Microbiology and Immunology (PMI) Year 2
Advisor: Dr. Douglas Reed

Influence of myeloid-derived suppressor cells on virulent Francisella tularensis pathology

Alexis McCollum, Jennifer Bowling, Eileen Barry, Douglas S.Reed

Francisella tularensis (F. tularensis) is an infectious, Gram-negative intracellular pathogen that causes pneumonic tularemia, a severe zoonotic disease with a high mortality rate, when inhaled. Francisella tularensis can rapidly disseminate from the lungs to other organs. Our lab has re-established the New Zealand White rabbit model of pneumonic tularemia to evaluate potential vaccines and understand tularemia pathogenesis. Rabbits exposed to virulent F. tularensis develop a fever, elevated erythrocyte sedimentation rates, and thrombocytopenia approximately 3 days after infection and succumb 5-7 days after infection. Analysis of transcriptomics data suggests the presence of myeloid-derived suppressor cells (MDSC) in lung, liver, and spleen tissue of rabbits at 3 and 5 days after infection with F. tularensis. MDSCs suppress inflammation and can trigger platelet activation and coagulation. MDSC have been found in the lungs of mice exposed to F. tularensis. I will characterize the myeloid cells in the lung, liver, and spleen of naïve and infected rabbits by utilizing flow cytometry and bulk RNA-Seq, to verify the presence of MDSC. Additionally, we will block MDSC generation to investigate the impact on F. tularensis pathogenesis. I will also evaluate whether compounds (such as antibodies and plasmin zymogens) that prevent thrombosis also prevent the loss of platelets in F. tularensis-infected rabbits and whether this changes pathogenesis and disease outcome in the host. This investigation will broaden our understanding of pneumonic tularemia pathology, provide insight into how host responds to F. tularensis infection, and can be utilized during the development of an effective vaccine.

POSTER #72

Medina-Sanchez, Luzmariel

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Program in Microbiology and Immunology (PMI) Year 3
Advisor: Reinhard Hinterleitner

Role of Gut Commensal Protist in Loss of Oral Tolerance to Dietary Antigens

Luzmariel Medina-Sánchez, Yanlin Zeng, Magdalena Siller, Pamela Brigleb, Terence Dermody, Marlies Meisel and Reinhard Hinterleitner

The mucosal immune system fosters a delicate regulatory environment which maintains oral tolerance to food. Tolerance to food antigens results via the induction of peripheral regulatory T cells (pTreg) and their regulatory suppressive functions. However, enteric viral infections can elicit pathological processes leading to the initiation of T helper 1 (Th1) immunity against dietary gluten resulting in loss of oral tolerance (LOT) and celiac disease. In this study we hypothesized that certain commensal gut microbes may have the capacity to protect from virus-mediated LOT. Here we show that the gut protist Tritrichomonas from the Parabasalia class, promotes oral tolerance and prevents viral-mediated LOT by promoting pTreg immunity to dietary antigens. Testing the protective effects of Tritrichomonas from reovirus mediated LOT we found that Tritrichomonas colonization was sufficient to block viral mediated Th1 immunity to dietary antigens and rescue pTreg suppression. Additionally, using gnotobiotic mice, we found that Tritrichomonas is sufficient to protect against reovirus mediated LOT in the absence of the microbiota. Mechanistically, we show that Tritrichomonas colonization restrains reovirus-induced inflammatory responses in dendritic cells and thus limit their ability to promote Th1 immune responses in vitro. Furthermore, to determine the relevance of these findings to celiac disease we tested oral tolerance to gluten in mice expressing the celiac disease predisposing HLA molecule DQ8 (DQ8tg mice). In summary, this study will provide insights to better understand the potential beneficial role of protist as a based therapeutic tool to prevent the development of celiac disease.

POSTER #73

Meyer, Mitchell

he/him/his
Program in Microbiology and Immunology (PMI) Year 4
Advisor: Will DePas

Identifying how Dos hypoxic dormancy impacts biofilm formation in nontuberculous mycobacteria (NTM) through regulation of mycomembrane components

Mitchell D Meyer, William DePas

Pulmonary infections by nontuberculous mycobacteria (NTM), especially Mycobacterium abscessus (MABS), are notoriously difficult to treat, demonstrating recalcitrance to antibiotic therapy. Biofilm formation allows chronic pulmonary pathogens to survive antibiotic therapy and is triggered by specific environmental cues. One such cue, low oxygen concentrations, is prevalent in CF sputum. NTM enter dormancy through the DosSR two-component system under anoxia, which changes cellular physiology and increases antibiotic tolerance. A critical early step in NTM biofilm formation is cell-cell adhesion, characterized by the formation of cellular aggregates in liquid culture. Cell surface remodeling is a known way that MABS adapt to the host lung, and previous studies have implicated regulation of glycopeptidolipids (GPLs) to be involved in biofilm formation. Here we hypothesize that under hypoxic conditions, activation of DosSR influences aggregation through cell wall modification. To see these system's impact on biofilm formation, we created a library of mutants of DosR regulon components and GPL biosynthesis genes. We use an in vitro aggregation assay to quantify changes in aggregation throughout culture maturity, allowing for exploration of early biofilm formation. In addition, use of a bioreactor system will help to quantify the impact of low-oxygen environments on NTM biofilm formation. Preliminary data indicates that NTM require oxygen to transition between an aggregated and planktonic state. In addition, mutation of the dosR hypoxic response regulator results in disrupted regulation of aggregation. Finally, we have determined that a GPL deficient mutant of the model NTM Mycobacterium smegmatis constitutively aggregates, indicating GPLs involvement in dispersal.

POSTER #74

Michaca, Manuel

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Cell Biology (CBMP) Year 4
Advisor: Matthew Nicotra

Visualizing the expression of Alr1 in Hydractinia symbiolongicarpus
Manuel Michaca, Steven Sanders, Bradley Angstadt, Srija Konduru, Matthew Nicotra

Allorecognition is the ability of an organism to recognize self from non-self within the same species. Allorecognition is nearly ubiquitous in animals. In colonial marine invertebrates, where it regulates intraspecific spatial competition and prevents stem cell parasitism. In humans, allorecognition plays a large role in clinical implications, such as pregnancy and organ transplantations. Our lab aims to expand our knowledge of allorecognition by studying it in Hydractinia symbiolongicarpus. Our work has revealed that Allorecognition (Alr) 1 and Alr2 genes are determinants of responses in Hydractinia, where colonies matching at least one allele at both genes can fuse while complete mismatches lead to rejection. Matching Alr1 and Alr2 alleles homophilically bind across cells through their extracellular Ig domains. However, the intracellular events that take place after this binding are as yet unknown. To investigate this, a yeast two-hybrid screen was performed and three proteins that interact with Alr1's cytoplasmic tail were identified: Afadin, PICK1, and Syntenin. These PDZ proteins are present in vertebrates and regulate cell-cell adhesion, suggesting Alr1 may play a similar role in Hydractinia. Here we report the development of Alr1 antibodies and their use in immunolocalization experiments across different life stages of Hydractinia. Our results show that Alr1 localizes to cell membranes in all tissues, consistent with a constitutive role in cell-cell adhesion. This suggests part of the Hydractinia allorecognition system may have evolved from existing cell adhesion proteins.

Miller, Leigh

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Program in Microbiology and Immunology (PMI) Year 3
Advisor: John F. Alcorn

Influenza Induced Memory B cells are required for T cell activation and viral control but not Super-Infection Host Defense

Leigh M. Miller, Ellyse M. Cipolla, John F. Alcorn

Each year in the United States seasonal influenza contributes to significant morbidity among the elderly, children, and immunocompromised persons. Clearance of influenza virus is mediated by the innate and adaptive immune systems, leading to an inflammatory environment in which the lung epithelium becomes damaged. This process leads to dysregulation of the innate and adaptive immune systems, leading to increased susceptibility of developing secondary bacterial pneumonia. Secondary bacterial pneumonias, particularly those caused by methicillin resistant Staphylococcus aureus (MRSA), result in increased morbidity and mortality. In the context of influenza infection, B cells can function to produce antibody, generate cytokines to regulate the immune response, and act as antigen presenting cells to CD4+ and CD8+ T cells. In comparison to mice with acute influenza infection, heterosubtypic influenza memory experienced mice have lower inflammation and more balanced inflammatory mechanisms, leading to decreased lung damage and susceptibility to bacterial colonization. Contrary to what is seen within wild-type (WT) mice, our studies show that heterosubtypic influenza memory experienced μMT mice, which lack B cells, display increased lung injury following MRSA challenge, despite effectively clearing the virus. Additionally, we found that the influenza memory experienced μMT mice seem to experience increased immunosuppression during super-infection. Our preliminary findings with MD4 mice suggest that antibody independent functions are important for clearance of infection and immunoregulation during super-infection. In future studies, we aim to explore how antibody independent B cell functions impact clearance and recovery during memory super-infection.

POSTER #76

Mulla, Joud

she/her/hers
Cellular and Molecular Pathology (CMP) Year 2
Advisor: Melanie J Scott

The Effect of Caspase-11 in Trauma-Induced Coagulopathy (TIC)

Joud Mulla, Nijmeh Alsaadi, Zachary Secunday, Rohan Katti, Matthew D. Neal, Melanie J. Scott

Introduction: Trauma is one of the leading causes of death globally in people 50 years and younger. Hemorrhagic shock following trauma leads to hypoxia, and blood loss, all of which can lead to trauma-induced coagulopathy (TIC), resulting in multiple organ dysfunction. Caspase-4/11 is associated with disseminated intravascular coagulation (DIC) in sepsis by allowing tissue factor (TF) binding and initiation of the coagulation cascade. However, its role in TIC is unknown. We investigate the effects of caspase-11 on coagulation.

