Abstract Program

2026 Cell Biology and Physiology Research Day

Thursday, April 30th, 2026

UNC Medical Biomolecular Research Building (MBRB)
111 Mason Farm Rd Chapel Hill, NC 27599

Table of Contents

Name	CBP Affiliation	Abstract Title	Poster #
Emily Bulik-Sullivan	CBP curriculum PhD student	Elucidating the mechanism of ENaC activity reduction by molecular glue degrader drugs	21
Kevin Chen	CBP Biomedical MS student	Maternal Hyperglycemia and Congenital Heart Defects: Unaltered Mevalonate Pathway Enzyme Expression Despite Increased Prenylation Activity	U
Mady Chlebowski	CBP curriculum PhD student	Investigating neuroimmune signatures of peripheral neurodegeneration	50
Tanisha Choudhury	CBP Biomedical MS student	Investigating LPL Aggregate Composition in the Absence of LMF1	DD
Jessica Cote	Postdoc	Inclusion of an alternatively spliced microexon in clathrin is disrupted in muscular dystrophies and critical for skeletal muscle development	4
Joseph Crecelius	Postdoc	Enteroendocrine cells regulate intestinal epithelial barrier integrity by modulating sphingolipid metabolism and abundance	14
David Cully	Non-CBP curriculum PhD student	Protein Geranylgeranylation Regulates Cardiac Morphology and Contractility During Embryonic Development	62
Bailey de Jesus	CBP curriculum PhD student	Investigating the Roles of Actin-Binding Proteins in Apical Constriction	27
Dhanushi Dedakia	CBP Biomedical MS student	Role of GATAD2A in Structure and Recruitment of NuRD Complex	K
Hailey Dodson	CBP curriculum PhD student	iPSC-derived human intestinal organoids to investigate gastrointestinal pathophysiology in cystic fibrosis	38
Morgan Ellington	CBP Biomedical MS student	Targeting KIF20A: A Small-Molecule Inhibitor Approach to Overcoming Therapeutic Resistance	BB
Hannah Ellyson	CBP Biomedical MS student	The Role of BRD4 in Spermatogenic Development	C
Ruth Fletcher	CBP Biomedical MS student	Hemodynamic Regulation of Atrioventricular Morphogenesis.	E

Name	CBP Affiliation	Abstract Title	Poster #
Anca Frasineanu	CBP curriculum PhD student	Investigating the role of brain enriched E3 Ubiquitin Ligase TRIM9 in Alzheimer's Disease/Tauopathies	16
Matthew Geib	CBP Biomedical MS student	Evaluating Candidate Antifibrotic Treatments in an IPF-Relevant Fibroblast Model	Y
Meredith Gillis	CBP curriculum PhD student	Illuminating the Organelle Signature Underlying Cardiomyocyte Differentiation	18
Antoine Gleizes	Postdoc	TET1 SAFEGUARDS LINEAGE ALLOCATION IN INTESTINAL STEM CELLS	12
Amber Gomez	CBP curriculum PhD student	NFκB governs CD8 T cell fate during exhaustion	53
Lauren Griffith	CBP curriculum PhD student	Establishing Spindle Positions in Oriented Cell Divisions	26
Shenice Harrison	CBP curriculum PhD student	Elucidating the Role of Protein Myristoylation During Embryonic Heart Development	15
Lillah Hayes	CBP Biomedical MS student	Toward Filopodia Purification Using Nanoporous Membranes	A
Mark Hazelbaker	CBP curriculum PhD student	The ARP2/3 Complex Regulation of Clathrin Dynamics is Induced through ACK	49
Harold Heatherly	Lab staff	Disturbed flow impairs ATGL-dependent lipid droplet clearance in endothelial cells	67
Max Hockenberry	CBP curriculum PhD student	High-Resolution Single-Particle Imaging of Actin Dynamics at the Leading Edge	54
Katie Holmes	CBP curriculum PhD student	A biphasic model for the morphological development of astrocytes in white ma7er	55
Nicole Hondrogiannis	CBP curriculum PhD student	Sublethal DNA Damage in Neurons: Is the Apoptotic Pathway Even Activated?	52
Sherry Hsu	CBP curriculum PhD student	Tubulin acetylation governs organelle remodeling and lysosomal reformation during neuronal differentiation	31
Kaitlyn Humphreys	CBP Biomedical MS student	Validation of Transcription Factor Candidates as Regulators of Proteinase 3	Z

Name	CBP Affiliation	Abstract Title	Poster #
Madison Kalk	Non-CBP curriculum PhD student	Lymphatic-specific protein interactome of RAMP1 in migraine	61
Kendall Kanakanui	Non-CBP curriculum PhD student	Multi-gene biomarkers reveal spatial organization and subpopulation-specific damage response in intrahepatic cholangiocytes	57
Juliet King	CBP curriculum PhD student	Hemidesmosomes and Notch signaling regulate epidermal differentiation during embryogenesis	36
Alexander Kinrade	Lab staff	Tubulin polyglutamylation modulates Golgi morphodynamics and neurite branching during neuronal morphogenesis	68
Poonam Kumari	Postdoc	Anti-migraine drugs modulate serotonin receptor signaling in lymphatic endothelial cells	9
Michelle LaBella	CBP curriculum PhD student	The Role of TNIK and tPA in Tumor-Stroma Crosstalk, ECM Degradation and Metastasis in Pancreatic Cancer	56
Ashlyn Laidman	CBP curriculum PhD student	Mechanisms of Collective Cell Migration during Epicardial Formation	28
Ava Langaker	CBP Biomedical MS student	Novel Therapeutic Strategy to Increase Radiosensitivity of High-risk HPV+ OPSCCs	N
William Lewis	CBP Biomedical MS student	AI- Guided Screening to Predict and Validate Novel FBXW7 Substrates in the Ubiquitination Pathway	AA
Kevin Lima	CBP Biomedical MS student	Redundancy in Sensorimotor and Associative Networks Mediates Age-Related Decline in Walking Speed and Endurance	B
Kimberly Lukasik	CBP curriculum PhD student	TRIM9 switches melanoma cell morphological phenotypes	46
Yehonathan Malis	Postdoc	SECTOLD: a novel pathway for trafficking of secreted proteins to lipid droplets mediated by TMEM43	7
Frankie Marchan	CBP curriculum PhD student	Branched Actin Regulation of Endomembrane Compartments	34

Name	CBP Affiliation	Abstract Title	Poster #
Kayla Mason	CBP curriculum PhD student	X-inactivation Escapee Ddx3x Enhances Translation Initiation of mRNAs Essential for Female Heart Function	35
Ian McCabe	CBP curriculum PhD student	Characterization of Pancreatic Ductal Adenocarcinoma Fibroblasts	20
Abigail Meader	CBP Biomedical MS student	Characterizing HA15-induced ATF4 stress signaling relevant to HIV-1 reservoir persistence	CC
Elizabeth Moorman	CBP Biomedical MS student	Exploiting the dependence of TNBC cells on glucose and glutamine metabolism to induce cell death following treatment with small molecule ClpP activator TR107	S
Kirthika Mutthoju	CBP Biomedical MS student	Mapping the Secretome and studying the Role of Microglia in Cortical Development	X
Jesse Niehaus	Postdoc	Single-cell RNA-seq reveals cellular adaptations underlying opioid addiction Decoding the opioid system using single-cell transcriptomics.	5
Aaliyah O'Dell	CBP Biomedical MS student	Choice of AAV purification method impacts viral vector performance in-vivo.	V
Zalma Ontiveros	CBP Biomedical MS student	Understanding Medium-Driven Differences In Primary Human Bronchial Epithelial Cells Cellular Functions	D
Becky Patrick	CBP Biomedical MS student	TRIM9 Switches the Morphological Phenotype of Melanoma Cells	M
Olivia Perez	CBP Biomedical MS student	Investigating the Roles of Alternative Splicing in Cardiac Biology	F
Addison Pignetti	CBP Biomedical MS student	Selective Targeting of NRAS and KRAS Isoforms: Differential Effects of Compound 181 on Q61R and Wild-Type	R
Alex Powers	CBP curriculum PhD student	APOE interacts with COX-2 on lipid droplets to modulate inflammatory lipid signaling.	33

Name	CBP Affiliation	Abstract Title	Poster #
Gabrielle Puller	Non-CBP curriculum PhD student	Identifying small GTPase binding sites on the exocyst tethering complex	59
Kayala Purdie	CBP Biomedical MS student	Epithelial Lipid Droplet Accumulation Enhances Intracellular Stress Fiber Formation and RhoA Activity	Q
Mariana Quinonez Medina	CBP Biomedical MS student	Lymphatic CGRP-CALCRL Signaling Regulates Intestinal Immune Cell Tracficking During Injury.	P
Aashita Rajput	Lab staff	Vascular Plasticity in the Regenerating Liver	65
Alicia Ravens	Postdoc	Unbiased genetics identifies a glutamatergic signaling network as a mediator of daily sleep patterns	71
Ava Reeder	CBP Biomedical MS student	Media Optimization to Increase Differentiation of Secretory Cells in Intestinal Enteroids	I
Jake Roetcisoender	CBP curriculum PhD student	Interrogating the role of vascular smooth muscle cells in guiding kidney innervation and development	48
Violet Rowland	CBP curriculum PhD student	The Influence of Background Genetics on Susceptibility to Alcohol-Induced Birth Defects	29
Samantha Ryken	CBP curriculum PhD student	Calcium regulated plasma membrane expansion and remodeling during neuronal morphogenesis	47
Matheus Sadovsky	CBP curriculum PhD student	Palmitoylation dynamics regulate mitochondrial function and redox homeostasis in embryonic cardiomyocytes	37
Taraneh Sadritabrizi	CBP curriculum PhD student	Genetic Regulation of Fetal Alcohol Syndrome: Evaluation of Phenotypic Variations Utilizing a Multigenerational Mouse Model System	40
Jeanette Sako	CBP Biomedical MS student	Pharmacokinetic and Clinical Outcomes of Transdermal Buprenorphine Solution (Zorbium) in Long Evans and Sprague Dawley Rats	J

Name	CBP Affiliation	Abstract Title	Poster #
Annalee Schmidt	CBP curriculum PhD student	Fermentation-derived metabolites modulate host signaling pathways to control cellular aging	24
Stephen Serafin	Postdoc	Proximity proteomics of RAMP3 identifies MYO6 as a regulator of adrenomedullin signaling in lymphatic endothelial cells	11
Courtney Slesinger	CBP Biomedical MS student	Polygenic Background Modifies Sickle Cell Trait Effects on Hematologic Traits	H
Audrey Smith	Non-CBP curriculum PhD student	A humanized mouse model of Giant Axonal Neuropathy with early functional phenotypes	60
Christina So	CBP curriculum PhD student	Mechanism of Intracellular ANGPTL3 and ANGPTL8 Trafficking	44
Caden Sweet	CBP curriculum PhD student	Transcriptomic Profiling Reveals Altered Cellular Maturity in Necrotizing Enterocolitis	22
Adrien Tassou	Postdoc	Control of mechanical pain by μ-opioid receptor-expressing spinal pre-motor V1 neurons	6
Yanna Tian	Postdoc	b-arrestin 1/2 are essential for embryonic lymphatic vessel development	13
Jovan Tormes Vaquerano	Non-CBP curriculum PhD student	A high-throughput approach to measuring environmental and behavioral changes in mouse home cages	58
Kanesha Travis	CBP curriculum PhD student	Elucidating the role of endothelial Apelin signaling in liver regeneration upon chemical toxicity	23
Henry Uchenna	CBP curriculum PhD student	The Bifunctional Nature of Actin Capping Protein in Cell Division	39
Kylie VanDerMolen	Non-CBP curriculum PhD student	The scramblase RFT1/RFTH-1 maintains metabolic homeostasis via mTORC2-specific signaling	63
Xingren Wang	CBP curriculum PhD student	Identification of Genes Escaping X-Inactivation in Cardiomyocytes	25
Yuhao Wang	CBP Biomedical MS student	Investigating TAM Receptors' Role in CAF-Mediated Tumor Growth Regulation in PDAC	G

Name	CBP Affiliation	Abstract Title	Poster #
Luke Wickersham	CBP Biomedical MS student	Cataloging NTSR1 Expression Across the Mouse Nervous System for Pain and Opioid Use Disorder	W
Victoria Williams	CBP curriculum PhD student	Artificial tethering to the ER promotes lipid droplet growth via enhanced lipid channeling	17
Samara Williams	Undergraduate student	Enteroendocrine Cells and Intestinal Barrier Function in IL-$10^{-/-}$ Inflammatory Bowel Disease	69
Won Yoon	Lab staff	Spatiotemporal characterization of endothelial proliferation in liver regeneration post-CCl4 injury	64
Maria Clara Zanellati	Postdoc	Illuminating organelle dynamics during neuronal differentiation	1
Qingye Zeng	Postdoc	Lipid droplet accumulation in endothelial cells impairs mechanoadaptation by physically interfering with cytoskeletal remodeling	2
Matthew Zimmerman	CBP curriculum PhD student	Folate Receptor β Orchestrates Tumor-Associated Macrophage Immunosuppressive Functions	30

Toward Filopodia Purification Using Nanoporous Membranes

Lillah E. Hayes[1], Richard E. Cheney[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Filopodia are dynamic actin-based cellular protrusions cells use to probe their environment. Filopodia are crucial for processes like embryonic development, angiogenesis, and synapse formation. Other major organelles, such as mitochondria and cilia, have been successfully purified, allowing for identification of their molecular components by proteomics. However, filopodia have not yet been purified, and the identity of the full set of their components remains unknown.

A *PNAS* publication has reported that filopodia can insert into nanopores. We seek to leverage this and investigate if filopodia could be isolated by shearing off the cell bodies and subsequently extracting the filopodia from the pores. We find that CCL-2 HeLa cells can insert their filopodia into 0.2 um pores on anodic alumina oxide (AAO) membranes, and that the cell bodies can be removed by a stream of buffer. Filopodia remain in the pores and stain positive for F-actin; however, our previous attempts to elute them using pressure or centrifugation have been unsuccessful.

Here, we investigate whether silanizing the filters to make them hydrophobic and reduce non- specific protein binding might facilitate elution of filopodia by fluid flow of buffers or silicone oil. To test this, we developed a procedure to silanize AAO membranes using overnight vapor deposition of trichloro(perfluorooctyl)silane under vacuum. Although this succeeded in making the nanoporous filters hydrophobic, wetting the filters unexpectedly led to increased cell adhesion. Because nanoporous alumina provides a novel and attractive strategy for rapid isolation of filopodia from many different cell types, future work will be directed towards alternative elution techniques, such as electrophoresis.

Redundancy in Sensorimotor and Associative Networks Mediates Age-Related Decline in Walking Speed and Endurance

Kevin Lima[1], Jason R. Franz[2], Michael D. Lewek[3], Eran Dayan[4]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
2. Lampe Joint Department of Biomedical Engineering, The University of North Carolina at Chapel Hill and North Carolina State University, Chapel Hill, NC, USA
3. Division of Physical Therapy and Human Movement Science Curriculum, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA [4]Department of Radiology and Biomedical Research Imaging Center, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, USA

Age-related neurological changes are well documented, yet individuals of similar ages often exhibit markedly different functional levels. Typically, walking speed and endurance decline with advancing age, but some individuals can preserve their walking function well into later life. Those with preserved mobility are also more resilient to the onset of neurodegenerative disease. These individuals are hypothesized to possess a neural reserve that acts as a neuroprotective mechanism. In this study, diffusion MRI data from the Human Connectome Project-Aging (HCP-A) were used to generate structural connectivity matrices via deterministic tractography, from which graph-theoretical metrics including redundancy (a measure of reserve) were derived. Mediation analyses tested whether redundancy mediated the relationship between age and walking outcomes (speed and endurance).

Additional graph-theoretical metrics (clustering, betweenness centrality, and efficiency) were evaluated for comparison. Covariates included Montreal Cognitive Assessment (MoCA) score, education, dominant grip strength, and network-matched connectivity strength. Redundancy was the only metric found to demonstrate statistically significant mediating effects between age and walking outcomes. The networks demonstrating significant mediation largely overlapped between walking speed and endurance, with only minor differences across attention, somatomotor, and visual systems. Inclusion of redundancy increased the goodness of fit (R^2) of the age-walking relationship by approximately 9.4–16.5% for walking speed and 1.8–3.9% for walking endurance; this difference may reflect a baseline difference in correlation with age between the two measures. These results suggest that age-associated differences in walking speed and endurance are to an extent dependent on differences in reserve across select brain networks.

The Role of BRD4 in Spermatogenic Development

Hannah Ellyson[1], Prabuddha Chakraborty, PhD[1], Terry Magnuson, PhD[1,2]

1. Department of Cell Biology and Physiology, The University of North Carolina at Chapel Hill, NC
2. Department of Genetics, Chapel Hill, NC

BRD4 is a member of the BET protein family that acts as an epigenetic regulator controlling gene expression, cell growth, and the regulation of the cell cycle [1,2]. This study aims to define the role of BRD4 in germ cell development and meiotic regulation. Through prior research, another BET protein, BRDT, has been identified to be involved in transcription and histone removal in spermatids [3], but it is still largely unknown how BRD4 regulates meiosis and spermatogenesis. Using wild type mice, we observed stage-specific differences in the expression of two BRD4 isoforms, the long BRD4 isoform (BRD4-FL), and the short isoform BRD4-Isoform C (BRD4-IsoC), between spermatogonia and the substages of prophase I of meiosis. We characterized the localization of BRD4-FL and BRD4-IsoC in spermatogonia and primary spermatocytes, revealing dynamic BRD4 activity across key stages of spermatogenesis. We generated *Brd4 f/+* (wild type)*; Brd4 f/+, Stra8Cre* (heterozygous) and *Brd4 f/Δ, Stra8Cre* (knockout) mice and identified a marked reduction in round spermatid production
in *Brd4* knockout testes. Along with decreased mature spermatozoa development, *Brd4* knockout mice exhibited reduced testis size and increased germ cell death during the first wave of spermatogenesis, although synapsis of homologous chromosomes at pachynema appeared normal. To define the BRD4 interactome, we have immunoprecipitated both BRD4-FL and BRD-IsoC and identified interacting proteins by mass spectrometry. We detected proteins required for chromatin remodeling (SWI/SNF and INO80 subunits), epigenetic modification (PRC2 subunits), and regulation of chromatin structure (cohesins) controlling gene expression. Together, these data support a central role for BRD4 in coordinating spermatogenic gene regulation and chromatin organization, which is essential for spermatocyte development and preservation of male fertility in mice.

Understanding Medium-Driven Differences In Primary Human Bronchial Epithelial Cells Cellular Functions

Zalma J. Ontiveros[1,2], Megan M. Maeso[1], Patrick R. Sears[1], Scott H. Randell[1,2], Phillip W. Clapp[1,2]

1. Marsico Lung Institute/Cystic Fibrosis Research Center,
2. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hil, Chapel Hill, NC 27599

The Airway BioCore supports translational lung research by generating primary human airway epithelial cells differentiated at an air-liquid interface (ALI) to model in vivo physiology. The Core employs a non-proprietary, in-house Bronchial Epithelial Growth Medium (BEGM) as a cost-effective alternative to commercial formulations; however, its metabolic impact remains incompletely characterized. This study compared UNC BEGM with PneumaCult™-NGEx and PneumaCult™-Ex Plus to evaluate differences in mitochondrial function and epithelial performance.

Primary human airway epithelial cells were expanded to Passage 5 and differentiated at two stages—Passage 2 and Passage 5—using in-house UNC Basal ALI media. Epithelial integrity and function were assessed via transepithelial electrical resistance (TEER), ciliary beating frequency (CBF), and histology. Mitochondrial respiration during expansion was quantified using the Seahorse XF Cell Mito Stress Test, measuring oxygen consumption rate (OCR) as an indicator of metabolic activity. At Passage 2, epithelial integrity and function were comparable across media, with minor condition-specific variation. In contrast, mitochondrial respiration profiles differed, suggesting that expansion media modulate cellular energy utilization.

These findings establish a baseline bioenergetic profile for airway epithelial cells cultured in distinct media formulations and support continued optimization of non-proprietary, cost-effective systems to enhance reproducibility and physiological relevance in airway epithelial models.

Hemodynamic Regulation of Atrioventricular Morphogenesis.

Christina Ruth Fletcher[1], Kirsten Giesbrecht, PhD[1], Michael Bressan, PhD[1-2]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill
2. McAllister Heart Institute, University of North Carolina at Chapel Hill

Congenital heart defects (CHDs) are very common, affecting 1% of live births in the United States annually. Atrioventricular (AV) septal defects also known as endocardial cushion (AVEC) defects occur in 2 out of 10,000 live births. The AVECs are the precursor structures that give rise to the mitral and tricuspid valves and AV septum. However, the role hemodynamics play in AV cushion and septum development is not well understood. The goal of my project is to determine how altered hemodynamics affect AV septum development and morphology during heart morphogenesis through quantitative 3D analysis of the AV region morphology in the embryonic chick heart.

Blood viscosity of chick embryos was altered via micro injection of different molecular weight dextrans into early circulation. Dextrans are polysaccharides that expand the volume of blood plasma. Following dextran injection, the entire vascular network of the embryo was labeled with fluorescently conjugated lectin which binds to endothelial cells. Following labeling, we cleared and imaged the entire 3D geometry of each embryo using light sheet fluorescent microscopy. Images were segmented in IMARIS and analyzed using morphometric analysis, a statistical tool used to quantify phenotypical trends during development. Preliminary data of the AVECs has shown some extreme phenotypes where the experimental groups' AV channel has widened and lost the stereotyped flow channels that need to transport blood from the developing atria to developing ventricle. In addition, the inferior AVEC was shortened, and surface area of the forming cushions varied more compared to controls. These phenotypes were not completely penetrant as some specimens in experimental groups were able to maintain a normal AVEC phenotype. RNAscope was used to see how hemodynamic stress affected downstream genetic signaling. KLF2 and Cx-40, wall sheer stress sensing genes, and PECAM, an endothelial cell marker, were measured to ascertain if wall sheer stress increased with blood viscosity. Preliminary data suggests high KLF2 expression in the AVC that appeared to change in response to dextran injection.

Through understanding the effects of hemodynamic stress on the AVEC, further knowledge of mechanical disease pathways of CHDs can be illuminated.

Investigating the Roles of Alternative Splicing in Cardiac Biology

Olivia Perez[1], Gabrielle Bais[1,2,3], Jimena Giudice[2,4,5]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill(UNC-CH).
2. Curriculum in Cell Biology and Physiology, UNC- CH.
3. Integrative Vascular Biology Training Program, UNC-CH.
4. Curriculum in Genetics and Molecular Biology, UNC-CH. **5.** McAllister Heart Institute, UNC- CH.

Alternative splicing is a post-transcriptional RNA processing mechanism by which one gene gives rise to multiple, mature mRNA transcripts through the inclusion or exclusion of alternative exons, which are then translated to proteins. This process occurs in over 95% of genes in the human genome. Alternative splicing drives genetic diversity. Although the human genome contains approximately 20,000 genes, alternative splicing enables the production of up to 80,000 functional proteins, expanding the diversity of a seemingly limited genome. In the Giudice lab, we study how this regulation is controlled, particularly in proteins involved in trafficking and membrane dynamics, and investigate how alternative splicing shapes cell biology and physiology in normal development and disease. I focused on the alternative splicing mechanisms of FXR1, a muscle-enriched RNA-binding protein (RBP) that is essential for survival in multiple species.

FXR1 has multiple isoforms due to alternative splicing, especially in an intrinsically disordered and serine/arginine-rich region of the protein. These regions are critical, as they help proteins form biomolecular condensates, which organize RNA and proteins inside cells. Additionally, we studied the effects of RBPs on atrial and ventricular development splicing patterns. We identified alternatively spliced microexons (≤ 51 nucleotides), many of which are present in cardiovascular disease-associated genes. This led us to investigate the effects of alternatively spliced microexons in the developing atria that may contribute to cardiovascular disease.

Investigating TAM Receptors' Role in CAF-Mediated Tumor Growth Regulation in PDAC

Yuhao Wang[1,2], Nancy Porterfield Kren[2], Yuliya Pylayeva-Gupta[2,3]

1. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, 27599
2. Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, 27599
3. Department of Genetics, University of North Carolina, Chapel Hill, 27514

Pancreatic Ductal Adenocarcinoma (PDAC) represents one of the most aggressive malignancies, with a dismal 12% five-year survival rate and projected to become the second leading cause of cancer deaths within a decade. PDAC is characterized by the dense tumor microenvironment (TME) with abundant cancer-associated fibroblasts (CAFs) and immunosuppressive myeloid cells, which creates an immunological barrier for therapy. The preliminary data show the TAM receptors (Tyro 3, Axl, MerTK) play non-redundant functions in PDAC TME and dysregulation of TAM receptors can enhance tumor growth metastasis through oncogenic pathways. To characterize TAM receptor-dependent CAF functions that regulate tumor growth, we assessed proliferation assays and efferocytic capacity across various knockout cell lines. We find Axl-/- pancreatic stellate cells (PSCs) exhibit the highest proliferation rate, and pharmacologic TAM inhibition reduces proliferation across all genotypes. In addition, all TAM-deficient PSCs display reduced efferocytic capacity, with $MerTK^{-/-}$ PSCs showing the most profound defect and $Axl^{-/-}$ PSCs following behind. Since efferocytosis is essential for prevention of secondary necrosis, supporting resolution of inflammation, and establishment of tissue-repairing microenvironment, selective disruption of TAM signaling is likely to reshape stromal programs in PDAC. Our findings suggest that TAM receptors could be potential targets for remodulating tumor microenvironment with implications for drug delivery, immune infiltration, and fibrosis.

Polygenic Background Modifies Sickle Cell Trait Effects on Hematologic Traits

Courtney Slesinger[1], Bjoernar Tuftin[2], Micah R. Hysong[2], Laura M. Raffield[2]

1. Cell Biology and Physiology Biomedical Master's Program, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
2. Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Including ancestrally diverse populations in human genomics research is essential for improving the accuracy and equity of precision medicine. Sickle cell trait (SCT), caused by the rs334 variant in the β-globin gene, is common in individuals of African ancestry and has known effects on red blood cell (RBC) traits. However, the extent to which these effects are modified by polygenic background remains unclear. We analyzed data from the All of Us Research Program to evaluate associations between SCT and hematologic traits in a large, diverse cohort. We examined erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Polygenic risk scores (PRS) for hematologic traits were constructed using published genome-wide association study weights and adjusted for ancestry using principal components. Linear regression models assessed main effects and interactions between SCT and PRS. SCT was associated with lower MCV, MCH, and HCT, and higher MCHC and RDW, but showed no significant association with HGB. PRS were strongly associated with all traits, reflecting cumulative genetic effects. A significant interaction between SCT and PRS was observed for MCV, with SCT carriers showing consistently lower MCV and reduced PRS effect compared to non-carriers. No interactions were observed for other traits. These findings demonstrate that monogenic and polygenic factors jointly influence hematologic traits and highlight the importance of diverse populations in genetic studies.

Media Optimization to Increase Differentiation of Secretory Cells in Intestinal Enteroids

Ava Reeder[1] and Heather McCauley[1]

1. Cell Biology and Physiology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC

The intestinal epithelium is composed of a variety of absorptive and secretory cells, all of which play a major role in barrier composition and function. This study compares the efficiency of two different medias on secretory cell differentiation in human intestinal epithelial organoids (enteroids) through evaluation of gene expression and protein production. Simultaneously, these methods were used to determine if other secretory cell types increased to compensate for the lack of enteroendocrine cells (EECs) in NEUROG3-/- (KO) enteroids. Intestinal stem and proliferative cells were cultured as 3D enteroids or as 2D monolayers on Transwells using either current (old) differentiation media (ODM) or new IntestiCult™ Organoid differentiation media (NDM). Differentiation outcomes were assessed through qPCR analysis of MUC2 (goblet cells), CHGA (enteroendocrine cells), and LYS (Paneth cells), immunostaining, imaging, and transepithelial electrical resistance (TEER) measurements to evaluate barrier integrity. Results indicate that KO enteroids had increased MUC2 transcription and protein production compared to wild type, suggesting compensatory differentiation in the absence of EECs. ODM promoted higher Goblet cell expression in knockout models, while NDM increased lysozyme (LYS) expression and overall cell density. EECs make up 1% of intestinal epithelium and overall ODM and NDM changes may be subtle and difficult to detect. Ultimately, differences in secretory cell expression between media conditions was not statistically significant, whereas differences between wild-type and knockout groups were better pronounced. Monolayer studies confirmed comparable proportions of secretory cells between 2D and 3D systems, supporting model consistency.