Method: To induce coagulopathy, male C57BL/6J and caspase-11-/- mice, were subjected to polytrauma. The model consists of blind cardiac puncture (25% total blood volume loss), laparotomy with liver crush, and bilateral pseudofractures (hindlimb crush injury and injection of crushed bone solution from an age- and weight-matched syngeneic donor). Blood was collected at 3, 6 and 24hrs. Caspase-11, TF, and fibrin expression were measured by Western blot of liver and lung lysates. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen were measured.

Results: Polytrauma significantly increased aPTT levels (18.1±0.8 to 42.7±0.8 sec, $p<0.0001$) and rose PT levels (13.3±0.8 to 14.4±0.8 sec) after 24h, indicating TIC. Polytrauma induced caspase-11 in liver. After 24h of polytrauma, aPTT increased in WT, but did not increase in caspase-11-/- mice (42.7±0.8 v.s 10.2 ±1 sec, $p<0.0001$). There is no change between WT and caspase-11-/- in PT. Caspase-11 deficiency increased TF in lung and liver lysates and decreased fibrin levels in lung lysate at 6h in TIC compared to WT.

Conclusion: Caspase-11 may regulate coagulation profile in TIC and TF localization. Inhibition of caspase-11 may be a future therapeutic option for TIC

POSTER #77

Mushala, Bellina

she/her/hers
Molecular Pharmacology (MPHL) Year 4
Advisor: Iain Scott

The Therapeutic Potential of the Adropin-GPR19 Signaling Pathway in Metabolic-associated Fatty Liver Disease (MAFLD)

Bellina Mushala, Bingxian Xie, Michael Jurczak, and Iain Scott

Obesity is the second leading cause of preventable death in the United States, and serves as a fundamental risk factor in various pathologies including metabolic-associated fatty liver disease (MAFLD).The cellular and molecular mechanisms that drive obesity-related disease in different tissues are not fully elucidated, and this represents an impediment to the development of effective and efficient treatments. Adropin is a nutritionally regulated liver- and brain-derived peptide hormone that modulates metabolic homeostasis in several tissues. Adropin levels are significantly reduced in obese human subjects, and this decrease is linked to increased adiposity, impaired glucose homeostasis, and liver injury. Recent reports show that MAFLD patients exhibit a marked decrease in serum adropin levels, however the functional significance of this is unknown. Various studies suggest that a class A orphan G-coupled protein receptor, GPR19, mediates adropin signal transduction, therefore we hypothesize that GPR19 may be the putative cellular receptor that permits adropin function in the liver. To investigate this, we used a novel whole-body GPR19 knockout mouse to perform a preliminary characterization of the effects of GPR19 depletion on hepatic and whole-body physiology. Using in vivo metabolic approaches, we report that GPR19 KO mice exhibit glucose intolerance, altered hepatic energy metabolism, and histological liver injury. Together, these data suggest that GPR19 KO mice display pathogenic events that promote a MAFLD phenotype, indicating that GPR19 may be critical for the protective effects of adropin signaling in the liver. These findings highlight the adropin-GPR19 signaling pathway as a potential target for therapeutic interventions in MAFLD.

POSTER #78

Myers, Tracey

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Center for Neuroscience (CNUP) Year 4
Advisor: Michael Palladino

Investigation of Resveratrol Administration and Neuromuscular Pathology in a Novel Murine Model of Triosephosphate Isomerase Deficiency

Tracey D. Myers, Eric Gliniak, Carolyn Ferguson, Gregg E. Homanics, Michael J. Palladino

Triosephosphate Isomerase Deficiency (TPI Df) is a progressive multi-system disease that results in symptoms such as hemolytic anemia, progressive muscle weakness, neurological dysfunction, increased susceptibility to infection, and a markedly reduced lifespan with death typically occurring before the age of 8. At present, there are no treatments for TPI Df. The disease is fully recessive and arises due to point mutations within the TPI1 gene, with the most common disease-associated mutation being the TPI1[E105D] mutation. TPI Df has been understudied due to the extreme rarity of human patients and the previous lack of mammalian models of disease. Recently, we have created a novel model of TPI Df by modeling the TPI1[E105D] mutation within the mouse (Tpi1[E105D]). This is the first murine model of a Tpi1 mutation that results in a neuromuscular phenotype. This model creates a unique opportunity to learn more about TPI Df pathogenesis and also provides a model to evaluate potential therapies. At this time, it is not known whether the muscle weakness seen in disease has myogenic or neurogenic origins. To investigate this, the neuromuscular system is being evaluated within our murine model of TPI Df using histology. Additionally, previous work identified resveratrol as a potential protective compound in TPI Df, and the effects of acute resveratrol administration are being evaluated within TPI Df mice. This work provides the first insights into neuromuscular pathology in TPI Df, and preliminarily investigates a potential disease treatment. Future work will expand upon these studies.

Parikh, Avani Bharat

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Program in Microbiology and Immunology (PMI) Year 2
Advisor: Jason Lohmueller, PhD

Programming Universal Chimeric Antigen Receptor (CAR) T Cells Using Small Molecule Adaptors

Avani Parikh, Gianna Falcone, Alexander Deiters, Jason Lohmueller

Chimeric Antigen Receptor (CAR) T cell therapy is an adoptive cell therapy in which cells are genetically modified to express a receptor that binds and kills tumor cells via a specific target antigen. This approach has demonstrated clinical success in combating several hematologic B cell cancers, however, CAR T therapy has failed to effectively treat solid tumors due to several issues, including: heterogeneity in antigen expression, inability to identify tumor-specific antigens leading to on-target/off tumor toxicities, and an immuno-suppressive tumor micro-environment. To address these limitations, we are developing universal CAR T cells that target tumor antigens and antigens on immunosuppressive cells via small molecule adaptors. Instead of directly binding to a target antigen, our universal CAR contains a mutated O6-alkylguanine-DNA alkyl transferase, SNAPtag, and is co-administered with heterobifunctional small molecule adaptors containing a benzyl guanine (BG) motif which forms a covalent bond with SNAPtag, and a second antigen binding small molecule ligand. We expect T-cell activation and specific cytotoxicity when there is effective recognition of the adaptor-modified T cells to the targeted cell surface protein. We are presenting preliminary data on synthesis and in vitro functional testing for adaptors targeting Carbonic Anhydrase IX, a protein which is over-expressed on solid tumors, as well as designs to target several other tumor and immunosuppressive cell antigens individually or in combination. We expect small molecule adaptors to have several favorable characteristics compared to antibody adaptors, including superior pharmacokinetics and lower cost, altogether providing multiple opportunities to treat solid tumors.

POSTER #80

Phan, Alexandra

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Program in Microbiology and Immunology (PMI) Year 2
Advisor: Yuan Chang

HDAC regulation of MCV persistence

Alexandra Phan, Lindsey Robinson-McCarthy, Patrick Moore, Yuan Chang

Merkel cell polyomavirus (MCV) is an oncogenic virus and is the etiological agent of 80% of Merkel cell carcinoma (MCC) cases, an aggressive skin cancer that affects immunocompromised individuals. The MCV genome is made of two regions. Genes expressed from the early region regulate viral replication and the late region encodes structural proteins for virion assembly. MCV is thought to exist in a mostly latent state in which viral gene expression is suppressed. Polyomavirus late gene expression is typically believed to be dependent on genome replication. We used a fluorescent late gene reporter virus to investigate the regulation of MCV late gene expression. We found that inhibiting histone deacetylases (HDACs) with sodium butyrate in cells transfected with a replication-deficient mutant of MCV promotes late gene expression, suggesting a replication-independent mechanism of gene expression. We aim to understand how epigenetic modifications like histone acetylation regulate MCV gene expression. We hypothesize that HDACs repress MCV gene transcription to regulate MCV latency. We will determine the effect of global HDAC inhibition on MCV genome replication, transcription, and protein expression. We aim to understand the mechanism of HDAC-mediated gene repression in MCV using new generation HDAC inhibitors to identify specific HDACs required for MCV gene repression. The finding that HDACs can suppress MCV transcription provides an understanding of the mechanisms of viral persistence and may point to potential antiviral targets. HDAC inhibitors are often used in anti-cancer treatments and the finding that HDAC inhibition may lead to viral gene expression or reactivation may be an important consideration for cancer patients, especially those who are immunocompromised.

Phelps, Catherine

she/her/hers
Program in Microbiology and Immunology (PMI) Year 3
Advisor: Marlies Meisel

Defining mechanisms by which physical exercise mediates improved antitumor immunity and tumor suppression in ICI-resistant melanoma
Phelps CM, Shapira JS, McPherson AC, Rana M, Laughlin C, Meisel M.