Pharmacokinetic and Clinical Outcomes of Transdermal Buprenorphine Solution (Zorbium) in Long Evans and Sprague Dawley Rats

Jeanette Sako[1], Jeannette Delva-Wiley[1,2], Morika Williams[1,2,3]

1. Pain, Aging, and Interdisciplinary Neurobehavioral Lab, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA;
2. Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA;
3. Division of Comparative Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; UNC Neuroscience Center, Chapel Hill, NC, USA

Pain management in rats is essential for those kept as pets and also those vital to laboratory research, yet the standard form of pain medication for these rodents requires frequent dosing by injection and can lead to increased handling stress. Our lab is investigating the use of transdermal buprenorphine (TBS) as an effective and less invasive alternative to repeated injections in rats post-surgery. While previous research has shown that TBS can remain in the system at therapeutic levels for multiple days, there is more to explore in regards to its use in pair-housed animals. Because rats are social creatures, using a topical analgesic raises concerns about potential transfer between cage mates through grooming behaviors.

This study evaluated TBS across multiple administration methods and housing conditions (pair-versus single-housed) to assess both efficacy and safety. Outcome measures included indicators of analgesic effectiveness as well as physiological and behavioral effects. Some examples of the measures include pharmacodynamic behavioral tests, food intake, body weight, and fecal output. These findings aim to clarify whether TBS provides consistent analgesia while minimizing unintended exposure and adverse effects in socially housed rats.

Role of GATAD2A in Structure and Recruitment of NuRD Complex

Dhanushi Dedakia[1], Glory Dan-Dukor[2], Torry Li[3], David C. Williams Jr.[3]

1. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC 27599.
2. Department of Chemical Biology and Medicinal Chemistry, University of North Carolina, Chapel Hill, NC 27599.
3. Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599.

Hemoglobinopathies such as sickle cell disease and β-thalassemia arise from impaired adult hemoglobin function and remain major global health burdens. One therapeutic strategy is the reactivation of fetal hemoglobin (HbF), whose expression is silenced during development through epigenetic mechanisms. The Nucleosome Remodeling and Deacetylase (NuRD) complex contributes to this silencing by repressing γ-globin gene expression. Our lab investigates how MYND-domain proteins recruit NuRD to DNA to enforce gene silencing. My work focused on ZMYND8, a regulatory protein which recruits NuRD via interactions with the GATAD2A subunit. Disrupting such protein–protein interactions may therefore provide a path to block NuRD-dependent gene silencing and ultimately restore HbF production and alleviate disease severity.

This study examined two complementary objectives. First, characterizing the binding specificity of ZMYND8 and the structurally similar MYND-domain protein ZMYND11 to verify peptide selectivity. Second, studying interactions among NuRD subunits GATAD2A, CHD4 and CDK2AP1. Recombinant proteins and peptide constructs were purified using affinity and size-exclusion chromatography. Binding interactions were quantified by isothermal titration calorimetry (ITC).

ITC analysis identified a strong interaction between ZMYND8 and the bivalent peptide NCORx2A, while no detectable binding was observed between NCORx2A and ZMYND11. As functional control, ZMYND11 binding to EBNA2, a known high-affinity ligand, was measured and proper protein folding was confirmed. These findings demonstrate selective peptide recognition among MYND-domain proteins and establish a strong framework for future efforts to selectively disrupt NuRD-associated repressive pathways and advance HbF-reactivation strategies for hemoglobinopathies.

TITLE

Ethan Brown, BA[1], Natalia S. Akopyants[1], Corey M. Jania[2], Misty Good[1]

1. Department of Cell Biology and Physiology, UNC-Chapel Hill, Chapel Hill.

Necrotizing enterocolitis is a life-threatening gastrointestinal inflammatory disease, which affects about 7% of pre-term infants. The etiology of this disease is incompletely understood, and no targeted therapies are currently available. Rebamipide, a mucosal protective agent, has been shown to participate in several potential therapeutic pathways related to the pathogenesis of the disease. This study aims to analyze whether Rebamipide administration alleviates disease severity in mice participating in a standardized necrotizing enterocolitis model. Mice in the model were administered Rebamipide at a dosage of 3mg/kg once per day for 3 days, and then ileal samples were taken and qPCR inflammatory biomarker analyses were performed alongside Kaplan-Meier survivability analyses. Rebamipide, at these experimental conditions, did not show significant reduction of inflammatory biomarkers or increased survivability. Future work should include work looking at the efficacy of the drug at different dosages and with increased administration.

TRIM9 Switches the Morphological Phenotype of Melanoma Cells

Becky Patrick[1], Kimberly Lukasik[1], Stephanie L. Gupton[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Melanoma is the most aggressive form of skin cancer, driven in part by its ability to undergo phenotypic switching and adopt invasive, therapy-resistant states. TRIM9, a brain-enriched E3 ubiquitin ligase that is not present in normal melanocytes, is also expressed in melanoma. However, its role in regulating melanoma cell behavior remains unclear. Using TRIM9 knockout models, we examined how TRIM9 regulates cell morphology and motility through actin cytoskeletal dynamics in two melanoma cell lines. Loss of TRIM9 increased cell area and promoted a more elongated morphology.
TRIM9 deficiency increased the number of filopodia and decreased their length, while also increasing both the size and density of focal adhesions. These changes were accompanied by increased random migration, indicating enhanced motility. These results demonstrate that TRIM9 regulates cytoskeletal organization and cell behavior in melanoma.

Novel Therapeutic Strategy to Increase Radiosensitivity of High-risk HPV+ OPSCCs

Ava Langaker[1,2], Aditi Kothari[3], Sri Vemulamanda[3], Natalia Isaeva[2,3], Wendell G. Yarbrough[2,3]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2. Lineberger Comprehensive Cancer Center, UNC School of Medicine, Chapel Hill, NC 27599, USA
3. Department of Otolaryngology/Head and Neck Surgery, UNC School of Medicine, Chapel Hill, NC 27599, USA

In the United States, human papillomavirus (HPV) infection is associated with ~71% of new oropharyngeal squamous cell carcinoma (OPSCC) cases, and its incidence continues to rapidly rise. While patients with HPV-associated OPSCCs typically have a better prognosis as compared to patients with HPV-negative tumors, approximately 30% of these patients suffer tumor recurrences for which there are few effective therapeutic options. Recently, we identified two subtypes of HPV+ OPSCC: one with good prognosis and one with poor prognosis. The subtypes have distinct somatic and HPV genes expression patterns, HPV physical status, as well as mutation and methylation profiles. Intriguingly, all differences converge on intrinsic tumor NF-κB activity with constitutively active NF-κB in tumor cells responsible for radiation sensitivity and likely driving improved patient survival. This discovery pointed to the novel molecular target with potential to improve radiation outcomes for the treatment resistant subtype. Invoking a novel strategy to pharmacologically activate NF-κB in these tumors, preliminary studies have identified that the toll-like-receptor 5 (TLR5) agonist Entolimod activates NF-κB in high-risk HPV+ head and neck cancer cells and significantly improves the response of HPV+ OPSCCs to radiation. While Entolimod has excellent clinical potential, activation of NF-κB in HPV+ OPSCC via other toll-like receptors has not yet been studied and presents a notable research gap. In this project, we investigated the TLR1/2 agonist Diprovocim and the TLR4 agonist monophosphorylated lipid A (MPLA) in HPV+ OPSCC. We utilized murine HPV+ oral cancer cells that belong to the high-risk OPSCC subtype, SCC509 and SCC512. NF-κB activation following treatment with Diprovocim and MPLA was evaluated using luciferase reporter assays and Western blotting, while radiosensitivity was assessed via clonogenic survival assays.

N

Investigating Cerebrospinal Fluid Dynamics Across the Lifespan Using MRI-Based Imaging.

Hedille Al-Dhalimy[1], Neil Sud[1], Heather Ward[1], Rachel Smith[1], Zumin Chen[1], Mark Shen[1], and Dea Garic[1]

1. School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

The glymphatic system plays a vital role in supporting neural homeostasis, supporting waste clearance, distribution of growth factors, and overall brain health across the lifespan. Impaired cerebrospinal fluid (CSF) dynamics have been observed in multiple neurodevelopmental and neurological conditions across the lifespan, such as autism, Angelman syndrome, and neurodegenerative diseases. We hypothesize that both infants with neurodevelopmental differences and adults with neurological disorders will show altered CSF dynamics, indexed by slower CSF flow and elevated extra-axial CSF (EA-CSF) volumes, compared to age-matched controls. To test this hypothesis, we acquired MRI scans from infant and adult participants and quantified CSF flow and EA-CSF. Infant analyses include participants with Down Syndrome, Fragile X syndrome, Angelman syndrome, and typically developing controls at 6, 12, and 24 months of age. Adult analyses include participants with migraines taking anti-calcitonin gene- related peptide (CGRP) medication, those with migraines not taking anti-CGRP medication, and healthy adult controls. Using ITK-SNAP, we segment EA-CSF to measure distribution, volume, and CSF flow dynamics, while remaining blinded to diagnostic groups. These analyses aim to clarify how glymphatic function varies across developmental and neurological conditions and to evaluate whether EA-CSF differences may serve as early biomarkers for neurodevelopmental and neurological risk. Future work will expand participant samples and integrate additional MRI-based measures of CSF dynamics to strengthen these findings.

O

Lymphatic CGRP-CALCRL Signaling Regulates Intestinal Immune Cell Tracficking During Injury.

Mariana Quinonez Medina [1], Hannah Hrncir[1], Kathleen Caron[2]

Intestinal lymphatic vessels, known as lacteals, play essential roles in lipid absorption, immune surveillance, and maintenance of tissue homeostasis (Oliver et al. *Cell.* 2020 PMID: 32707093). It is well established that IBD is a systemic disease (REF). Evidence suggests that lymphatic dysfunction contributes to inflammatory bowel disease and impaired recovery following mucosal injury (REF). The CALCRL signaling pathway has been shown to play a role in modulating intestinal injury response in goblet and immune cells. CALRCL signaling has also been shown to be critical for lymphatic function. However, its role in intestinal immune cell trafficking remains incompletely understood.
We hypothesize that lymphaVc CGRP signaling influences intesVnal injury responses. To test this, we performed flow cytometry on different immune compartments in control and CGRPGOF animals. We also developed imaging approaches to study immune–lacteal interacVons.
We found that CGRP may promote clearance of immune cells from the lamina propria to the mesenteric lymph node while at the same time enriching for 'gut homing' LPAM1+ T cells.
Interestingly, we found expression of the classical 'gut homing' surface protein MAdCAM-1 on some lacteals in the intestinal villus. This is notable because lymphatics do not play a role in immune cell delivery but are classically believed to promote immune cell clearance. While preliminary, these data suggest that lymphatics play active roles in regulating immune cell trafficking in the intestine. Future work is required to determine how CGRP-CALCRL signaling regulates intestinal lymphatic function.

Epithelial Lipid Droplet Accumulation Enhances Intracellular Stress Fiber Formation and RhoA Activity

Kayala Purdie[1], Harold Heatherly[2], Evan Freeman[3], Qingye Zeng[2], and Boa Kim[2]

1. Department of Cell Biology and Physiology, University of North Carolina School of Medicine,
2. Department of Pathology & Laboratory Medicine and McAllister Heart Institute,
3. Department of Biology, University of North Carolina at Chapel Hill

Lipid droplets (LDs) are dynamic intracellular organelles playing a foundational role in the storage of triacylglycerols (TAGs), which are enclosed within a phospholipid monolayer embedded with Perilipin proteins. Along with storing TAGs, LDs are ubiquitous in cells important in supporting cell health by regulating lipid homeostasis and alleviating intracellular stress. LD metabolism is regulated by diacylglycerol acyltransferase (DGAT) the rate-limiting enzyme for LD synthesis and adipose triglyceride lipase (ATGL) the rate-limiting enzyme for LD hydrolysis. However, dysregulation of LD metabolism can contribute to multiple disease pathologies within the body. For instance, excess LD accumulation in kidney tissue can contribute to chronic kidney disease (CDK) by stimulating inflammation and intracellular stress. We are interested in how excess LD accumulation can influence intracellular mechanisms within epithelial cells. Here we aimed to validate an in-vitro system of excess LD accumulation by using two mammalian cell lines, Madin-Darby Canine Kidney (MDCK) and Caco-2. We showed LD accumulation in-vitro via the addition of oleic acid (OA) and subsequently the downstream eXects of excess LD accumulation. OA treated MDCK cells showed that OA was readily taken up and stored as LDs via western blot and immunocytochemistry (ICC). Using ICC, we showed that LD accumulation led to increased RhoA activity and increased stress fiber formation in epithelial cells in-vitro.

Q

Selective Targeting of NRAS and KRAS Isoforms: Differential Effects of Compound 181 on Q61R and Wild-Type

Addison Pignetti[1], Leiah Carey[2], Mohammad Ashhar Khan[2], Sharon Campbell[2,3]

1. UNC-CH Dept. of Cell Biology and Physiology
2. UNC-CH Dept. of Biochemistry and Biophysics
3. Lineberger Comprehensive Cancer Center

RAS proteins are membrane-associated small GTPases that transmit extracellular signals into the cell to regulate pathways involved in cell growth. As proto-oncogenes, RAS genes are frequently mutated in human cancers. The Campbell lab is pursuing a drug discovery project to develop inhibitors that bind to NRAS. Early compounds show promising binding to NRAS and weaker interactions with KRAS.

This study further characterizes compound binding to both wild-type KRAS and the oncogenic KRAS Q61R mutant. Using two-dimensional $^{1}H–^{15}N$ heteronuclear single quantum correlation (HSQC) nuclear magnetic resonance (NMR) spectroscopy, we assessed the isoform, mutation, and state specificity of Compound 181.

Our results show that Compound 181 preferentially binds NRAS over KRAS in both Q61R mutants and wild-type proteins, confirming isoform-specific interactions. The compound exhibited weaker chemical shift perturbations in the inactive state compared to the active state. Strong NMR perturbations were observed in NRAS wild-type and Q61R in both active and inactive states. KRAS wild-type and Q61R showed weaker perturbations overall; however, stronger perturbations were observed for the active state of the KRAS Q61R mutant relative to wild-type.

These findings provide confidence that Compound 181 may be a promising drug candidate for NRAS-driven melanomas.

R

Exploiting the dependence of TNBC cells on glucose and glutamine metabolism to induce cell death following treatment with small molecule ClpP activator TR107

Elizabeth C. Moorman1,2,3, Sabrina C. Daglish2,3, Grace E. Moore2,3, Lee M. Graves2,3

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill
2. Department of Pharmacology, University of North Carolina at Chapel Hill
3. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

Mitochondrial caseinolytic protease P (ClpP) regulates mitochondrial proteostasis and degrades misfolded proteins as part of the ClpXP complex. Hyperactivation of ClpP can selectively target and damage cancer cells because of their heavy reliance on mitochondrial metabolism to support their uncontrolled proliferation. The Graves Lab has established that treatment with the small molecule ClpP agonist TR107 induces senescence in Triple Negative Breast Cancer (TNBC) cells. In an eRort to further our research, I investigated how the altered metabolism of cancer cells could be exploited to cause cell death in these senescent cells. One of the hallmarks of cancer cells is their diRerential metabolism and nutritional demands compared to normal cells. Most notably, they heavily rely on glucose for energy production by glycolysis, and glutamine, which acts as an antioxidant to combat ROS. To directly investigate the eRects of nutritional conditions on senescent TNBC, I first made cells senescent by incubating MDA MB-231 cells with TR107 for 48 hrs. After this time, TR107 was removed and the senescent TNBC cells were exposed to diRerent glucose or glutamine concentrations. Cell growth inhibition and cell death were measured by Hoechst and propidium iodide staining and the dead/live cell ratio determined using a Celigo cell imager. The results of my studies demonstrate that glucose or glutamine starvation can induce cell death in TNBC cells after TR107-induced senescence. Thus this research suggests that the anti-cancer eRicacy of ClpP agonists may be influenced by altering the nutritional conditions under which they are applied.

Demethylation Impacts on Human Papillomavirus Protein Expression

Lizzie Adelman[1], Shelby Sundstrom[2], Cary A. Moody[2,3,4]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill,
2. Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill,
3. Department of Microbiology and Immunology, University of North Carolina at Chapel Hill,
4. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

High-risk human papillomaviruses (HPVs) are DNA viruses responsible for cervical and other anogenital cancers, with viral replication and oncogene expression tightly regulated by host epigenetic mechanisms. DNA methyltransferase 1 (DNMT1) maintains DNA methylation during replication and plays a critical role in HPV genome amplification and transcriptional control. My project focus was to investigate how DNMT1 influences HPV protein expression and endogenous retroviral element (ERV) activation. HPV31+ epithelial cells were either maintained in an undifferentiated state or induced to differentiate with high calcium conditions. Cells were treated with the DNMT1 inhibitor decitabine or cycloheximide (CHX) to inhibit protein synthesis. Protein levels were assessed by western blotting, and mRNA expression was quantified using RT-qPCR. Dysregulating DNMT1 with decitabine increased ERV protein expression, reactivating its function of inducing an immune response. Similarly, CHX treatment reduced UHRF1 (a DNMT1 recruiter) and DNMT1 protein levels in undifferentiated and differentiated 9E cells. Inhibition of DNA methylation, and thus a decrease in DNMT1 protein levels led to an upregulation of ISG56, an inhibitor of viral replication. These findings demonstrate that DNMT1 supports HPV oncogene expression while repressing ERV and ISG56 activation. Loss of DNMT1 leads to ERV reactivation and altered innate immune signaling, highlighting its role in viral persistence. DNMT1 appears particularly important in undifferentiated HPV-positive cells, linking epigenetic regulation to HPV pathogenesis. This work identifies DNMT1 as a potential therapeutic target and underscores the importance of DNA methylation in regulating viral-host interactions. It also suggests a future direction for understanding why DNMT1 dependence may be greater in undifferentiated compared to differentiated HPV-positive cells.

T

Maternal Hyperglycemia and Congenital Heart Defects: Unaltered Mevalonate Pathway Enzyme Expression Despite Increased Prenylation Activity

Kevin Chen[1,3], David Cully[1,2,3], Whitney Edwards[1,3]

1. Department of Cell Biology and Physiology,
2. UNC Curriculum in Genetics and Molecular Biology,
3. McAllister Heart Institute

Congenital heart defects (CHD) represent the most common type of birth defect, affecting around 1% of live births and often requiring expensive medical management. Although genetic factors contribute substantially, maternal hyperglycemia has been associated with a 2–5-fold increased incidence of CHDs. Pregestational and gestational diabetes have also been linked to abnormal embryonic development: hyperglycemia impairs neuronal maturation and cognition, increases the risk of congenital malformations, and predisposes individuals to metabolic diseases. Our lab investigates lipidation, a post-translational modification that is dynamically regulated during heart development. Protein prenylation, a subtype of lipidation derived from the mevalonate pathway, is the attachment of farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) to proteins. Previous research has implicated that geranylgeranylation is critical for proper cardiac morphogenesis. Hyperglycemia may upregulate mevalonate pathway activity and increase isoprenoids via glucose-to-Acetyl-CoA metabolism. Evidence indicates that hyperglycemia impairs cardiomyocyte maturation, proliferation, and morphogenesis, and our lab has observed increased prenylation activity in cardiomyocytes cultured under high-glucose conditions. Despite these documented outcomes, the molecular mechanisms through which elevated glucose disrupts cardiac development remain insufficiently characterized. We demonstrate that both the mRNA and protein of some mevalonate pathway enzymes are modestly upregulated or remain unchanged during hyperglycemic embryonic cardiac development. We also show that cardiomyocytes treated at higher glucose concentrations exhibit a slight decrease at both the mRNA and protein levels of the same enzymes. As a result, in the future, we will examine metabolites of the mevalonate pathway to identify if hyperglycemia increases metabolic flux, rather than the expression of the enzymes themselves.

Choice of AAV purification method impacts viral vector performance in-vivo.

Aaliyah O'Dell[1], Martin Bohlen[2], Kimberly Ritola[1]

1. University of North Carolina at Chapel Hill, Neuroscience Center, Chapel Hill, United States,
2. Duke University, Neurobiology, Durham, United States

Adeno-associated virus (AAV) is a critical viral vector for basic research as well as clinical gene therapy applications. Expression of the AAV payload depends on complex protein-protein interactions between the host cell and virus particle. The ability of AAV to target many cell types stems from its inherent genetic diversity. In addition to the natural diversity of AAV particles, AAV vectors when produced in laboratory environments using various purification strategies are introduced to signature protein and chemical contaminants which are delivered into the target animal along with virus particles. Variability in AAV vector performance between vector preparations and between animal models is common. Here we show how variations in AAV production impact their performance in vivo with the end goal to create highly effective viral/gene therapy vectors, specialized for their end use across animal models.

We see previously described differences between purification strategies, in GC/ml, protein contaminants, and empty to full vector ratios. Among different purification strategies AAV titer varied slightly. Listed in descending order of average titer CsCl gradient, iodixanol gradient, then column purification. Protein content was visualized using SDS-Page gels and quantified using a Badford assays, an obvious difference between purification types was detected, with CsCl retaining more non-viral proteins post purification followed by iodixanol and column purification. We saw a slight decrease in contaminating protein with an additional round of CsCl purification, however it was not consistent. TEM and qPCR reinforced the expected differences in the empty:full ratios for each purification strategy. CsCl purification consistently resulted the highest percentage of full particles. This data is conserved across multiple rounds of CsCl purification. Iodixanol had the next highest percent full, then column purification. Differences in in vitro and in vivo performance were simil

Cataloging NTSR1 Expression Across the Mouse Nervous System for Pain and Opioid Use Disorder

Luke P. Wickersham[1-3], David F. Lee[1-3], Rhys Rudiger[1-3], Grégory Scherrer[1-3]

1. Department of Cell Biology and Physiology, UNC Neuroscience Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA;
2. Department of Pharmacology, The University of North Carolina at Chapel Hill, Chapel Hill 27599, NC, USA;
3. UNC Neuroscience Center, The University of North Carolina at Chapel Hill, Chapel Hill 27599, NC, USA;

In the U.S millions of adults live daily with chronic pain, and high-impact chronic pain that restricts daily activity. Chronic pain can be clinically treated with opioids known to provide potent analgesia, yet they carry substantial risk. In recent years alone, millions of Americans have reported misusing prescription opioids, and hundreds of thousands have died from opioid overdoses. Opioids act on MOR, a GPCR responsible for providing the analgesic effects, but are clinically limited by the risk of addiction, dependence, and adverse effects of respiratory depression and overdose. Neurotensin receptor 1 (NTSR1) is a non-opioid GPCR. Using small molecule selective agonists like SBI-553 we are able to trigger NTSR1 β-arrestin agonism and G-protein antagonism. We then determined that NTSR1 β-arrestin biased agonism has novel analgesic and OUD attenuating effects. NTSR1 selective agonism has shown to mitigate chronic opioid withdrawal symptoms, opioid-induced conditioned place preference, and serve as a potent antinociceptive analgesic. To interrogate the NTSR1 system, we sought to faithfully categorize expression across the mouse nervous system. We did this experiment in the previously published and validated NTSR1-Venus reporter mouse line, primarily through multiplex immunofluorescence (mIF) and fluorescence in-situ hybridization (FISH) to determine expression and localization. By elucidating NTSR1 expression throughout the nervous system, we hoped to help determine its role in central pain circuits as well as its expression levels in spinal cord lamina and dorsal root ganglion (DRG). We have identified NTSR1 expression in key regions associated with central pain circuits including key pain regions encoding the emotional processing of pain and reward such as the VTA and medial thalamus. In future directions we plan on investigating the expression of NTSR1 in satellite glial cells and the co-localization matching CGRP and PKCg within the lumbar spinal cord expressing populations.

Mapping the Secretome and studying the Role of Microglia in Cortical Development.

Kirthika Mutthoju[1], Eva Anton[1],

1. Department of Cell Biology and Physiology, UNC School of Medicine

The brain cerebral cortex performs neural computations underlying sensory processing, decision making, and elements of personality. These higher level processes are supported by the organized layers of neurons of the cortex that arise during embryonic development. This development and organization of the cortex is influenced by interactions between various cortical cell types, including microglia. Microglia are the resident immune cells of the brain with main functions consisting of surveillance, phagocytosis and secretion. They also play an important role in neurodevelopment, contributing towards the development and organization of the cerebral cortex, however the mechanism by which microglia influence cortical development is not fully understood. To investigate microglial contributions to cortical development, we utilize a conditional knockout (cKO) mouse model where Ift88 is selectively deleted in the microglia. Ift88 is a gene critical for protein secretion and necessary in maintaining primary cilia. This Ift88 cKO mouse brain displayed disrupted early cortical development with disorganized axon branching, progenitors and altered microglia distribution. This project aims to delineate the underlying mechanisms of microglia-mediated cortical development through comparing the proteomic difference between Ift88 cKO and wild-type and map the secretome of microglia using Cre-inducible BirA*G3 mouse line for biotin tagging of protein in the microglial ER for streptavidin affinity purification.
After mass spectrometry proteomic and mRNA seq analysis between control and Ift88 cKO, Slit2, a protein important in axon branching and neuronal migration, was identified to be upregulated in Ift88 cKO mice. This finding was validated using western blot and analyzed conforming to a significant increase in Ift88 cKO .BirA*G3-ER mouse biotinylation was validated using western blot to confirm biotinylated protein only when Cre was induced (Cre+) and BirA+. Optimization of the in vivo proximity labelling led to water being the best route of administration of better pulldown for mass spectrometry. These results step further into an avenue for investigating and mapping of the microglial secretome to understand underlying mechanisms of the role of microglia in cortical development.

Evaluating Candidate Antifibrotic Treatments in an IPF-Relevant Fibroblast Model

Matthew Geib[1], Jim Hagood[1],

1. Department of Pediatrics (Pulmonology), UNC, Chapel Hill, Mary Ellen Jones

Idiopathic pulmonary fibrosis (IPF) is a fatal fibrotic lung disease driven in part by persistent fibroblast activation and myofibroblast differentiation. Transforming growth factor beta (TGF-β) is a key mediator of this profibrotic response and is commonly used to model fibroblast activation in vitro. Here, we used a long-course TGF-β assay in HNL32A fibroblasts to evaluate candidate antifibrotic treatments in an IPF-relevant system. Following TGF-β stimulation, cells were treated with sThy-1, nintedanib, and nintedanib-related analogues, then assessed by immunofluorescence, LDH assay, and RT-qPCR. This study establishes an in vitro model for evaluating how candidate therapies modulate TGF-β-driven fibroblast activation in IPF.

Y

Validation of Transcription Factor Candidates as Regulators of Proteinase 3

Kaitlyn Humphreys, Patrick Taus, Dominic Ciavatta, Ronald Falk

1. Department of Cell Biology and Physiology, UNC-Chapel Hill, NC

Anti-neutrophil cytoplasmic antibody (ANCA)- associated vasculitis (AAV) is a group of disorders caused by autoinflammation of small blood vessels and commonly affect renal vasculature. Currently, the standard of care for this disease relies on non-specific immunosuppressive therapies, which can increase susceptibility to secondary infections. Our research focuses on the genetic mechanisms of AAV, with a focus on the regulation of autoantigen Proteinase-3 (PRTN3), expressed on the surface of circulating neutrophils. In addition to its role in AAV, PRTN3 also plays a key role in Acute Myeloid Leukemia tumorigenesis through multiple signaling pathways. A CRISPRi screen has identified several regulators of PRTN3, where myelopoetic stem cell development regulators GATA2 and SPI-1, have been validated as regulators of PRTN3. We are investigating GATA2 activation of secondary regulators across multiple AML cell lines, focusing on CEBPE and MYB as potential coregulatory factors of PRTN3 expression in neutrophils. Understanding the genetic drivers of PR3 is essential to optimizing treatment plans for patients with both ANCA vasculitis and AML.