Metastatic melanoma is among the deadliest forms of cancer due to high rates of resistance to first-choice treatments, including immune checkpoint inhibitor (ICI) therapy. As global melanoma incidence remains high, it is imperative that novel factors to improve treatment efficacy be explored. External factors such as physical exercise have been shown to exert antitumor effects and improve ICI responsiveness in patient studies, but the mechanism by which this occurs remains undefined. Here, we present exciting preliminary data showing that physical exercise restrains ICI-resistant melanoma tumor growth in a manner which requires adaptive immunity and the microbiota. On a cellular level, we identified that physical exercise facilitates a significant systemic expansion of interferon-gamma producing CD8 and CD4 T cells and suppresses Foxp3+ regulatory T cells. Further, we identify that exercise induces significant changes to the gut and tumor microbiome composition through 16s rRNA sequencing and a live culturomics approach. We hypothesize that physical exercise promotes anticancer immunity in ICI-resistant melanoma by enriching antitumor commensal bacterial species in the gut and/or tumor microbiome that promote antitumor activity of T cells. In ongoing and future analyses, we will determine exercise-induced compositional and transcriptomic changes to intratumoral immunity and assess the requirement and sufficiency of different T cell subsets and the tumor microbiota to confer tumor suppression. Results of this study will identify immunologic- and microbial-based therapeutic targets that can be deployed as adjuvants to increase sensitivity to existing anti-cancer immunotherapies in melanoma and possibly other cancers.

POSTER #82

Powell, Juliana

she/her/hers
Molecular Genetics and Developmental Biology (MGDB) Year 5
Advisor: Yoel Sadovsky & Adrian Morelli

Small extracellular vesicles from plasma of women with preeclampsia increase arterial tone in mouse mesenteric arteries

Juliana Powell, Robin Gandley, Emily Lackner, Adrian Morelli, Carl Hubel, & Yoel Sadovsky

Preeclampsia (PE) is a common disease of pregnancy, characterized by high blood pressure and proteinuria, and when exacerbated, leads to multi-system dysfunction. Extracellular vesicles (EVs) are lipid-bilayer nano-particles released from cells. They are involved in cell-cell communication and transport of diverse types of cargo molecules. Small extracellular vesicles (sEVs, exosomes) are defined by their size and formation within endocytic multivesicular bodies. Whereas placental trophoblasts, a key player in the maternal-fetal interface, are known to produce sEVs, their function in pregnancy remains unknown. Here, we focused on sEVs released to the maternal circulation, and hypothesized that sEVs from pregnant women with PE play a role in regulation of vessel tone. Upon exposure of isolated mouse mesenteric arteries ex vivo to sEVs isolated from the plasma of healthy pregnant women vs pregnant women with PE, we found an increase in arterial constriction in arteries that were exposed to sEVs from women with PE. This effect was not observed using plasma from women with PE that was depleted of EVs or using only microvesicles from plasma of women with PE. Vessels exposed to sEVs from women with PE were also more resistant to methacholine-stimulated relaxation. We used immunofluorescence to detect sEVs within endothelial cells, supporting the role of these cells in mediating sEV impact on vessel tone. Together, these data suggest that sEVs from pregnant women play a role in regulation of arterial pressure in health and disease.

POSTER #83

Rafael Guimaraes, Thais

she/her/hers
Center for Neuroscience (CNUP) Year 4
Advisor: Amantha Thathiah

G protein-coupled receptor kinase 2 modulates tau phosphorylation and aggregation in vitro

Thais Rafael Guimaraes, Amantha Thathiah

Aggregation of the hyperphosphorylated tau protein in neurofibrillary tangles (NFTs) is a pathological hallmark of Alzheimer's Disease (AD) brains. Several kinases contribute to the pathological phosphorylation of tau; however, kinase-targeted therapies for AD have failed in clinical trials due to low efficacy and severe side effects. G protein-coupled receptor (GPCR) kinases (GRKs) have been implicated in neurological disorders, such as Parkinson's Disease, via phosphorylation of non-GPCR substrates, e.g., α-synuclein. Previously, we showed that GRK2 is abundantly expressed in neurons, positively correlated with soluble tau levels, and associated with NFTs in the human AD brain. Therefore, we hypothesized that GRK2 can directly modify tau phosphorylation and aggregation. To test this hypothesis, we first showed that genetic deletion of Grk2 induces global changes in the tau phosphoproteomic profile, while GRK2 overexpression increases tau phosphorylation (pTau) at a disease-relevant site (PHF1). Moreover, we find that GRK2 modulates pTau in a kinase activity-independent manner, via other major tau kinases (e.g., ERK, GSK3β). Furthermore, we used an inducible optogenetic system (optoTAU), which allows for control of the expression and temporal aggregation of tau, to show that optoTAU aggregation is reduced in Grk2-deficient cells. Lastly, Grk2 genetic deletion protects the cells from aggresome formation upon proteasome inhibition, suggesting the involvement of GRK2 in regulating proteasome-mediated tau degradation. Collectively, these studies causally implicate GRK2 as a multifactorial modulator of tau pathology through changes in phosphorylation, aggregation, and degradation of tau and support further investigation of therapeutic interventions against GRKs in AD.

POSTER #84

Raja, Sripriya

she/her/hers
Molecular Pharmacology (MPHL) Year 4
Advisor: Ben Van Houten

Understanding the role of UV-DDB in the SMUG1-mediated repair of the oxidative DNA lesion, 5-hydroxymethyl-2-deoxyuridine

Sripriya Raja1,2,3, Sunbok Jang2,3, Vera Roginskaya2,3, Bennett Van Houten2,3
1Graduate Program in Molecular Pharmacology, 2Department of Pharmacology and Chemical Biology, and 3UPMC-Hillman Cancer Center, School of Medicine, University of Pittsburgh

UV-DDB conventionally works as the first responder for the removal of UV-induced damage during global genome nucleotide excision repair. However, we have previously described a role for UV-DDB in the base excision repair (BER) pathway by showing UV-DDB can enhance OGG1, APE1, and MUTYH activity. To test whether UV-DDB has a wider role in BER, we have analyzed SMUG1. The oxidation of thymidine creates 5-hydroxymethyl-2-deoxyuridine (5-hmdU), which is removed by single-strand selective monofunctional DNA glycosylase (SMUG1). SMUG1 is product inhibited, binding more avidly to abasic sites than 5-hmdU. Biochemical experiments indicate UV-DDB stimulates SMUG1 4-5-fold. Immunofluorescence of cells treated with 5-hmdU (5μM for 15 min), leads to discrete formation of co-localizing DDB2-mCherry and SMUG1-GFP foci. Moreover, we see faster recruitment of DDB2, preceding SMUG1 after damage with 5-hmdU. Interestingly, we see strong colocalization of both SMUG1 and UV-DDB. We further hypothesize UV-DDB has several roles during the SMUG1 mediated BER of 5-hmdU including: facilitating damage recognition, promoting glycosylase turnover from abasic sites, and in PARP1 dissociation from single strand breaks to encourage efficient repair. Like previous studies, we confirmed that siRNA-mediated knockdown of SMUG1 decreases 5-hmdU toxicity. Additionally, the double knockdown of SMUG1 and DDB2 reduces 5-hmdU toxicity, suggesting a role for UV-DDB in 5-hmdU repair. Finally, we demonstrate robust PAR accumulation after 5-hmdU treatment (5μM for 15 min) in the presence of SMUG1. Suggesting excessive PARylation by poly-(ADP-ribose) polymerase 1 may lead to a bioenergetic collapse during the SMUG1 mediated repair of 5-hmdU. Supported by NIH R35ES031638.

POSTER #85

Rein, Hayley

she/her/hers
Molecular Pharmacology (MPHL) Year 5
Advisor: Dr. Kara Bernstein

Determining the Consequences of Ovarian Cancer Derived RAD51C Mutations

Rein H. L., Russell R. A. and Bernstein K. A.

Approximately 50% of high grade serous ovarian cancers (HGSOC) are deficient in the homologous recombination (HR) pathway. HR is a high-fidelity DNA double-strand break repair pathway, which uses a homologous template for repair. The central protein of this process, RAD51, is tightly regulated by proteins including BRCA2, PALB2, and the RAD51 paralogs. The RAD51 paralogs, including RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 are ancient gene duplications of the ATPase RAD51 that have evolved distinct functions from RAD51. Collectively, RAD51C and RAD51D are mutated in 8% of HR-deficient familial HGSOC. Although the RAD51 paralogs were discovered 20+ years ago their exact function in HR, and how mutations in these proteins contribute to HGSOC is largely unknown. We have identified 26 missense mutations in RAD51C identified in ovarian cancer patients through literature searches and cancer databases such as COSMIC. Each of these missense variants are characterized as variants of unknown significance (VUS). Therefore, their contribution to disease progression and their response to HR-deficient targeted therapies has yet to be determined. To examine RAD51C function, we analyzed these 26 variants for altered RAD51C protein interactions with its binding partner RAD51D by yeast-3-hybrid analysis. We found that five of these RAD51C mutants exhibit a reduced interaction with RAD51D. Of these, we identified one mutant that is HR deficient using a sister chromatid recombination (SCR) assay in human cell line U2OS. By characterizing the activity of these RAD51C variants we will uncover how these RAD51C variants contribute to HR-deficiency in HGSOC.

POSTER #86

Ricci, Morgan

she/her/hers
Cell Biology (CBMP) Year 4
Advisor: Gerry Hammond

Investigating the Subcellular Regulation of the PI3K Pathway

Morgan Ricci and Gerry Hammond, PhD

Phosphoinositide 3-kinase (PI3K) initiates phosphatidylinositol 3,4,5-triphosphate (PIP3) and subsequent phosphatidylinositol 3,4-biphosphate (PI(3,4)P2) synthesis. PIP3 and PI(3,4)P2 direct protein kinase B (Akt) signaling which is upregulated in breast cancer (BC). PI3K inhibitors have recently been used as a BC therapy but due to disruption of physiological Akt signaling, have led to development of serious adverse effects. PI3K activity has been indicated at both the plasma membrane (PM) and endosomal membranes. However, to date the spatiotemporal dynamics of PI3K have not been defined.