AI- Guided Screening to Predict and Validate Novel FBXW7 Substrates in the Ubiquitination Pathway

William Lewis[1,2], Michael J. Emanuele, PhD[2], Alison Graff[2]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC.
2. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

The FBXW7 gene functions as a tumor suppressor and is mutated in many cancer types. Because FBXW7 helps regulate protein degradation through the ubiquitination pathway, identifying additional FBXW7 substrates may improve our understanding of how disrupted protein turnover contributes to cancer development. In this project, AlphaFold 3, an AI-based protein modeling program, was used to screen for potential FBXW7 substrates by comparing predicted interactions between FBXW7 and candidate proteins in phosphorylated and unphosphorylated states. Candidate proteins were prioritized based on increased ipTM scores after phosphorylation and the presence of a phosphorylation motif predicted to interact with the WD repeat region of FBXW7. Selected candidates were then tested experimentally using GFP-tagged plasmid transfection with and without FBXW7 expression. Mean GFP fluorescence was used as an initial readout to evaluate whether candidate proteins may be affected by FBXW7-dependent ubiquitination and degradation. To further validate these findings, western blot analysis was performed to measure candidate substrate protein levels more directly and determine whether co-expression with FBXW7 reduces substrate abundance. This combined approach provides a workflow for integrating computational protein-structure prediction, fluorescence-based screening, and biochemical validation to identify and characterize potential new FBXW7 substrates.

Targeting KIF20A: A Small-Molecule Inhibitor Approach to Overcoming Therapeutic Resistance

Morgan Ellington[1], Abhishikt David Solomon[2], and Aimin Peng[2,3]

1. Department of Cell Biology and Physiology, School of Medicine, The University of North Carolina at Chapel Hill, NC 27599, USA
2. Department of Biomedical Sciences, Adams School of Dentistry, The University of North Carolina at Chapel Hill, NC 27599, USA
3. Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, NC 27599, USA

Microtubule (MT) components have been studied for their involvement in cancer progression with several MT agents in clinic. MT regulators such as kinesins are ATP-dependent biological motor proteins that play a vital role in intracellular transport, cell division, and mitotic progression. Because MT regulators are overexpressed in multiple cancer types, they serve as promising therapeutic targets. Within the Kinesin family, KIF20A gene is responsible for organizing central spindle assembly formation, yet minimal investigation has been conducted regarding its potential as a target for combination therapy in cancer treatments. This study focused on evaluating the efficacy of kinesin inhibitor, Paprotrain, in modulating tumor response and investigating the mechanism in which they synergize with DNA-damaging chemotherapy agents and PARP inhibitors.

An initial screening of Kinesin inhibitors identified Paprotrain, a KIF20A inhibitor, as an effective compound in decreasing cancer cell viability. Subsequent analyses concentrated on potential drug response with chemotherapeutic agents (Doxorubicin and Etoposide) and PARP inhibitors (NMS and Olaparib) in combination with Paprotrain, which demonstrated an enhanced additive effect. To demonstrate the effect of Paprotain in combination with chemotherapeutic agents and PARP inhibitors, we utilized in vitro 3D spheroid cell viability assays, 2D cell viability assays (CCK-8), and trypan blue exclusion assays. Combination treatments displayed strengthened anti-tumor effects in comparison to monotherapy, as shown by reduced spheroid size and cell viability. Furthermore, synergy analysis using ZIP modeling further validated enhanced interactions between DNA-damaging agents and PARP inhibitors with kinesin inhibition.

Henceforth, kinesin inhibitors, such as Paprotrain, represent a new intervention in anti-cancer therapies that may overcome resistance in several tumors where MT regulators, including kinesins, are overexpressed. Therefore, we identified Paprotrain as a new candidate that holds translational potential along with current therapeutic cancer treatments.

Characterizing HA15-induced ATF4 stress signaling relevant to HIV-1 reservoir Persistence

Abigail Meader[1], Hongjie Chen[1], Waqas Ahmed[1], Yuyang Tang[1], Guochun Jiang[1,2]

1. Department of Medicine and the UNC HIV Cure Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA 27599
2. Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA 27599

Human Immunodeficiency Virus-1 (HIV) persists in long-lived cellular reservoirs despite antiretroviral therapy (ART). Stress-based cure strategies aim to eradicate these reservoirs, but the signaling mechanisms underlying their effects remain poorly defined. The integrated stress response (ISR) has been linked to HIV transcription through the induction of the transcription factor ATF4, which in turn is recruited to the HIV promoter to induce HIV transcription from latency. Here, we examined how the latency reversal agent HA15 influences ATF4-associated stress signaling and whether some protein kinases modulate this response. Using a 2D10 Jurkat latency model and primary human $CD4^+$ T cells, cells were treated with HA15 alone or in combination with a kinase inhibitor and analyzed by western blotting, phosphatase validation, and flow cytometry to assess stress signaling, viral transcriptional activity, and cell viability. HA15 increased ATF4 protein levels and altered phosphorylation-dependent regulation of multiple signaling proteins, consistent with activation of ISR-associated pathways. Inhibition of one kinase further enhanced ATF4 accumulation and reshaped its phosphorylation patterns, indicating that this kinase activity regulates the strength and pattern of HA15-induced stress signaling. Together, these findings provide a mechanistic context to inform future studies targeting HIV reservoir formation and persistence through modulation of stress-associated signaling pathways.

CC

Investigating LPL Aggregate Composition in the Absence of LMF1

Tanisha Choudhury[1], Ming Wu[1], Saskia Neher[1]

1. Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Hypertriglyceridemia, characterized by elevated plasma triglyceride levels, affects approximately 1 in 5 adults in the United States and is a major risk factor for cardiovascular disease and acute pancreatitis.[1] Lipoprotein lipase (LPL) is the primary enzyme responsible for clearing triglycerides from the bloodstream; consequently, mutations impairing its folding or secretion are leading causes of the disease. LPL maturation occurs in the endoplasmic reticulum (ER), where it forms five critical intramolecular disulfide bonds. This process requires Lipase Maturation Factor 1 (LMF1), an ER-resident chaperone that recruits an essential network of oxidoreductases. In the absence of LMF1, the ER environment becomes more oxidized and loses the capacity to resolve mispaired disulfide bonds, resulting in the formation of disulfide-linked LPL aggregates and impaired secretion.[2]

The molecular composition of these aggregates remains unknown; specifically, which proteins are covalently trapped with LPL and whether the Unfolded Protein Response (UPR) can remodel these interactions. To investigate this, we expressed His-tagged LPL in LMF1-deficient HEK293 cells and utilized large-scale Ni-NTA affinity purification to isolate LPL and its associated interactors. Cells were treated with the small-molecule UPR modulators to selectively induce ER proteostasis pathways.

Preliminary analysis via western blotting and Coomassie staining reveals that the oxidoreductases ERp44 and PRDX4 co-purify with LPL aggregates. Interestingly, UPR activation remodels these interactions: ERp44 signal increases under reducing conditions following UPR treatment, suggesting active recruitment, while its absence under non-reducing conditions suggests it is released from covalent entrapment within the aggregate. These findings suggest a thiol-mediated interaction between the ERp44-PRDX4 complex and LPL aggregates that is sensitive to ER stress modulation. Future mass spectrometry analysis will provide a comprehensive proteomic profile of the aggregates, offering mechanistic insight into how LMF1 loss disrupts ER redox homeostasis.

DD

Illuminating organelle dynamics during neuronal differentiation

Maria C. Zanellati[1], Z. Coman[1], D. Bhowmik[1], C.S. Hsu[1], J. Burgess[3], R. Basundra[2], S. Rhoads[1], S. Yeung[3], M. Deshmukh[2], Sarah Cohen[1]

1. UNC - Sarah Cohen Lab, Chapel Hill, NC
2. UNC - Mohanish Deshmukh Lab, Chapel Hill, NC
3. Stanford University, Stanford, CA

Organelles undergo dramatic changes in shape, position, dynamics, and interactions with other organelles (together termed morphodynamics) in response to changing environmental or developmental conditions. We used multispectral imaging live cells to visualize organelle morphodynamics along neuronal differentiation. We transfected induced pluripotent stem cells (iPSCs) and iPSC-derived cortical neurons (iNeurons) with genetically encoded organelle markers and collected multispectral z-stack images at five-time points throughout neuronal differentiation and maturation: iPSCs, and iNeurons at day 7, day 14, day 21, and day 28. Raw images were then subjected to linear unmixing and run through a Napari-InferSubC image analysis pipeline for segmentation and analysis of approximately 5000 morpho-metrics including organelle volume, size, shape, and number; number and volume of the contacts (2 to 4-way). We found that the cell body (soma) contracts during differentiation from an iPSC to an iNeuron. We observed dramatic remodelling of organelles during differentiation, instances are increased mitochondria and ER volumes, decreased lysosomal size, and increased in peroxisome, lysosomes and mitochondria number. We also observed an increase in total number of the contacts and specifically an increase in higher order contacts (3- and 4-way contacts) as iNeurons matured. Tether proteins can modulate contacts between organelles. We found that expression of VAPB and ACBD5, which mediate endoplasmic reticulum (ER)-peroxisome contacts, increase as iPSCs differentiate into iNeurons and correlate significantly with our number of the contacts. This contact is implicated in the production of plasmalogens, a subclass of glycerophospholipids which are essential for the growth and maintenance of synapses in the nervous system. We observed an increase in multiple plasmalogen classes as iNeurons matured, and this increase was abolished when VAPB/ACBD5 were knocked down, confirming that ER-peroxisome contacts modulate plasmalogen synthesis during neuronal differentiation. Our results suggest that extensive rewiring of organelle contacts is necessary to support metabolic changes throughout differentiation, and that the resulting organelle signature is required to sustain neuronal functions.

Lipid droplet accumulation in endothelial cells impairs mechanoadaptation by physically interfering with cytoskeletal remodeling

Qingye Zeng[1], Harold Heatherly[1], Evan Freeman[2], Schuyler Bowers[1] and Boa Kim[1]

1. Department of Pathology & Laboratory and McAllister Heart Institute, Chapel Hill, NC, USA
2. Department of Biology, University of North Carolina Chapel Hill, Chapel Hill, NC, USA

Lipid droplets (LDs) are small intracellular organelles that accumulate in endothelial cells following high-fat intake. Excessive LDs have been recently associated with vascular diseases such as hypertension and atherosclerosis. Nevertheless, how LDs exert their deleterious action at the molecular level, and whether they have a causative role in vascular dysfunction remain unclear. Here, we show that LD accumulation disrupts vascular barrier function and impairs endothelial mechanoadaptation by physically obstructing microtubular remodeling. Endothelial cells accumulating LDs fail to elongate and align with flow, display increased VE-cadherin internalization and junctional irregularities, and exhibit increased permeability in vitro and in vivo. Mechanistically, LD accumulation impairs cytoskeletal dynamics by interfering with microtubule extension, which is essential for the coordinated mechanical adaptations to shear stress observed in endothelial cells. This interference leads to the release of the microtubule-associated RhoA activator GEF-H1, resulting in increased actomyosin tension and junctional destabilization. Normalizing LD burden restores microtubule dynamics, suppresses RhoA-ROCK activation, and rescues endothelial flow-mediated remodeling and barrier function. Overall, our findings reveal an unexpected biophysical mechanism by which LDs exert detrimental effects in endothelial cells. More broadly, we introduce these small lipid storing organelles as a mechanistic link between metabolic regulation and endothelial mechanotransduction, providing insight into how high-fat intake contributes to the pathogenesis of diverse vascular diseases.

Developmental sleep disruption dysregulates synaptic translation

Anantha. J[1], Lord. J.S[1], Joyce K.K[1], Grizzard J.S[1], Mordant. A.L[2], Herring. L.E[2], Diering G.H[1]

1. Department of Cell Biology and Physiology, School of medicine, UNC Chapel Hill, NC 27599.
2. UNC Proteomics core facility, Department of Pharmacology, UNC Chapel Hill, NC 27599.

Across the lifespan, sleep and synaptic development (both synaptogenesis and synaptic maturation) exhibit a harmonious relationship. During the juvenile developmental phase, synaptogenesis and rapid eye movement sleep are at their highest. With progression into adolescence, slow wave sleep (SWS or non(n)REM) peaks with concurrent reduction in synaptogenesis. Throughout much of adulthood, synaptic numbers and sleep amounts remain stable. This close proportional relation between sleep and synapse numbers across life presents several nodes of vulnerability across life and specifically during development. Sleep disturbance and insomnia are a key feature of neurodevelopmental synaptopathies such as autism. Our previous work established that a heterozygous deletion of the 22nd exon Shank3$^{(+/-)22ex}$ in mice results in enhanced vulnerability to early-life sleep disruption (ELSD), resulting in life long behavioral deficits. These findings emphasize the causal role of developmental sleep disruption in long lasting effects on behavior and brain function, thus, highlighting an interaction between developmental sleep disruption and genetic vulnerability. However, the alterations in synaptic proteome in consequence to ELSD remain unknown, this knowledge is crucial since synapses are assemblies of proteins and protein production and supply are required for synaptogenesis. To address this knowledge gap, we have made advances by innovatively combining the ELSD paradigm in the Shank3$^{(+/-)22ex}$ model with synapse fractionation and proteomics. Synapse fractions were isolated from cortex, hippocampus and striatum from WT and Shank3$^{(+/-)22ex}$ animals subjected to ELSD, or control treatment. We observed that the hippocampal and striatal synapses were most significantly affected in both WT and Shank3$^{(+/-)22ex}$ in consequence to ELSD. Our gene ontology analysis revealed that the proteins that change significantly in response to ELSD in the hippocampal and striatal synapse fractions of Shank3$^{(+/-)22ex}$ are involved in processes critical for protein translation. Issues with protein translation are implied in ASD, here we observe that ELSD resulted in alteration in proteins that regulate translation in Shank3$^{(+/-)22ex}$ mice. We hypothesize that ELSD mediates long lasting behavioral effects through the modulation of synaptic translational events in Shank3$^{(+/-)22ex}$.

Inclusion of an alternatively spliced microexon in clathrin is disrupted in muscular dystrophies and critical for skeletal muscle development

Jessica L. Cote[1], Adam J. Black[1], R. Eric Blue[1], Matheus Sadovsky[1,2], Sumaya Addish[1,2], J. Ashley Ezzell[1,3], Olivia A. Perez[1], Allie Mills[4], Laura Herring[4], Jimena Giudice[1,2,5,6]

1. Department of Cell Biology and Physiology, The University of North Carolina School of Medicine, Chapel Hill, NC 27599
2. Graduate Curriculum in Cell Biology and Physiology, The University of North Carolina School of Medicine, Chapel Hill, NC 27599
3. Histology Research Core Facility, Department of Cell Biology and Physiology, The University of North Carolina School of Medicine, Chapel Hill, NC 27599
4. Michael Hooker Metabolomics and Proteomics Core, Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
5. McAllister Heart Institute, The University of North Carolina School of Medicine, Chapel Hill, NC 27599
6. RNA Discovery Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Alternative splicing differentially includes or skips certain regions of a pre-mRNA and thus generates two or more distinct, mature transcripts from one gene. The RNA research community has become increasingly interested in a group of microexons – typically defined as exons that contain 3 to 51 nucleotides – whose alternative splicing (inclusion or skipping) gives rise to substantial biological consequences, particularly during the development of specific tissues. The tissues that exhibit the highest levels of alternative microexon inclusion are the brain and skeletal muscles. However, most microexon research has been focused on brain while skeletal muscles have been largely ignored. Here we show that the inclusion of an alternatively spliced 21-nucleotide microexon in clathrin heavy chain, which is highly enriched in skeletal muscles in a developmental stage-specific and evolutionarily conserved manner, is disrupted in early-onset muscular dystrophies. Using our novel CRISPR-Cas9-generated mouse model, we demonstrate that the inclusion of the microexon during mouse postnatal development is a critical molecular event for skeletal muscle function. At the cellular level, we show that the microexon modulates the remodeling of highly specialized, curved membrane structures that are exclusive to striated muscles.

Single-cell RNA-seq reveals cellular adaptations underlying opioid addiction Decoding the opioid system using single-cell transcriptomics.

Jesse K. Niehaus[1], Adrien Tassou[1], Hannah Koolpe[2], Hannah C. Nourie[2], Dong-Wook Kim[3] , Hongkui Zeng[3], Grégory Scherrer[1,2]

1. Department of Cell Biology and Physiology, UNC-Chapel Hill, Chapel Hill, NC 27514
2. Neuroscience Center, UNC-Chapel Hill, Chapel Hill, NC 27514
3. Allen Institute for Brain Science, Seattle, WA 98109

Opioids produce both pain relief and undesirable side effects, such as euphoria and respiratory depression, by binding the mu opioid receptor (MOR; encoded by *Oprm1*) in different brain regions. MOR signaling inhibits neurons through Gi-coupled GPCR signaling. Activating *Oprm1*-expressing neurons in distinct brain regions using opto- and chemogenetics resolves opioid-induced effects, but targeting endogenous receptors is difficult because co-expressed druggable targets are unknown. Here, we used single-cell RNA-sequencing (scRNA-seq) to generate an atlas of druggable targets in *Oprm1*-expressing neurons throughout the brain. Of these targets, the excitatory Gq-coupled GPCR lysophosphatidic acid receptor 1 (*Lpar1*) is co-expressed by *Oprm1*+ neurons in the ventral tegmental area, a brain region related to opioid reward. Systemically administering an LPAR1 agonist reduced opioid reward behaviors in preliminary studies. Chronic opioid addiction causes cellular and molecular changes throughout the brain, but the cell types and molecular pathways susceptible to these changes are unclear. To investigate cell type-specific changes underlying chronic opioid use, we performed scRNA-seq on eight brain regions in mice modeling opioid dependence and withdrawal to generate the largest single-cell atlas of opioid addiction to date. Interestingly, *Oprm1*-expressing neurons were particularly resistant to chronic opioid-induced transcriptional changes, suggesting that alternative cell types may be more effective as drug targets in treating opioid use disorder. We determined that cortical and thalamic glutamatergic neurons are particularly susceptible to transcriptional changes during opioid dependence and withdrawal, respectively. We identified that chronic opioid dependence alters molecular pathways related to circadian and neural activity, whereas acute withdrawal incurs signatures related to mitochondrial dysfunction and neurodegeneration. Together, these data provide a rich resource to identify cell-type-specific druggable targets and biological processes associated with opioid addiction.

Control of mechanical pain by μ-opioid receptor-expressing spinal pre-motor V1 neurons

Adrien Tassou[1-3], Karen Huang[1-3], Jesse Niehaus[1-3], Valentina Morales[1-3], Bowen Zheng[1-3], Jonathan Saju[1-3], Sy'Keria Garrison[1-3], Dong-Wook Kim[4], Hongkui Zeng[4], Grégory Scherrer[1-3,5^]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
2. UNC Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
3. Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.
4. Allen Institute for Brain Science, Seattle, WA, USA.
5. Lead contact.

The spinal cord integrates nociceptive signals from sensory neurons and relays them to the brain, and is a critical site of action for opioid analgesics acting through the μ-opioid receptor (MOR). These functions are classically attributed to neurons in the dorsal horn, whereas ventral horn circuits are primarily associated with motor control. However, MOR expression has also been reported in the ventral horn for more than three decades, yet its functional significance has remained unclear. Here, we combined spinal cord single-nucleus RNA sequencing, mouse genetics, light-sheet microscopy, slice electrophysiology, chemogenetics, and behavioral approaches to map MOR expression and function across ventral spinal neuron populations. Unexpectedly, we reveal that MOR-expressing V1 premotor neurons, previously linked exclusively to locomotion, form reciprocal connections with the dorsal horn and directly contribute to mechanical pain. Conditional MOR deletion in V1 neurons completely blocked pain recovery from peripheral inflammation, and chemogenetic experiments showed that activity in MOR-expressing V1 neurons is both necessary and sufficient for mechanical pain control. In contrast, MOR-expressing V2a neurons exclusively project within ventral circuits, without dorsal projections, and mediated opioid-induced muscle rigidity, a major side effect of opioid treatment. Together, these findings reveal an unappreciated role for ventral spinal circuits in pain regulation, challenge the functional segregation of dorsal and ventral spinal domains, and provide a cellular framework for both beneficial and adverse opioid actions in the spinal cord.

SECTOLD: a novel pathway for trafficking of secreted proteins to lipid droplets mediated by TMEM43

Yehonathan Malis[1], Maria Clara Zanellati[1], Venkata Reddy Chirasani[2,3], Gregory E Miner[1], Sarah Cohen[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
2. Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States
3. R. L. Juliano Structural Bioinformatics Core, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

We recently discovered that multiple secreted proteins, including apolipoprotein E (APOE) and cyclooxygenase 2 (COX2), can be redirected from the secretory pathway to lipid droplets (LDs) during lipogenesis. This is consistent with other secreted proteins, such as APOB and cathelicidin, all of which are endoplasmic reticulum (ER) luminal proteins that eventually interact with the cytosolic side of the LD. At the site of LDs, these secreted proteins serve different functions including modulating inflammatory lipid signaling. Here, we propose to name this rerouting the secreted-to-LD (SECTOLD) pathway. Our unpublished immunoprecipitation/proteomics study revealed that, among several proteins, the cardiomyopathy-associated ER protein TMEM43 strongly interacts with APOE during induced LD biogenesis in astrocytes. TMEM43 is an integral membrane protein with four transmembrane domains located in the ER, functioning as a nuclear envelope stabilizer. We found that during lipogenesis, the secreted-to-LD translocation of APOE was abolished upon depletion of TMEM43 using siRNA. Interestingly, trafficking of the classic ER-to-LD cargo protein diacylglycerol acyltransferase 2 (DGAT2) was not reduced by TMEM43 knockdown. TMEM43 depletion also affected LD number and size. Structural modeling of TMEM-43 suggests the hypothesis that this protein can form an oligomeric assembly at the ER-LD interface to mediate sorting of secreted proteins to a subset of LDs. These results reveal a role for TMEM43 in LD metabolism, associated with the sorting of inflammatory mediators to the LD surface membrane. With its roles in nuclear membrane stabilization, lipid homeostasis, and the recruitment of disease-relevant factors to LDs, TMEM43 emerges as a central protein implicated in chronic inflammation and numerous cardiac and neurodegenerative diseases.

The deletion of the pro-apoptotic *Bax* gene modulates alcohol-induced apoptosis

Dina I. Abu Rabe, Jackson R. Gerdes and Scott E. Parnell

1. Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC

Prenatal alcohol exposure (PAE) during early gestation can cause significant cognitive and physical defects, including the craniofacial malformations associated with fetal alcohol syndrome (FAS). During gastrulation, alcohol targets the ectoderm which forms the brain, eyes and the face. Our previous study showed that deletion of the *Bax* gene in mice significantly reduced embryonic cell death (apoptosis) and the incidence of craniofacial malformations. The current study focuses on understanding the mechanisms by which prenatal alcohol exposure during the gastrulation stage leads to brain and craniofacial defects. We established a *Bax*-knockout murine embryonic fibroblasts (MEFs) NIH3T3 cell line using CRISPR-Cas9 technology. Western blot, PCR, and DNA sequencing were used to confirm the deletion of the *Bax* gene. Furthermore, MEFs were isolated and cultured from embryos derived from crosses of heterozygous *Bax*-/+ male and female mice. The effects of alcohol (50 mM,100 mM, and 200 mM) on the apoptosis and ROS of the *Bax* wild-type, *Bax*-knockout, and the transformed MEFs were evaluated using the TUNEL assay, MitoSox staining and RT-PCR. The impact of pharmacological BAX inhibition (using 5uM Eltrombopag) on alcohol induced apoptosis was examined in NIH3T3 cells using TUNEL assay. Interestingly, the treatment of *Bax* wild-type MEFs cells with ethanol significantly (p-value = 0.0001) induces apoptosis and ROS in a dose-dependent manner. However, the deletion of the *Bax* gene completely mitigates ethanol-induced apoptosis and ROS compared to the wild-type cells, indicating a role of *Bax* in alcohol-induced apoptosis and ROS release. The caspase 3 expression was significantly (p-value < 0.0001) lower in *Bax* knockout cells treated with 100 mM and 200 mM ethanol compared to the *Bax* wild-type NIH3T3 cells. The inhibition of BAX protein using 5μM Eltrombopag significantly (p-value = 0.0001) reduced alcohol-induced apoptosis in NIH3T3 cells. Overall, these results confirm that the *Bax* gene modulates the effect of alcohol exposure.

Anti-migraine drugs modulate serotonin receptor signaling in lymphatic endothelial cells

Poonam Kumari[1], D. Stephen Serafin[1], Yanna Tian[1], Monserrat Avila-Zozaya[1], Aftyn Trumbo[2], and Kathleen M Caron[1].

1. Department of Cell Biology and Physiology,
2. Biomedical and Biological Sciences Program, University of North Carolina, Chapel Hill, NC

Migraine is a chronic neurovascular disease characterized by recurrent severe headache attacks, accompanied by omiting, photophobia and nausea. Sumatriptan and Lasmiditan are both acute anti-migraine drugs acting through distinct serotonin receptor mechanism. Sumatriptan belongs to triptans which act as an agonist on 5HT1B/1D receptors, reducing trigeminovascular activation, calcitonin gene -related peptide (CGRP) and glutamate release, inhibiting nociceptive signaling, and producing vasoconstriction that contributes to its clinical efficacy but limits its use in migraine patients with cardiovascular disease. Lasmiditan is a selective 5HT1F receptor agonist novel antimigraine drug which suppresses migraine pain without vasoconstriction, mainly by reducing trigeminovascular system activation and CGRP-mediated signaling. Lymphatic endothelial cells may represent an underappreciated site of serotonergic signaling in migraine therapeutics. Here, we use spatial transcriptomics to identify multiple serotonin receptor transcripts in mouse lymphatic vessels. Further our data indicates that human dermal lymphatic endothelial cells (HDLECs) express serotonin receptors 5-HT1F, 5-HT1B, and 5-HT1D mRNAs, as confirmed by qPCR. In HDLECs treated with serotonin, lasmiditan, and sumatriptan, the ONE-GO assay which measures endogenous GPCR activity showed increased Gαi1 signaling, and GloSensor assay which measures the cAMP activity demonstrated reduced forskolin-stimulated cAMP activity, together indicating activation of inhibitory Gαi-coupled pathways. Our preliminary data also showed that sumatriptan increased the formation and remodeling of button junctions and that button junction formation was reduced in the presence of CGRP. In contrast, lasmiditan did not show any change in the Ve-cadherin junction remodeling compared to vehicle, but in the presence of CGRP, we observed more zipper junctions. These results suggest that serotonin receptor signaling in HDLECs is not only transcriptionally present but also pharmacologically functional, and that this signaling contributes to Ve-cadherin junction remodeling. This expands current understanding of migraine pharmacology by identifying the lymphatic endothelial as a potential cellular target for migraine therapeutics.

Identifying ADGRF5 as a central receptor of lymphatic endothelial function

Monserrat Avila-Zozaya, D. Stephen Serafin, Yanna Tian, Elizabeth S Douglas, Erika N Chan, Xiaolei Liu, Douglas G Tilley and Kathleen M Caron.