The purpose of this study is to demonstrate where PI3K is recruited and how PIP3/PI(3,4)P2 are intercellularly distributed and their subsequent impacts on Akt signaling. To do this, we have endogenously tagged the PI3K/Akt pathways major signaling components with mNeonGreen (mNG) and employed these genomically edited cell lines alongside our labs lipid biosensors to measure the protein and lipid dynamics with live cell microscopy. We have shown that while PIP3 levels drop to baseline minutes after stimulation, subsequent 5'-phosphatase mediated PI(3,4)P2 synthesis is sustained, thereby indicating PI(3,4)P2 may be the key mediator in long-term-oncogenic Akt signaling. We hypothesize that PI3K is recruited to the PM where it colocalizes with clathrin-coated structures and synthesizes PIP3, this is followed by sustained PI(3,4)P2 synthesis at the PM that can be endocytosed and direct long-term Akt activation from both the PM and endosomes.

Completion of this work will demonstrate PI3K recruitment and localization and determine where PI(3,4)P2 synthesis is sustained and whether it is sufficient to drive long-term Akt signaling.

Roberts, Sean

he/him/his
Immunology (IMM) Year 6+
Advisor: Mark Shlomchik

Brain Acid Soluble Protein 1 Regulates Germinal Center B Cell Development and Function

Sean Roberts, Daniel J. Wikenheiser, Derrick J. Callahan & Mark J. Shlomchik

Long-lived humoral immunity is essential for protection against pathogens. Germinal centers (GC) are specialized sites in lymphoid tissues where antigen-specific B cells undergo proliferation, affinity maturation, and differentiation into high-affinity long-lived plasma and memory B cells following immunization or infection. Although germinal center B cells are crucial to produce high-affinity memory cells, the specific signals and mechanisms that are involved in their activation and differentiation into long-lived memory B cells and plasma cells during immunization is not completely understood. Here, we show that Brain Acid Soluble Protein 1 (BASP1) is abundantly expressed in germinal center B cells. To test the functional role of BASP1 in regulating GC B cells, we retrovirally transduced B cells with short-hairpin RNA against Basp1 and adoptively transferred them into B cell receptor (BCR)-restricted hosts. Following immunization with the model antigen Nitrophenylacetyl Chicken Gamma-Globulin (NP-CGG), we found significantly fewer splenic germinal center B cells in Basp1-shRNA expressing cells. To conditionally target BASP1 in B cells during peak GC responses, we generated BASP1fl/flhCD20TamCre mice. Following NP-KLH immunization and tamoxifen treatment, BASP1fl/flhCD20TamCre mice had reduced GC B cells. Finally, we demonstrate that BASP1 regulates the proliferation and survival of GC B cells by measuring EdU uptake and caspase expression in tamoxifen-treated mice. Together, these data suggests that BASP1 plays a B cell-specific role in regulating germinal center responses.

POSTER #88

Rokes, Alecia

she/her/hers
Program in Microbiology and Immunology (PMI) Year 4
Advisor: Vaughn Cooper

Differences antimicrobial resistance evolution between strains of A. baumannii highlight the importance of evolutionary history in adaptation

Alecia B. Rokes, Alfonso Santos-Lopez, Vaughn S. Cooper

Antimicrobial resistance (AMR) is a rapidly worsening global health issue. Of serious concern is Acinetobacter baumannii, a nosocomial and highly multi-drug resistant (MDR) pathogen. Although resistance is often acquired through common mechanisms, it remains unclear how genetic background affects AMR evolvability. History is an under appreciated force influencing evolution and can have lasting effects on the genome that alters paths available for adaptation to antibiotic stress.

We are testing the effect of history on the evolution of AMR by utilizing strains with different backgrounds and resistance profiles. ATCC 17978 is highly adapted to laboratory conditions, whereas AB5075-UW is a more recently isolated MDR clinical strain. To test the effect of strain genetic background on AMR adaptation we propagated both strains in increasing concentrations of tigecycline. Whole population genome sequencing revealed that individual mutations and broader population dynamics differed between strains, while mechanistic pathways conferring drug resistance were conserved. Both strains acquired mutations in genes related to drug efflux, however, the specific pumps mutated differed. ATCC 17978 primarily acquired mutations disrupting the gene adeL, the regulator for the adeFGH efflux pump. AB5075-UW primarily acquired point mutations within the adeRS-ABC efflux system. Ongoing RNA-seq analysis on ancestral and evolved populations will identify additional regulatory and gene expression differences between strains.

The differences in efflux mutations indicate that even under strong selective forces strain history can influence the course of evolution. The influence of history could have implications for the predictability of AMR evolution and clinical translatability.

Runk, Breonna

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Program in Microbiology and Immunology (PMI) Year 2
Advisor: Terence Dermody

Elucidating the binding interface between mammalian orthoreovirus and paired immunoglobulin-like receptor B

Breonna Runk, Pengcheng Shang, Joshua D. Simpson, Melanie Köhler, David Alsteens, Liya Hu, B.V.V Prasad, Terence Dermody

Receptor interactions are essential for viral infection and pathogenesis. Mammalian orthoreovirus (reovirus) infects a wide range of mammals, including humans. Reovirus targets specific subsets of neurons in the central nervous system (CNS) of newborn mice, leading to lethal encephalitis. However, receptors dictating reovirus infection in the CNS are unknown. Our lab recently identified paired immunoglobulin-like receptor B (PirB) as a reovirus receptor. Following peroral or intracranial reovirus inoculation, PirB-/- mice display increased survival and attenuated encephalitis relative to wild-type mice. The PirB ectodomain consists of six, concatenated immunoglobulin-like domains, termed D1 through D6. Reciprocal exchange of PirB domains with sequences of the paralog PirA, which does not bind reovirus efficiently, demonstrated that the PirB D3-D4 region is required for reovirus binding and infectivity, suggesting that this region is targeted by reovirus for attachment. The goal of this research is to elucidate molecular details of the reovirus-PirB binding interface. Single reciprocal domain swaps of PirA and PirB will be engineered and analyzed to identify the domain required for reovirus binding. Residues in the PirB domain (D3, D4, or both domains) required for reovirus binding and infectivity will be identified using structure- and sequence-guided mutagenesis. A better understanding of the reovirus-PirB binding interface will expand our knowledge of reovirus entry mechanisms and tropism for neurons.

POSTER #90

Sankar, Rithika

she/her/hers
Integrative Systems Biology (ISB) Year 2
Advisor: Dr. Sarah Hainer

FACT maintains pluripotency through gene distal regulation and coordinated co-localization with chromatin regulatory factors

Rithika Sankar*, Dave Klein*, Sarah Hainer

The FACT complex is a conserved and essential histone chaperone, studied for its roles in replication, transcription, and repair. It was previously thought to be essential for cell growth and proliferation, given its role in regulating passage through the nucleosomal roadblock for RNA Pol II and replication machinery. However, FACT was found to be dispensable for viability and cell growth of most adult non-cancerous mammalian tissue. Depletion of FACT in undifferentiated cells results in loss of pluripotency, yet the mechanism through which FACT aids in orchestrating pluripotency remains unclear. To determine the mechanism, we generated an AID-tagged version of SPT16, a subunit of FACT, for rapid depletion. Depletion of SPT16 led to increased nucleosomal occupancy at loci bound by master pluripotency factors while simultaneously downregulating both mRNA transcripts and enhancer transcripts associated with Pou5f1, Sox2, and Nanog. Chromatin profiling for SOX2 after SPT16 depletion revealed reduced occupancy suggesting that FACT regulates expression and localization of pluripotency factors. Next, we investigated how FACT localization correlates with additional pluripotency factors and chromatin regulatory factors. We identified that FACT co-localizes with several important factors including BRG1, EP400, while binding adjacent to CHD1, CHD2 and INO80. Our results suggest that potential disruption of recruitment and retention of factors may aid in changes observed at the nucleosomal and transcriptomic levels after FACT depletion. In conclusion, we find that FACT maintains pluripotency through a complex combination of gene distal regulation and coordinated functioning alongside chromatin regulatory factors.

Savransky, Sofya

she/her/hers
Molecular Pharmacology (MPHL) Year 3
Advisor: Jean-Pierre Vilardaga

Spaciotemporal GPCR signaling via cAMP controlled by glycosaminoglycans
Sofya Savransky, Jean-Pierre Vilardaga

The parathyroid hormone (PTH) type 1 receptor (PTHR) is the canonical class B G-protein coupled receptor (GPCR) for PTH and PTH-related protein (PTHrP). Both hormones display similar pharmacological properties for activating G-protein signaling pathways, but promote distinct physiological outcomes. While PTH acts in an endocrine manner to maintain calcium and phosphate ions homeostasis in the blood, PTHrP exerts its effects in an autocrine and paracrine manner to regulate growth and development of bone and mammary glands, as well as placental calcium ion transport. We have demonstrated that PTH promotes sustained cAMP signaling from internalized PTH-PTHR complexes in early endosomes, while PTHrP promotes sustained cAMP signaling via active PTHrP-PTHR complexes localized to the cell plasma membrane. Our goal is to determine the molecular and cellular mechanisms by which spaciotemporal cAMP specificities of PTH and PTHrP differ. Our pilot data revealed that glycosaminoglycan (GAGs), essential components of the extracellular matrix (ECM), regulate the actions of PTH and PTHrP in time and space. We hypothesize that GAGs control PTHR signaling via direct interactions with the receptor and/or ligands. Promising results suggest that GAGs such as heparan sulfate and hyaluronin, mediate ligand and location biased PTHR signaling. If confirmed, these findings will provide insight into the biased signaling mechanisms of PTHR and contribute to a better understanding of how to target PTHR for the treatment of bone and mineral diseases.

Schall, Terra

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Center for Neuroscience (CNUP) Year 4
Advisor: Yan Dong

Investigating the neural representations of cocaine vs. sucrose in the nucleus accumbens

Terra Schall, King Lun Li, Yan Dong

Drug craving and relapse are prominent in substance use disorders (SUD) and are often instigated by re-exposure to the cues previously associated with drug taking, even after prolonged drug abstinence. Rodent studies reveal that drug-induced changes in principal medium spiny neurons (MSNs) within the nucleus accumbens (NAc) play a critical role in cue-induced drug seeking. One such critical change is cocaine-induced generation of AMPA receptor (AMPAR)-silent excitatory synapses, which rewire the NAc circuit and are proposed as key synaptic substrates in forming NAc ensembles that support cocaine-associated memories at the circuit level. However, it is unclear if silent synapses form in response to natural rewards or if silent synapses are a unique synaptic substrate generated in response to cocaine exposure. Our current study is designed to examine the roles of silent synapses and NAc ensembles in sucrose vs. cocaine seeking. Our results show that silent synapses are generated at different magnitudes in the NAc following cocaine vs. sucrose self-administration. Furthermore, we detected cocaine-cue and sucrose-cue ensembles in the NAc, each of them comprising of a small population of NAc neurons displaying temporally contingent activities with cue-induced operant responding. Collectively, these preliminary findings provide evidence for sucrose-cue vs. cocaine-cue ensembles in the NAc and set up the study to determine the role of silent synapses in forming these reward-specific ensembles.

Schneider, Nathan

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Center for Neuroscience (CNUP) Year 3
Advisor: Ross Williamson

Extratelencephalic neurons encode learned stimulus categories and behavioral choice
Nathan A. Schneider, Tomas Suarez Omedas, Rebecca F. Krall, Ross S. Williamson

Auditory-guided behavior is ubiquitous in everyday life, whenever sound is used to guide our decisions and actions. Nestled among several populations, extratelencephalic (ET) neurons reside in the deep layers of auditory cortex (ACtx) and route auditory information to sub-cortical targets associated with decision-making. To investigate the role of ET neurons in auditory-guided behavior, we developed a head-fixed choice task, where mice categorized the rate of sinusoidal amplitude-modulated (sAM) noise bursts as either high or low to receive a water reward. We used two-photon calcium imaging alongside selective GCaMP8s expression to monitor the activity of ET and layer (L)2/3 intratelencephalic (IT) populations. Clustering analyses of ET populations revealed “categorical” firing patterns (i.e., neurons that responded best to low or high sAM rates). This categorical selectivity was not present early in training; rather, ET neurons shifted their response profiles dynamically across learning to reflect these discrete perceptual categories. Using dimensionality reduction methods, we found that ET population activity also reflected behavioral choice, regardless of reward outcome. Neural decoding analyses confirmed that behavioral choice could be robustly predicted from ET activity. Both choice and categorical selectivity were notably lessened in the L2/3 IT population, hinting at a unique ET role. Critically, ET categorical selectivity was only evident during active behavioral engagement and disappeared during passive presentation of identical stimuli. This suggests that categorical selectivity is shaped via top-down inputs that act as a task-dependent filter, and that ACtx ET neurons selectively propagate behaviorally-relevant signals brain-wide to influence behavior.

POSTER #94

Scott, Ellen

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Program in Microbiology and Immunology (PMI) Year 4
Advisor: Dario Vignali

Interplay of IL-12 and IFNg to induce Treg fragility within the tumor microenvironment
Ellen N. Scott, Angela M. Demske, Creg J. Workman, Dario A.A. Vignali

Regulatory T cells (Tregs) are able to suppress the anti-tumor T cell response through use of direct and indirect mechanisms. However, under some circumstances Tregs can lose their suppressive activity while retaining expression of their master transcription factor, Foxp3, a phenomenon our lab has termed Treg fragility. A hallmark of Treg fragility is production of IFNg, a pleiotropic cytokine necessary for response to cancer immunotherapies such as anti-PD1. However, it is unknown the exact mechanism that leads to Treg fragility as well as the consequences of Treg-produced IFNg.

By using a novel transgenic mouse with a conditional deletion of IL-12Rb2 (Il12rb2.Thy1.1L/LhNGFR Foxp3Cre.YFP), in Tregs we were able to test the effect of IL-12 on Treg function and response to immunotherapies during tumor progression. We have found that anti-PD1 therapy modestly increase IFNg and IL-12 levels within the tumor. In contrast, when using an IL-12 inducing therapies there were larger amounts of IL-12 intratumorally along with very large increases of IFNg levels in tumors and serum of wildtype mice. However, tumor growth was not affected in mice with IL-12R deficient Tregs after therapy, and these Tregs remained sensitive to fragility induction. These data may indicate that the increase in IFNg induced by immunotherapy may circumvent the IL-12 signaling loss in our mouse model and induce Treg fragility. While the mechanism of how IL-12 is effecting these changes still unknown, this evidence suggests that IL-12 can indirectly induce fragility in Tregs and therefore augment the TME and the response to immunotherapy.

POSTER #95

Somoulay, Xayathed

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Program in Microbiology and Immunology (PMI) Year 4
Advisor: Terence Dermody

ATXN2L interacts with reovirus nonstructural protein μNS at viral factories and is required for viral replication

Xayathed Somoulay and Terence Dermody

RNA viruses have evolved mechanisms to induce the biogenesis of intracellular viral factories (VFs) that serve as sites of viral genome replication and particle assembly. VF formation is essential for replication of most RNA viruses. However, little is known about VFs formed by mammalian orthoreovirus (reovirus), a double-stranded RNA virus that causes age-restricted disease in a broad range of mammals and is associated with loss of immunological tolerance to dietary antigen. Reovirus nonstructural protein μNS nucleates formation of VFs, but its precise function is not clear. During reovirus replication, viral structural and nonstructural proteins as well as viral mRNAs are recruited to VFs. However, the landscape of cellular factors recruited to VFs and their function in genome synthesis and particle assembly is unknown. To identify host factors recruited to VFs during reovirus replication, we conducted a BioID screen for host interactants of μNS. Using STRING functional enrichment analysis of candidates identified by μNS BioID, we observed enrichment of proteins involved in stress granule assembly, including ATXN2L, a stress granule nucleating factor. Using siRNA-mediated gene silencing, we observed diminished reovirus titers in ATXN2L siRNA-treated cells compared with control siRNA-treated cells. We conducted co-immunoprecipitation assays and observed an interaction between ATXN2L and μNS. Using immunofluorescence microscopy, we found that ATXN2L co-localizes with μNS at the periphery of VFs. Together, these data suggest that ATXN2L interacts with μNS at VFs, where it aids in efficient replication of reovirus.

POSTER #96

Spahr, Kellie

she/her/hers
Program in Microbiology and Immunology (PMI) Year 2
Advisor: Dr. Greg M Delgoffe

Exhausted T cells are characterized by inappropriate lipid accumulation

Kellie Spahr1,2,3 Nicole Scharping1,2,3, Greg M Delgoffe1,2
1Tumor Microenvironment Center, UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA 2Department of Immunology, University of Pittsburgh, Pittsburgh, PA 3Program in Microbiology and Immunology, University of Pittsburgh, Pittsburgh PA

The efficacy of immunotherapy depends on the presence and persistence of functional immune cells within the tumor. While tumor-specific T cells can be activated and infiltrate the tumor microenvironment, they are quickly rendered dysfunctional by the combination of chronic antigen stimulation, hypoxia, and nutrient scarcity. We have also shown that T cell exhaustion and metabolic insufficiency are driven by mitochondrial stress1. Interestingly, we and others have observed that CD8+ T cells accumulate large lipid stores as they progress towards exhaustion2. Using an in vitro model of chronic stimulation and hypoxia, we sought to define the role of lipid metabolism in the progression of CD8+ T cell exhaustion. Inhibition of citrate transport from the mitochondria via SLC25A1 in CD8+ T cells resulted in reduced lipid accumulation and reduced expression of inhibitory receptors known to be upregulated on exhausted T cells. We also observed increased production of inflammatory cytokines in response to TCR restimulation. Our results indicate a role for mitochondrial citrate flux in the accumulation of cytosolic lipids and progression of CD8+ T cell exhaustion. We propose that as exhausted T cells experience mitochondrial stress and perform less oxidative phosphorylation, they shuttle excess citrate to the cytosol where it fuels production of acetyl-CoA and de novo fatty acid synthesis. We aim to clarify whether lipid accumulation in these cells contributes to their dysfunction or represents an untapped source of carbon that may be the key to their reinvigoration.