Adhesion receptors are critical regulators of lymphatic endothelial cell integrity and function. They control crosstalk between the endothelium and microenvironment, vascular permeability, inflammation, and lymphangiogenesis. Disruption of these mechanisms impairs lymphatic growth, function, and membrane integrity, leading to lymphatic-associated disorders, such as lymphedema. There is currently a lack of effective therapies targeting the lymphatic system; therefore, identifying key regulators of the lymphatics has high therapeutic potential.
Adhesion G protein–coupled receptors (aGPCRs) are members of the GPCR superfamily and are recognized as regulators of tissue architecture and cellular dynamics. Through a large extracellular region enriched with adhesion domains, aGPCRs control cell–cell and cell–matrix interactions. In addition, these receptors translate biochemical and mechanical cues into intracellular responses through structural rearrangements of their seven-transmembrane domain. In our current work, we propose that *ADGRF5*, an aGPCR, functions as a central regulator of lymphatic endothelial cell homeostasis by regulating adhesive and mechanical signals. We found that *ADGRF5* was highly expressed in lymphatic endothelial cells (LECs). Knockdown of *ADGRF5* resulted in significant alterations in cell-matrix and cell-cell adhesion components, including diminished adhesion to fibronectin, reduced VE-cadherin and elevated paxillin protein levels which affected cellular permeability. *ADGRF5*-deficient LECs exhibited a robust upregulation of ERK and AKT phosphorylation, accompanied by high cellular proliferation. Interestingly, mechanical stimulation induced upregulation of *ADGRF5* in LECs, and the knockdown of *ADGRF5* resulted in a higher upregulation of KLF4 expression under shear stress. Additionally, preliminary tail microlymphography data from mice lacking *adgrf5* in LECs showed a delay in tracer clearance along the tail lymphatic capillaries compared to wild-type controls. Collectively, our findings identify *ADGRF5* as a previously unrecognized regulator of lymphatic endothelial biology and provide important insight into the largely unexplored role of adhesion GPCRs in the lymphatic endothelium.

Proximity proteomics of RAMP3 identifies MYO6 as a regulator of adrenomedullin signaling in lymphatic endothelial cells

D. Stephen Serafin[1], Monserrat Avila-Zozaya[1], Poonam Kumari[1], Natalie R. Harris[1], and Kathleen M. Caron[1*]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill; 111 Mason Farm Road, Chapel Hill, North Carolina, USA 27599.

The receptor activity-modifying protein 3 (RAMP3) heterodimerizes with the G-protein coupled receptor (GPCR), calcitonin receptor-like receptor (CLR), to form an adrenomedullin (AM)-receptor. RAMP3 allosterically regulates CLR to coordinate signaling, internalization, and endosomal recycling after stimulation by the pro-lymphangiogenic peptide, AM. Importantly, the host of cellular machinery that RAMP3 engages to regulate CLR remains unknown. Here, we utilize proximity proteomics to identify the RAMP3 interactome and characterize how it changes in response to AM ligand. The unconventional myosin VI (MYO6) protein, an actin-based molecular motor, was identified as a proximal RAMP3-CLR interactor. We then interrogate within primary lymphatic endothelial cells the role of MYO6 on AM-mediated signaling and functional cellular outcomes. We found that loss of MYO6 inhibits AM-mediated GαS, ERK, and AKT activation while simultaneously increasing cAMP accumulation as well as CREB and PKA activity. We further show that loss of MYO6 has broad cellular outcomes on AM-mediated adherens junction remodeling, focal adhesion assembly, and migration. This RAMP3-CLR interactome shines

TET1 SAFEGUARDS LINEAGE ALLOCATION IN INTESTINAL STEM CELLS

Gleizes Antoine[1], Janto Nicolas[2], Bombin Sergei[2], Patel Krupa[2], Sun Siyang[1], Rao Vivek[2], Ari Gurel[2], Fonseca Maria[1], Yao Bing[2], Gracz Adam[1]

1. University of North Carolina, Chapel Hill, NC, USA
2. Emory University, Atlanta, GA, USA

Background : Intestinal stem cell (ISC) differentiation is genetically controlled by factors including DNA methylation, chromatin accessibility, and gene expression. The chromatin-modifying enzyme TET1 catalyzes DNA demethylation to regulate transcription and sustain WNT pathway during intestinal development as shown in *Tet1*-null mouse. We hypothesized that *Tet1* regulates adult ISC biology through epigenomic mechanisms.
Aims : This project aims to define *Tet1* requirement for intestinal epithelial cell differentiation and underlying chromatin regulatory mechanisms.
Methods : The role of TET1 was assayed using a conditional and inducible *Villin-CreER* mouse model for *Tet1* knock-out (Tet1iKO). Cell state and behavior were evaluated at the cellular and transcriptomic level respectively by immunofluorescence and single-cell RNA sequencing. Cell differentiation was functionally challenged in Tet1iKO organoids using growth factor treatment. Epigenomic mechanisms were assayed by transcriptomic (RNA-seq), chromatin accessibility (ATAC-seq) and methylomic (5mC/5hmC-seq) in ISCs.
Results : Tet1iKO mice exhibited elevated numbers of absorptive enterocytes and goblet cells as well as a reduction in ISCs and progenitor cells *in vivo*. Pseudotime analysis of scRNA-seq demonstrated early bias towards absorptive transcriptional signatures in the absence of *Tet1*. Interestingly, Tet1iKO phenotype revealed environment dependent and silent in absence of stimulation in a high-level barrier facility. Tet1iKO organoids were phenotypically identical to control in basal culture conditions. However, they exhibited a significant shift in cellular differentiation towards secretory progenitors upon growth factor challenge. TOBIAS prediction for transcription factors binding from ATAC-seq revealed a positive enrichment for CTCF in Tet1IKO ISCs. Methylome sequencing revealed increase in 5hmC opposed to a decrease in 5mC over transcription factor-specific loci such as CTCF.
Conclusion : *Tet1* loss sensitizes ISC to differentiation signals. As Tet1iKO phenotype is silent in absence of stimulation, we propose that *Tet1* loss is priming gene expression in ISC by modifying DNA methylation and CTCF
binding.

β-arrestin 1/2 are essential for embryonic lymphatic vessel development

Yanna Tian[1], D. Stephen Serafin[1], Monserrat Avila-Zozaya[1], Alyssa M. Tauro[1], Natalie M. Torres-Valle[1], Bryan M. Kistner[1], Danielle M. Dy[1], Elizabeth S. Douglas[1], Kathleen M. Caron[1]

1. [1]Department of Cell Biology and Physiology, the University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

β-arrestins are ubiquitously expressed cytosolic adaptor proteins that regulate G protein-coupled receptor-dependent and -independent pathways essential for numerous physiological functions. This study investigated the role of β-arrestin1 and -2 in embryonic lymphatic vessel development and survival by generating and characterizing mice with lymphatic, tamoxifen-inducible loss of the genes encoding β-arrestin-1 and -2 (*Arrb1/2*$^{\square\square iLEC}$). At embryonic day15.5 (E15.5), *Arrb1/2*$^{\square\square iLEC}$ embryos exhibit profound hydrops fetalis and increased embryonic mortality compared to control *Arrb1/2*$^{fl/fl}$ embryos. Edematous *Arrb1/2*$^{\square\square iLEC}$ embryos, which were more often represented by the female sex, showed growth restriction and decreased lymphatic endothelial cell (LEC) proliferation in the jugular lymphatic sac compared to controls. In vitro knockdown of β-arrestin1 in LECs increased proliferation and increased activation of AKT, while knockdown of β-arrestin2 decreased proliferation and decreased activation of both ERK and CREB. *Arrb1/2*$^{\square\square iLEC}$ embryos also exhibited dilated dermal lymphatics with decreased continuous VE-Cadherin adherens junctions compared to controls. These results were recapitulated in vitro in β-arrestin1 and/or -2 knockdown human LECs, which showed a decrease in membrane VE-Cadherin and β-catenin levels, and prevention of adrenomedullin-induced linearization of VE-cadherin at endothelial cell–cell junctions. Collectively, these results demonstrate that loss of β-arrestin1/2 in lymphatics causes hydrops fetalis, mid-gestational growth arrest and embryonic demise associated with reduced LEC proliferation and disrupted VE-Cadherin adherens junctions.

Enteroendocrine cells regulate intestinal epithelial barrier integrity by modulating sphingolipid metabolism and abundance

Joseph Crecelius[1], Saanvi Gupte[1], Avery Staub[1], Keila S. Espinoza[2], Jennifer Nwako, Justin M. Snider[3,4], Ashley J. Snider[3,4], Anne-Marie Riedman[1], Heather McCauley[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599
2. Department of Physiology, University of Arizona, Tucson, AZ 85721, USA
3. School of Nutritional Sciences and Wellness, University of Arizona, Tucson, AZ 85721, USA
4. University of Arizona Cancer Center, University of Arizona, Tucson, AZ 85721, USA

Enteroendocrine cells (EECs) are specialized hormone-producing cells in the gastrointestinal tract that react to metabolites and microbes to maintain homeostasis. EECs produce over 20 hormones that regulate various cellular processes such as cellular respiration, glucose uptake, and hunger responses. We have previously established EEC-deficient mouse and human intestinal enteroids (NEUROG3 knockout) to explore novel roles for EECS in their local environment. One major finding was that intestinal epithelial barrier rigidity and function were disrupted, resulting in increased barrier permeability. We hypothesize that EECs regulate intestinal barrier structure by releasing hormones that stabilize tight junction proteins in sphingolipid-rich plasma membrane microdomains, such as lipid rafts.

We compared NEUROG3-/- mouse tissue to wildtype using lipidomic MS/MS and found a significant increase in the production of short-chain ceramide species (16 carbons). These short-chain ceramides were produced through de novo synthesis of ceramides, while other de novo longer-chain ceramides, such as ceramide 24:1, were not. This suggests that alterations in ceramide pathway regulation occur in the absence of EEC-derived hormones, which may contribute to changes in intestinal barrier permeability. We compared control human intestinal enteroids with those harboring a genetic loss of *NEUROG3* (EEC-deficient). We isolated detergent-resistant membranes (DRMs), such as lipid rafts, from the remainder of the cell lysate to examine tight junction protein localization. In EEC-deficient cells, there was a shift of proteins associated with lipid rafts, such as CAV1 and Claudin-1, away from more soluble DRMs to more of the total protein fraction. Conversely, we observed an increase in Claudin-2 in DRMs, which is more closely associated with porous tight junctions. Treatment with ceramide-16 and ceramide-24:1 altered the localization of all proteins but did not rescue CAV1 in the higher fractions compared with vehicle-treated EEC-KO cells. We also used EEC hormones PYY and OCT (somatostatin analog), which activated the PI3K/AKT and mTOR signaling pathways, which are upstream regulators of sphingolipid metabolism and abundance. These findings in human enteroids correlate with increased barrier permeability in EEC-deficient mice.

Elucidating the Role of Protein Myristoylation During Embryonic Heart Development

Shenice Harrison [1,2], Khalid Tejan-Sie [1,2], Gregory Miner [1,2], Whitney Edwards [1,2]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2. McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Congenital heart diseases (CHDs) affect nearly 1% of all live births. However, the etiology remains unknown in more than 50% of case, highlighting the need to uncover additional molecular mechanisms responsible for heart development. Disruptions in sarcomere formation contribute to CHDs, yet the regulatory pathways governing this process remain elusive. Protein myristoylation is a lipid modification in which an N-myristoyltransferase (NMT1) attaches myristic acid to the N-terminal glycine of proteins, regulating protein localization, interactions, and stability. Prior studies suggest that NMT1 activity is required for CM differentiation *in vitro* and work in *Caenorhabditis elegans* show that protein myristoylation is required for sarcomere formation and muscle function. *Therefore, we hypothesize that protein myristoylation is critical for sarcomere formation and function during cardiogenesis*. In studies conducted in our lab, quantitative proteomic profiling and immunohistochemical analyses revealed that NMT1 is abundantly expressed in the developing heart and is enriched in the myocardium. Furthermore, we show that pharmacologic inhibition of NMT1 in embryonic CMs disrupts sarcomere organization and reduced sarcomere length, establishing a functional requirement for myristoylation in sarcomere assembly. To define the *in vivo* role of myristoylation in heart development, we generated a novel CM-specific NMT1 knockout model and preliminary phenotypic analyses support a role for NMT1 myocardial development. Additionally, our preliminary bioinformatic analysis suggest that myristoylation may regulate sarcomere formation and contractility by facilitating localization and function of costamere associated proteins. These findings provide new insight into how myristoylation regulates sarcomere formation and may uncover molecular mechanisms and therapeutic targets for CHDs.

Investigating the role of brain enriched E3 Ubiquitin Ligase TRIM9 in Alzheimer's Disease/Tauopathies

Anca G. Frasineanu[1], Surbhi Cooduvalli[2], Todd J. Cohen[3,4], Stephanie L. Gupton[1,5,6]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, NC 27599
2. Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, NC 27599
3. Neurology, University of North Carolina at Chapel Hill, NC 27599
4. Duke-University of North Carolina Alzheimer's Disease Research Center (ADRC), 2424 Erwin Road, Durham, NC 27705
5. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC 27599
6. Neuroscience Center, University of North Carolina at Chapel Hill, NC 27599

In the last decade, we have demonstrated that the brain-enriched E3 ubiquitin ligase TRIM9 regulates cytoskeletal dynamics, membrane remodeling, and netrin-dependent signaling pathways in all stages of neuron development, including dendritic spines maturation. TRIM9 is enriched within the post-synaptic density (PSD), and its protein levels are maintained throughout adulthood. Our proximity labelling experiments to characterize the TRIM9 interactome identified several proteins implicated in synaptic function and Alzheimer's disease (AD), including Tau. Here, we investigate the role of TRIM9 in tau-mediated neurodegeneration, using the P301S (PS19) mouse model and primary neuronal cultures, to understand the mechanistic role that TRIM9 plays in the adult brain. Broadly, our data implicate TRIM9 in several aspects of neuronal degeneration, including cytoskeletal modification and aggregation, mitochondrial dysfunction, and neuroinflammation. Briefly, Iba1:CD68+ microglia are enriched in the hippocampus, entorhinal cortex, and amygdala of 6 and 8 month old *Trim9*$^{-/-}$*:PS19* mice compared to *Trim9*$^{+/+}$*:PS19* mice. Pathological tau (p-Tau stained with AT8) accumulation was significantly higher in the hippocampus and cortex of *Trim9*$^{-/-}$*:PS19* mice compared to *Trim9*$^{+/+}$*:PS19* mice, and significantly mislocalized to the PSD in six month cortex. Quantitative PSD proteomics of cortical PSD fractions suggests that impaired mitochondrial function in *PS19* mice may be exacerbated in the absence of *Trim9*. Ongoing experiments are exploring mitochondrial morphology, dynamics, and TRIM9 localization to mitochondria in cultured neurons with or without mitochondrial stress, and mitochondrial respiration in brain and culture. The novel partial TRIM9 localization to mitochondria could represent an exciting potential therapeutic target for Alzheimer's disease and related tauopathies.