POSTER #97

Staggers, Sophia

she/her/hers
Molecular Biology & Structural Biology (MBSB) Year 2
Advisor: Stella Sun

Exploring Flagellar Attachment Protein Sorting in Trypanosoma brucei using Cryo-electron Tomography (ET)

Sophia R. Staggers and Stella Y. Sun

Trypanosoma brucei is a single-celled eukaryotic parasite that causes African Sleeping Sickness in humans, a debilitating illness that can lead to death if left untreated. T. brucei expresses a singular flagellum throughout its life cycle that adheres to the cell body along its length. This characteristic is crucial for whole-cell motility and proliferation. A group of proteins called the flagellar attachment zone (FAZ) is involved in flagellum adherence to the cell body. The specific roles and organization of these FAZ proteins are still largely unknown. Two members of the FAZ, flagellar adhesion glycoprotein (FLA1) and FLA1-binding protein (FLA1BP) are intermembrane glycoproteins that are both required for flagellum adhesion to the cell body in T. brucei. While the localization of FLA1 and FLA1BP within the FAZ is generally known, the sorting process of these proteins in the cell is still unclear. A key player in T. brucei cellular function is the flagellar pocket (FP), a bulbous structure located around the base of the flagellum that is the only part of the cell membrane that performs endocytosis and exocytosis. In this study, we used cryo-electron tomography (ET) combined with molecular cell biology to elucidate FLA1-FLA1BP sorting in the FP and provide insights into other T. brucei structures involved in protein sorting into flagellum. Our investigation will generate fundamental insight into the molecular mechanism of membrane protein entrance for flagellar adhesions, a fascinating yet poorly characterized protein trafficking and sorting process that is required for flagellum-driven cell migration, which are necessary for therapeutic intervention development.

POSTER #98

Sui, Justin

he/him/his
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Corrine Kliment

Macrophage inflammatory and metabolic function is dependent upon Adenine Nucleotide Translocase 1 (ANT1) in COPD

Justin Sui, Jian Shi, Sruti Shiva, and Corrine R. Kliment

Chronic obstructive pulmonary disease (COPD) is characterized by macrophage-driven tissue destruction and chronic inflammation. While macrophage phenotypes in advanced human COPD have been explored, the initiating aberrant transcriptional changes that occur in lung macrophages leading to COPD remain unknown. Adenine nucleotide translocase 1 (Ant1) is an ATP transporter critical in mitochondrial metabolism. We hypothesize that loss of ant1 in macrophages results in metabolic dysfunction and changes in inflammatory pathways impacting early COPD pathogenesis that propagates through later disease stages. To identify cell-specific molecular pathways early and late in COPD, we performed single-cell RNA sequencing on wildtype and ant1-null mice exposed to CS for 2 and 6mo compared to air-exposed controls. The Agilent Seahorse XFe96 analyzer was used to measure mitochondrial and glycolytic metabolism in isolated wildtype and ant1-null bone marrow-derived (BMDM) and alveolar macrophages. Wildtype alveolar macrophages exhibited higher oxygen consumption rates and extracellular acidification rates compared to ant1-null cells, suggesting that wildtype cells are more metabolically active. Loss of ant1 protects against emphysematous tissue destruction in a mouse model of COPD with a reduction in macrophage accumulation. Wild-type mouse alveolar macrophages upregulate inflammatory pathways at both 2- and 6-months smoke treatment, while this does not occur in ant1-null mice. These findings highlight the importance of temporal assessment in identifying initiating pathways that lead to COPD development and implicate modulation of metabolic pathways as a promising therapeutic avenue for treating COPD.

POSTER #99

Tennant, Bill

he/him/his
Cellular and Molecular Pathology (CMP) Year 5
Advisor: Chris Donnelly

Phosphorylation state of TDP-43 alters both liquid-liquid phase separation and nuclear/cytoplasmic localization

Tennant, WM, Mann, JR, Donnelly, CJ

TDP-43 is a predominantly nuclear DNA/RNA binding protein (RBP) where it plays a key role in RNA processing. In ALS, TDP-43 is depleted from the nucleus and mislocalized to the cytoplasm and forms hyperphosphorylated and p62 positive aggregates that strongly correlate with CNS degeneration. The upstream mechanisms leading to TDP-43 proteinopathy and its contribution to neurotoxicity remain unresolved and existing approaches to model this pathological event are unreliable. We recently developed a optogenetic technique to generate TDP-43 inclusions (optoTDP43) in human cells employing photokinetic protein clustering. We show that oligomerization of the C-terminal LCD of TDP-43 promotes aggregation. While hyperphosphorylation of TDP-43 aggregates is a hallmark pathology of ALS, the actual physiological and pathological effects of phosphorylated TDP-43 remain unknown. Using the aforementioned optoTDP43 system, we have generated a phosphomimetic (S to D) and phosphomutant (S to A) construct at 5 distinct sites that are phosphorylated in ALS patient tissue. When pulsed with blue light, we find that the both the phosphomimetic and phosphor-null condition, mimicking hyperphosphorylation and hypophosphorylation respectively, more readily separate into droplets when compared to the wild-type protein. Furthermore, these droplets are more stable and persist longer than the hyperphosphorylation and wild-type counterparts. Continued studies of full length overexpression models also show disrupted nuclear expression and mislocalization to the cytoplasm. This altered phase transition and mislocalization of TDP-43 suggests both a physiological and pathological role for phosphorylation as a regulator of TDP-43 behavior.

POSTER #100

Thosar, Sanjana

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Molecular Genetics and Developmental Biology (MGDB) Year 5
Advisor: Patty Opresko

Telomeric oxidative base damage promotes the alternative lengthening of telomeres pathway

Sanjana Thosar, Patty Opresko

Telomeres protect the ends of linear chromosomes from being aberrantly detected as sites of chromosome breaks. The guanine rich sequence renders telomeres highly susceptible to oxidative base damage, particularly the formation of the common lesion 8-oxoguanine (8-OxoG). This lesion is repaired by the base excision repair (BER) pathway via specific recognition of 8-OxoG by the DNA glycosylase OGG1. Using our recently published chemoptogenetic tool that induces 8-OxoG specifically at the telomeres, we showed that telomeric 8-OxoG alone, is sufficient to drive replication stress in immortalized non-cancer cells but not in telomerase positive HeLa cells. 5-15% of cancers maintain their telomeres by a homologous recombination (HR) based mechanism called alternative lengthening of telomeres pathway (ALT). ALT telomeres maintain a steady state level of replication stress to facilitate homology directed telomere extension. In this study we propose that the base lesion 8-OxoG when produced at the telomeres regulates cell growth by modulating ALT. An acute 20-minute exposure to induce telomeric 8-oxoG increased various markers of ALT activity such as ALT associated PML bodies (APBs), c-circles and telomere sister chromatid exchanges (TSCEs) and this increase was augmented when OGG1 was depleted, further emphasizing the role of 8-OxoG in modulating ALT. This acute induction of telomeric 8-oxoG further activated the ATR/Chk1 response to replication stress. These studies will elucidate a potential role for the base lesion 8-OxoG as a positive regulator of replication stress driven ALT.

POSTER #101

Troutman, Kayla

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Cell Biology (CBMP) Year 5
Advisor: Marijn Ford

The dual role of Pib2 in reactivation and inhibition of vacuolar TORC1 in S. cerevisiae
Kayla K. Troutman, Natalia V. Varlakhanova, and Marijn G. J. Ford

The target of rapamycin complex 1 (TORC1) is a highly conserved kinase complex that is critical for control of cell growth and autophagy. TORC1 integrates cellular nutrient status, and its activity is regulated by several upstream signaling pathways. TORC1 can be inhibited by nutrient starvation or with rapamycin and can be reactivated by amino acids. In Saccharomyces cerevisiae, TORC1 reactivation is critically dependent on Pib2, however, the molecular mechanisms of TORC1 reactivation remain poorly understood. Pib2 localizes to both the vacuole and endosomes and is a glutamine sensor, which activates TORC1 through a direct interaction. Pib2 contains several conserved regions and domains. Through systematic mutagenesis and functional dissection of S. cerevisiae Pib2, we have identified regions that are essential for TORC1 reactivation and Pib2 subcellular localization. We have also identified key residues in the inhibitory regions of the Pib2 N-terminus which prevent TORC1 reactivation. Here we use rapamycin exposure assays and live-cell confocal imaging to show that Pib2 helical region E and the tail motif, are vital for TORC1 reactivation, whereas the N-terminal regions A and B have TORC1 inhibitory functions. Furthermore, we show that while the Pib2 FYVE domain is critical for vacuolar localization, it is unexpectedly not required for recovery from rapamycin induced growth arrest. Using fusion proteins which specifically target Pib2 to the vacuole or endosomes, we show that vacuolar localization of Pib2 is essential for the reactivation of TORC1 and cell growth following rapamycin exposure. Here we have demonstrated that Pib2 plays a unique role as a dual modulator of TORC1 activity in S. cerevisiae.

POSTER #102

Waxman, Susannah

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Cellular and Molecular Pathology (CMP) Year 4
Advisor: Dr. Ian Sigal

Quantifying Astrocyte Morphology in the Collagenous Lamina Cribrosa

Susannah Waxman1; Marissa Quinn1; Cara Donahue2; Louis Falo2,3; Daniel Sun4; Tatjana Jakobs4; Ian A. Sigal1,3

Purpose: Astrocytes in the lamina cribrosa (LC) play important roles in cell signaling and extracellular matrix remodeling, impacting the physiology of neural tissues necessary for vision. Although LC astrocyte morphology is known to undergo substantial changes throughout the pathogenesis of glaucoma, techniques to visualize and evaluate collagenous LC astrocyte morphology are limited. We hypothesized that multicolor DiOlistic labeling allows visualization and quantitative evaluation of collagenous LC astrocyte morphology.
Methods: Gold particles were coated with all combinations of three fluorescent lipophilic dyes (DiI, DiD, and DiO) to create 7 different groups of microcarriers. Coronal vibratome sections were obtained through the LC of goat, sheep, and pig eyes (N = 16 eyes) at 150 μm thickness. Dye-coated microcarriers were delivered into sections with a gene gun. Dyed cells were imaged via confocal microscopy. 3D models of 56 dyed astrocytes were created from Imaris image segmentations. Morphological features of models were quantified for LC astrocyte characterization.
Results: Somas and branched processes of astrocytes labeled with all 7 combinations of dyes were visualized. Label density allowed segmentation and 3D model construction of individual astrocytes within LC pores. Average astrocyte branch number, hierarchy, length, thickness, and straightness were 132.0±46.1, 7.4±4.0, 11.2±10.7μm, 1.9±1.2μm, and 0.9±0.1, respectively.
Conclusions: Multicolor DiOlistic labeling in vibratome sections of the collagenous LC is a suitable technique for visualization and quantitative analysis of astrocyte morphological features. Healthy astrocyte morphologies can later be compared with those in glaucomatous LCs to better understand the role of astrocytes in pathogenesis.

POSTER #103

van der Geest, Rick

he/him/his
Cellular and Molecular Pathology (CMP) Year 6+
Advisor: Janet Lee

BATF2 mediates pro-inflammatory cytokine production in macrophages and enhances early host defense against pulmonary Klebsiella pneumoniae infection

Rick van der Geest, Hernán F. Peñaloza, Zeyu Xiong, Shekina Gonzalez-Ferrer, Xiaojing An, Huihua Li, Hongye Fan, Mei Hulver, Mohammadreza Tabary, Seyed Mehdi Nouraie, Yanwu Zhao, Yingze Zhang, Will Bain, and Janet S. Lee

Background – We previously identified the transcription factor BATF2 among the top upregulated genes in LPS-treated human alveolar macrophages. Here, we assessed the signaling pathways that mediate the induction of BATF2 expression in response to LPS stimulation, and we determined the role of BATF2 in the host response to pulmonary infection with Klebsiella pneumoniae (Kp) – a Gram-negative pathogen that is a common cause of hospital-acquired pneumonia.

Approach & Results – Evaluation of Batf2 gene expression in Myd88-/- and Trif-/- macrophages in vitro showed that Kp-induced Batf2 expression requires TRIF but not MyD88 signaling. Analysis of WT and Batf2-/- macrophages by RNA-seq and ELISA identified a reduced pro-inflammatory response in Kp-treated Batf2-/- macrophages, which was characterized by markedly lower pro-inflammatory cytokine production compared to WT macrophages (IL-6: -15%, $p<0.001$; CCL5: -32%, $p<0.001$; CCL4: -37%, $p<0.05$; IL-12p40: -31%, $p<0.001$). In vivo, Kp-infected Batf2-/- mice displayed a 6-fold higher ($p<0.01$) lung bacterial burden compared to WT mice, as well as increased dissemination of Kp to the spleen (5-fold, $p<0.05$) and liver (6-fold, $p<0.05$), indicating an impaired host defense in BATF2-deficient mice. Importantly, analysis of the bronchoalveolar lavage fluid (BAL) of Kp-infected WT and Batf2-/- mice by multiplex immunoassay showed that BAL pro-inflammatory cytokine levels were markedly lower in Batf2-/- mice compared to those in WT mice (IL-5: -51%, $p<0.05$; IL-12p40: -41%, $p<0.05$; IFN: -33%, $p<0.01$; CCL5: -60%; $p<0.01$).

Conclusion – Our data show that BATF2 is important for an effective pro-inflammatory cytokine response in macrophages during pulmonary Kp infection, and that as such, BATF2 contributes to the host defense against Kp.

POSTER #104

Vergara, Kevin

he/him/his
Program in Microbiology and Immunology (PMI) Year 4
Advisor: Maninjay Atianand

Defining the mechanism of LUCAT1 mediated immune gene regulation in macrophage
Kevin Vergara, Sneha Lal, Richard T. Cattley, William Hawse, Maninjay K. Atianand

Long noncoding RNAs (lncRNA) are transcripts larger than 200 nucleotides that play crucial roles in many cells and tissues by regulating gene expression in an RNA-dependent manner. The molecular basis of their cellular functions, however, remains poorly understood. Here we seek to determine the mechanism of action of LUCAT1 (Lung cancer associated transcript 1) lncRNA, which we have discovered as a crucial regulator of macrophage inflammatory response. LUCAT1 promotes the expression of many immune- and inflammation-associated genes including DUSP1, DUSP5, and NT5E at the transcriptional level. Using in vitro binding assays with biotinylated RNA and nuclear lysate, followed by quantitative mass spectrometry, we have identified several putative LUCAT1-ineracting nuclear proteins including an RNA binding protein RBMX. We have generated RBMX-mutant THP1 cells via CRISPR-Cas9 to test the functional consequences of LUCAT1-RBMX interaction. These cells show significantly lower expression of a subset of LUCAT1 regulated genes in resting and TLR4-activated conditions, indicating that LUCAT1 and RBMX are functionally coupled. Ongoing experiments are aimed at further characterization of the LUCAT1-RBMX axis, and defining the composition and function of the LUCAT1 RNP complex in macrophages. These studies will advance our current understanding of the biological roles of lncRNAs in immune cells, and their mechanism of action in general.

POSTER #105

Warunek, Jordan

he/him/his
Program in Microbiology and Immunology (PMI) Year 3
Advisor: Heth Turnquist

Recognition of Donor MHCI Disrupts Recipient Reparative Macrophage Differentiation and Wound Repair

J. Warunek, L. Mathews, M. Oberbarnscheidt, F. Lakkis, H. Turnquist

Injury is inherent to organ procurement and transplantation (Tx). Typically, graft infiltrating monocytes differentiate into CD86hiLy6chi pro-inflammatory or CD206+CD301+ reparative macrophages (MΦs) in response to local signals and cytokines to orchestrate tissue repair. However, Tx creates a novel situation where non-self, major histocompatibility (MHC) molecules will persist at injury sites. Monocytes express paired-immunoglobulin-like receptors (PIRs) that can distinguish non-self from self MHCI to stimulate alloimmunity. If non-self MHCI impacts MΦ-mediated tissue repair is unknown. We tested the hypothesis that MΦ recognition of non-self MHCI alters their differentiation and function to limit effective repair. Wildtype, Rag2-/-γc-/-, and Pira-/- C57BL/6 MΦ differentiation was assessed after exposure to allogeneic (BALB/c; H-2d; Allo) or syngeneic (C57BL/6; H-2b; syn) materials in vitro and in vivo using flow cytometry. The function of syn and allo exposed MΦs were assessed in a wound healing assay utilizing IncuCyte live-cell imaging. Syn materials created local environments dominated by CD206+ CD301+ reparative MΦs, while allo generated phenotypically pro-inflammatory Ly6chiCD86hi MΦs in both WT and Rag2-/-γc-/- B6 recipients. Recombinant H-2Dd (Allo) alone stimulated pro-inflammatory MΦ differentiation in vitro. Pro-inflammatory MΦ generation in response to non-self MHCI was not mediated via PIR-A as Pira-/- MΦs responded similarly. Exposure to allo, but not syn, modulated MΦ functional capacity for repair. Our data provide evidence for the intersection of innate allorecognition and repair pathways. This suggests that MΦ responses to MHCI via presently undefined receptor(s) may contribute to persistent clinical problems, like graft fibrosis and vasculopathy.

Weckerly, Claire

she/her/hers
Cell Biology (CBMP) Year 4
Advisor: Gerry Hammond

Characterization of the membrane binding of NIR1-LNS2 for its use as a phosphatidic acid biosensor

Claire Weckerly1, Michael Airola2, Taylor Rahn2, Jeremy Baskin3, Gerry Hammond1
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Phosphatidic acid (PA) is a plasma membrane-localized lipid with broad cellular functions. It facilitates membrane bending, recruits enzymes to cellular membranes, and acts as a precursor for other lipids. Dysregulation of PA has been implicated in neuronal diseases, viral entry, oncogenic signaling, and cardiovascular diseases. Despite its importance, there is a lack of tools available for live-cell imaging of PA. The Nir family (Nir1, Nir2, and Nir3) of lipid transfer proteins has a conserved C-terminal LNS2 domain which has been suggested to bind to PA. However, the role of PA in Nir membrane recruitment is still unclear.

The purpose of this work is to determine how PA regulates Nir1-LNS2 localization in order to utilize this domain as a novel genetically encoded lipid biosensor for PA. We hypothesize that PA is sufficient for the membrane-binding of Nir1-LNS2, and that Nir1-LNS2 localization depends specifically on PA. To test this, we acutely manipulated PA levels by enzyme overexpression or pharmacological agents while imaging NeonGreen-Nir1-LNS2 localization using confocal and TIRF microscopy.

So far, this work has demonstrated that Nir1-LNS2 becomes plasma membrane-localized after inducing PA production, and it recruits more robustly than the established biosensor Spo20-PABD. Nir1-LNS2 plasma membrane localization depends on PA, as PA depletion resulted in a reduction of Nir1-LNS2 at the plasma membrane.

Completion of this project will determine the role of PA in Nir1-LNS2 localization and providing important insight into how the Nir family is regulated. Additionally, this work will lead to the development of a robust new biosensor for PA, which will facilitate future studies of this important lipid within live cells and various disease models.

POSTER #107

Wedn, Abdall

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Molecular Pharmacology (MPHL) Year 2
Advisor: Steffi Oesterreich

The differential expression and activation of cAMP/PKA/CREB pathway in invasive ductal and lobular breast tumors

Abdalla Wedn, Susrutha Puthanmadhom-Narayanan, Adrian Lee, and Steffi Oesterreich

Invasive lobular cancer (ILC) is the second most common subtype of breast cancers after invasive ductal cancer (IDC) but associated with worse outcomes. Despite the known molecular differences between ILC and IDC, ILC treatment is the same as stage matched IDC making the development of ILC targeted therapies an urgent need. Therefore, this study was conducted to identify pathways that are deferentially active in ILC which could be therapeutically targeted. Differential gene expression and pathway analysis between luminal A ILC and IDC patients in SCAN-B, TCGA and METABRIC datasets revealed ILC related upregulation of Gs-alpha-subunit (Gas), cyclic adenosine 3',5'-cyclic monophosphate (cAMP) and Protein Kinase A (PKA) mediated signaling. Additionally, Weighted Gene Correlation Network Analysis revealed that Gas, cAMP and cAMP-response element binding protein (CREB) pathways are positively correlated with lobular histology. Besides, gene set enrichment analyses in SCAN-B database confirmed the enrichment of these pathways in ILC patients. In-vitro studies showed higher levels of CREB phosphorylation in ILC (MM134, SUM44PE, BCK4, WCR25) and ILC like (CAMA1, MPE600, ZR75-30) cell lines after Forskolin treatment, a compound that increases intracellular cAMP, compared with IDC (MCF7, T47D7, EFM19, BT474). Importantly, the enhanced activation of PKA pathway following Forskolin has also been observed in ILC patient-derived organoids (PDOs) compared with IDC organoids. Nevertheless, knockout of CDH1 gene in IDC cells failed to affect Forskolin evoked CREB phosphorylation. Collectively, the data point to an upregulated cAMP/PKA/CREB machinery in ILC that is unrelated to loss of E-Cadherin in these tumors. This could serve as basis for development of novel therapies for ILC tumors

POSTER #108

Wilson, Sierra

she/her/hers
Cellular and Molecular Pathology (CMP) Year 3
Advisor: Andrew Duncan

Diploid hepatocytes resist acetaminophen-induced acute liver injury and drive compensatory regeneration

Sierra R. Wilson, Evan R. Delgado, Madeleine P. Leek, Kero Kamel, Patrick D. Wilkinson, Frances Alencastro, Bharat Bhushan, Silvia Liu, Joseph Locker, Andrew W. Duncan

The functional differences between diploid and polyploid hepatocytes are poorly understood. We hypothesize that diploid hepatocytes drive rapid regeneration in the context of acute liver injury. To study ploidy populations in vivo, we utilized mice with a liver-specific knockout of E2f7 and E2f8 (LKO) that are functionally normal but are depleted of polyploid hepatocytes. Acute liver injury was induced in LKO and control mice with 300 mg/kg acetaminophen (APAP) and harvested over 0-96H. Elevated liver enzymes, necrosis, and apoptotic hepatocytes revealed that while APAP damaged both treatment groups, control livers were significantly more damaged than LKO. Reduced damage and accelerated liver healing in the LKO model could be caused by gene expression effects associated with E2f7/8 loss or by enrichment of diploid hepatocytes. To discriminate between these possibilities, we first analyzed gene expression by RNA-sequencing in LKO and control mice after APAP injury. Differential gene expression was observed over the time course, which could contribute to varied sensitivity to APAP. Second, we focused on genes expression differences only by knocking out E2f7/8 in adult livers. These mice had normal ploidy and responded to APAP equivalent to control. Finally, the response to APAP by WT ploidy populations was studied in vitro. Both populations were equally damaged, but diploids showed enhanced proliferation. These data suggest that the response to APAP overdose in the LKO model is controlled by variations in gene expression and the enrichment of diploid hepatocytes. In conclusion, diploid hepatocytes are a driver of compensatory regeneration after acute liver injury, which underscores a novel role for hepatic ploidy populations.

POSTER #109

Ye, Chenxian

she/her/hers
Program in Microbiology and Immunology (PMI) Year 2
Advisor: Greg Delgoffe

Low Glucose Avidity Favors Suppressive Capacity of Treg cells

Chenxian Ye, McLane J. Watson, Greg M. Delgoffe

Regulatory T (Treg) cells are key mediators in maintaining immune homeostasis and preventing autoimmune responses. However, in tumor immunity, Treg cells play a detrimental role in antitumor immune responses. High accumulation of Treg cells in the tumor microenvironment (TME) correlates with rapid tumor progression and poor outcomes. TME is characterized by tumor hypoxia, acidity, and metabolic dysregulation. Previous studies have shown that the altered glucose metabolic landscape of TME is associated with increased intratumoral Treg cell activity. But the specific mechanisms by which glucose metabolism regulates Treg cells have not been identified yet. We measured the glucose uptake ability of Treg cells in the TME and periphery tissues based on fluorescent glucose tracer GlucoseCy5. We observed the presence of Treg subsets with low and high glucose avidity, but high glucose avid (GlucoseCy5high) Treg cells were least enriched in the TME. Interestingly, GlucoseCy5high Treg cells exhibited a reduced suppressive capacity compared with GlucoseCy5low Treg cells. GlucoseCy5low Treg cells also maintained higher suppressive capacity when cultured in the high-glucose medium in vitro. Furthermore, we found that GlucoseCy5low Treg cells had a higher level of BCl6 which represses the gene program of the glycolysis pathway. In the future, we will focus on the links between intrinsic glucose avidity with Treg suppressive function. And we will specifically investigate the role of BCL6 in controlling Treg glucose metabolism and Treg cell function. Our study will provide insights into the development of potential Treg cell-based strategies to improve antitumor therapy.

POSTER #110

Yeh, Hsuan

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Cellular and Molecular Pathology (CMP) Year 1
Advisor: Roderick Tan

Effect of Relaxin on Age-related Renal Fibrosis

Hsuan Yeh, Roderick Tan

Healthy aging results in renal function decline and corresponding histologic features, including tubulointerstitial fibrosis. Relaxin, an insulin-like hormone, has demonstrated antifibrotic effects in various rodent models of cardiovascular and renal diseases. Whether it can suppress age-related renal fibrosis and the possible mechanisms of its action are unknown. This study attempts to examine whether relaxin has antifibrotic effects in aged rat kidneys and whether it interacts with Wnt signaling pathways or angiotensin II receptors (AGTRs) using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assays to determine the change of levels of mRNA expression of the related genes. Age-induced fibrosis was minimally observed as quantified by the selected markers in our experiment. Relaxin was not found to significantly reduce fibrosis in aged rats. Neither Wnt signaling molecules nor AGTRs showed significantly different expression between treatment versus non-treatment groups. Despite the overall negative results described in this interim report, we could not exclude all possibilities that relaxin has potential roles in age-related renal disease. More studies to validate our findings and experimental optimization is required.

POSTER #111

Zdinak, Paul

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Program in Microbiology and Immunology (PMI) Year 3
Advisor: Dr. Alok Joglekar

Signaling and antigen-presenting bifunctional receptors paired with computational prediction for TCR-directed antigen discovery in type 1 diabetes

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Elucidating the epitopes targeted by CD4+ T cells in type 1 diabetes (T1D), and in general, has remained a challenge due to the technical limitations of conventional MHC class-II antigen discovery methods. Therefore, we have developed a method of CD4+ TCR-directed antigen discovery which allows for de novo identification of T cell specificity using our lab's signaling and antigen-presenting bifunctional receptors (SABRs), augmented by computational prediction of TCR similarity. SABRs are peptide-MHC complexes paired with intracellular signaling domains which provide a detectable output downstream of recognition by a cognate TCR. To demonstrate our method, we performed single cell RNA sequencing of pancreatic islet infiltrating T cells from 6-, 8- , and 10-week-old female Non-Obese Diabetic (NOD) mice. NOD mice share numerous autoantigens which have been translated to discovery in human T1D patients. Additionally, we constructed a SABR library of 4,075 I-Ag7-restricted (NOD MHC class-II) epitopes based on published datasets and used this library to screen the top 40 clonally expanded TCRs. SABR screening alone yielded the epitope specificities for 10 of the TCRs de novo. Furthermore, computational prediction identified 5 unique TCRs predicted to bind the same epitopes, which were validated experimentally. Therefore, we have demonstrated a novel and powerful method of TCR-directed antigen discovery which can be used to profile CD4+ T cells for translation to diagnostic, preventative, and therapeutic efforts.

POSTER #112

Zhang, Robert

he/him/his
Program in Microbiology and Immunology (PMI) Year 3
Advisor: Dr. Melissa Kane

Genetic differences between 129S substrains affect antiretroviral immune responses
Robert Z. Zhang, Vincent Mele, Lia Robben, Melissa Kane

Inbred mouse lines vary in their ability to mount protective antiretroviral immune responses, and even closely related strains can exhibit opposing phenotypes upon retroviral infection. Here, we found that 129S mice inherit a previously unknown mechanism for the production of anti-murine leukemia virus (MLV) antibodies and control of infection. The resistant phenotype is controlled by two dominant loci that are independent from known MLV-resistance genes. We also show that production of anti-MLV antibodies in 129S7, but not 129S1 mice is independent of interferon gamma (IFNγ) signaling. Thus, our data indicate that 129S mice inherit an unknown mechanism for control of MLV infection and demonstrate that there is genetic variability in 129S substrains that affects their ability to mount antiviral immune responses.