Artificial tethering to the ER promotes lipid droplet growth via enhanced lipid Channeling

Victoria Williams[1], Gregory Miner[1], Sarah Cohen[1]

1. Department of Cell Biology and Physiology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599

Eukaryotic cells compartmentalize biochemical processes through membrane-bound organelles that coordinate their functions at membrane contact sites (MCSs). Lipid droplets (LDs) are dynamic lipid storage organelles key to cellular metabolic and signaling functions, including those dysregulated in disease states. Proteins key to LD function can target to LDs through the ER, from which LDs originate, at ER-LD MCS. To further characterize the function of these MCSs and how they influence cellular lipid distribution in LD, we have used our Contact-FP dimerization-dependent fluorescent protein (ddFPs) system to label MCS in live cells. This system pairs a weakly fluorescent A monomer with a non-fluorescent B monomer to produce a bright fluorescent signal when the two monomers dimerize, to be detected using confocal microscopy. Using ddFPs targeted to ER and LDs, we effectively and specifically label ER-LD MCSs in live cell. This tool can also induce MCSs through transfection of high amounts of ddFP. We have found that inducing such contacts increases LD size, changing the distribution of intracellular lipid in a way that differs from lipid droplet biogenesis induced by the common dietary fatty acid oleic acid (OA). We then interrogated the mechanism for this size increase, looking at movement of triglyceride synthesis enzyme DGAT2 to the LD and the movement of supplemented fluorescent fatty acids onto existing LD. We found that while inducing contacts increases the proximity of DGAT2 in the ER to the LD, it does not lead to the re-targeting of DGAT2 from the ER to the LD seen in OA supplemented conditions. These data suggest that recruitment of DGAT2 to the LD likely does not modulate the increase in LD size. In contrast, supplementation of fluorescent fatty acid analogue FLC12 shows increased movement of FA from the ER to the LD at ddFP-induced contact sites. Together, these data suggest that this artificial tether system can facilitate the movement of lipid but not proteins from the ER to the LD which lead to increased LD size. Our data indicate that inducing a single MCS via an artificial tether can alter cell metabolism in a specific and targeted manner.

Illuminating the Organelle Signature Underlying Cardiomyocyte Differentiation

Meredith F. Gillis[1], Matheus Sadovsky[1], Greg Miner[1], Whitney Edwards[1], and Sarah Cohen[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

Cardiomyocyte differentiation from pluripotent stem cells requires extensive metabolic and structural remodeling to support their specialized contractile function. The transition from glycolytic progenitors to oxidative cardiomyocytes involves coordinated changes in mitochondrial structure and activity, ER specialization into the sarcoplasmic reticulum with advanced Ca^{2+} handling, and specialized cytoskeletal assembly. To meet these changing demands, dynamic inter-organelle communication is required. While individual contact sites are transient, the global interactome remains stable within a given cell type but differs between cell types; suggesting the interactome is reflective of the functional needs and identity of the cell. However, how these and other organelle contact sites are established, regulated, and remodeled during cardiomyocyte differentiation remains unknown.

Mitochondria-ER contacts are the most studied contact site in cardiomyocytes due to their role in regulating mitochondrial dynamics, and their misregulation contributes to cardiovascular diseases. Yet, these studies have ignored the role of other contact sites in supporting cardiomyocyte physiology. Here, we use multispectral live-cell imaging to simultaneously visualize seven organelles, coupled with computational image analysis to quantify organelle morphology, distribution, and inter-organelle contacts. Using this systems-level approach, we identified changes in the organelle interactome during cardiomyocyte differentiation. Preliminary data revealed increased two- and three-way contacts among lipid droplets, mitochondria, ER, Golgi, and lysosomes. These results indicate dynamic lipid flux, metabolic coupling during mitochondrial maturation, high membrane and protein trafficking demand, and elevated organelle turnover. Further interrogation of these contacts and cardiomyocyte-specific regulators will advance disease modeling, regenerative medicine, and therapeutic target discovery.

Hyperlicensing: a path to replication stress tolerance?

Jack Bennett[1], Arvin Talwar[2], Jean Cook[1]

1. Department of Cell Biology & Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.
2. Department of Biochemistry & Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.6

Origin licensing, or the loading of MCM helicases onto chromatin, is a G1-restricted process that is critical for successful DNA replication in the subsequent S phase. When cells are insufficiently licensed (underlicensed), they are highly sensitive to replication stress and susceptible to genome instability. Here we describe an unstudied form of origin licensing, hyperlicensing, defined by excessive loading of MCM complexes onto chromatin. By utilizing a CDK2 inhibitor to extend the G1 phase or a cell line overexpressing a stabilized licensing factor (*CDC6*), cells are able to reach a hyperlicensed state prior to S phase entry. We used quantitative flow cytometry and immunoblotting to define hyperlicensing and uncover the impacts of enhanced origin licensing in untransformed and transformed cells. This enhanced origin licensing may serve as a mechanism for cells to manage replication stress and confer genomic protection.

Characterization of Pancreatic Ductal Adenocarcinoma Fibroblasts

Ian C McCabe[1], Ngan N.K Van[2], Sandra A Zarmer[3], Joseph F Kearney[4], Laura Peng[2,3], Jen Jen Yeh[2,3,4]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC
2. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC
3. Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC
4. Department of Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with a 5-year survival rate of just 9%. PDAC tumors are composed of tumor epithelial cells and a dense fibrotic stroma made up of cancer associated fibroblasts (CAFs), immune cells, and extracellular matrix (ECM). CAFs have been shown to promote tumor growth and invasion through mechanisms linked to secretion of soluble factors and ECM deposition and remodeling, however, ablation or broad targeting of CAFs can have a similar eVect – suggesting that these cells exist in the TME as distinctive subpopulations. Through bulk transcriptomic analysis of patient tumors, we have identified two prognostic molecular subtypes of CAFs: tumor restrictive (restCAFs) and tumor promoting (proCAFs), the latter of which shows worse overall survival among patients.

We hypothesized that patient derived proCAFs would promote a more aggressive tumor phenotype in vitro compared to restCAFs. Interestingly, tumor cells stimulated with restCAF conditioned media displayed increased invasion through transwell compared to proCAF conditioned media. As CAFs are major contributors to ECM deposition, we derived cell free matrix from both CAF subtypes. We found that proCAF matrix appears more disorganized and promotes tumor cell migration compared to restCAF matrix. Overall, both proCAFs and restCAFs can promote an aggressive tumor phenotype in vitro, but through diVerent mechanisms. Our next step is to evaluate how proCAF and restCAF co-injected with tumor organoids eVects tumor growth and invasion in vivo.

Elucidating the mechanism of ENaC activity reduction by molecular glue degrader drugs

Emily Bulik-Sullivan[1,2], Jacob R. Galiger[2], Rhianna Lee-Ferris[1,2], Kiana Venable[2], Scott Lyons[3], Angie Mordant[3], Lihua He[1,2], Laura Herring[3], Scott H. Randell[1,2]

1. Department of Cell Biology & Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 27599
2. Marsico Lung Institute/Cystic Fibrosis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 27599
3. UNC Metabolomics and Proteomics Core Facility, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 27599

Some cereblon-based molecular glue degrader drugs (MGDs), including CC-90009, SJ6986, and MRT-2359, reduce epithelial sodium channel (ENaC) activity, which is a potentially mutation-agnostic avenue for treating cystic fibrosis (CF) and other muco-obstructive lung diseases. [1,2,3] The mechanism by which these drugs reduce ENaC activity is poorly understood, but is known to be dependent on cereblon. [4]
Here, we investigated whether additional, previously untested MGDs reduce ENaC activity in the respiratory epithelium. To do so, primary human airway epithelial cell (HAEC) air-liquid interface (ALI) cultures were treated with a suite of MGDs for 24 hours, after which ENaC activity was measured using a transepithelial current clamp amplifier (TECC). [1] We then treated primary non-CF HAEC ALI cultures for either 6 or 18 hours with 0.1 μM CC-90009 or 0.1% DMSO and performed global LC-MS/MS using a Thermo Vanquish Neo/Orbitrap Astral. Finally, we identified candidate proteins that may be involved in CC-90009's mechanism of ENaC activity reduction from our proteomics data, performed CRISPR-Cas9 knockdown of these candidates in ALI cultures, treated with either CC-90009 or DMSO, and assessed the resulting impacts on ENaC activity using TECC. [4]
Of the MGDs tested here, only CC-90009 reduced ENaC activity. After both 6 and 18 hours of CC-90009 exposure, the most-reduced proteins were GSPT1/eRF3a and GSPT2/eRF3b, the known substrates of CC-90009. As expected, the abundance of more proteins was significantly changed after 18 hours of exposure to CC-90009 than after 6 hours. We knocked down six candidate proteins (APP, CLSTN1, ETF1, PAIP2, SNX17, and STXBP4) identified via LC-MS/MS, but none of these knockdowns reduced ENaC activity in the absence of CC-90009. Further inquiry is therefore required to uncover this potentially novel and druggable mechanism of ENaC activity reduction.

Transcriptomic Profiling Reveals Altered Cellular Maturity in Necrotizing Enterocolitis

*Caden W. Sweet[1,2], *Mickael Meyer2, Keith Breau2, Joseph Burclaff2, Dani Sklar3, Catherine Rizzuto3, Misty Good3,4, Scott T. Magness1,2,4

1. Department of Cell Biology and Physiology,
2. CGIBD,
3. Department of Pediatrics,
4. Department of Medicine | UNC Chapel Hill
   *Equal Contribution

Necrotizing Enterocolitis (NEC) is a disease of premature infants characterized by an aberrant inflammatory response leading to intestinal epithelial necrosis shortly after birth, though its underlying mechanisms remain unclear1. Diagnostic biomarkers of NEC are critical, as the mortality rate nears 40% if not treated early2. Given that NEC primarily affects premature infants, cellular immaturity likely contributes to disease severity; however, current literature largely focuses on epithelial immaturity3,4. Thus, we profiled cell-type-specific maturity states across the intestine to better characterize disease phenotypes. Using flash-frozen intestinal resections, we isolated and sequenced nuclei from fetal, NEC, and healthy infant tissue. Leiden clustering was then used to identify distinct cell types across the full-thickness intestine. The inclusion of fetal samples in our dataset enabled characterization of developmental programs and establishment of a baseline for cell-type-specific maturity. Differential gene expression between fetal and healthy infant samples defined maturity signatures, which were then compared to NEC. Interestingly, epithelial cells in NEC generally exhibited mature expression profiles, whereas underlying mesenchymal populations including endothelial cells, fibroblasts, and neurons, appeared less mature. This suggests that immaturity in non-epithelial compartments may contribute to NEC pathogenesis and warrants further investigation.
Differential gene expression analysis was then used to find novel genes and pathways that are dysregulated between NEC and healthy infant samples. Understanding cell-type-specific alterations in maturity, as well as transcriptional programs and pathways in NEC, provides a foundation for future functional studies to support development of improved biomarkers and therapeutic strategies.

Elucidating the role of endothelial Apelin signaling in liver regeneration upon chemical toxicity

Kanesha Travis[1], Aashita Rajput[1], Won Yoon[1], Emily Hernandez[1], D. Berfin Azizoglu[1,2]

1. Department of Cell Biology and Physiology, UNC-CH, North Carolina, Chapel Hill 27599
2. McAllister Heart Institute, UNC-CH, North Carolina, Chapel Hill, 27514

The liver is a highly vascularized organ and is capable of full regeneration unlike other mammalian organs. While the roles of epithelial cells in liver regeneration have been extensively studied, how the vasculature of the liver responds to injury and facilitates regeneration remains mostly unknown. My work will investigate how the liver vasculature responds to injury and facilitates regeneration through the Apelin-APJ pathway in mice, using carbon tetrachloride (CCl4)-mediated liver injury that mimics acetaminophen overdose in patients. Apelin is a relatively newly identified angiogenic ligand that signals through the G-coupled receptor APJ and activates PI3K/AKT/mTOR and MAPK/ERK pathways involved in cell proliferation, cell survival, and cell cycle control. Apelin-APJ signaling plays crucial roles in vasodilation, inflammation, and angiogenesis. Thanks to these roles, the Apelin-APJ system is thought to have therapeutic potential in various diseases, including liver fibrosis; yet, the precise cell types and molecular pathways remain uncharacterized, particularly in the context of liver regeneration. Recently, our lab has found that injury to the liver induces Apelin expression in a subset of vascular endothelial cells. My preliminary lineage-tracing results suggest an expansion of the Apelin-expressing endothelial cells over the regeneration period. This is in line with data from our lab and others showing endothelial cell proliferation in liver injury. I hypothesize that Apelin-APJ signaling promotes endothelial proliferation and expansion through the PI3K/AKT/mTOR and/or MAPK/ERK pathways to facilitate new blood vessel growth during liver regeneration. I will test this hypothesis by 1) characterizing the expression of Apelin and APJ in injured livers and analyzing the expansion behaviors of Apelin- and APJ-expressing endothelial cells, 2) genetically inhibiting the expansion of Apelin-expressing endothelial cells and assessing the impact on liver regeneration, and 3) genetically ablating the receptor APJ in endothelial cells and assessing the impact on liver regeneration. Together, my studies will determine how endothelial Apelin-APJ signaling participates in liver regeneration and provide insight into the mechanisms of vascular recovery upon liver injury.

Fermentation-derived metabolites modulate host signaling pathways to control cellular aging

Annalee Schmidt[1], Rachel DuMez-Kornegay[1], Rob Dowen[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Caloric restriction initiates conserved catabolic responses that promote longevity, yet whether bacterial-derived metabolites modulate these pathways remains unclear. Fermentation by gut microbes is essential for intestinal health, as fermentation products serve as primary energy sources for intestinal epithelial cells. During aging and disease, fermentative bacteria decline, leading to metabolic stress in intestinal tissues. Despite this, fermentation-derived metabolites remain an underexplored class of regulators of host metabolism and aging. Kombucha Tea is a dietary product containing an active microbial community that produces diverse fermentation products, providing a tractable system to examine how these molecules influence host metabolism and aging. We developed a method to feed *C. elegans* either actively fermenting Kombucha Tea-associated microbes (KTMs) or a non-fermenting mixture of the same species (KTM-M). Only ingestion of fermenting KTMs induced metabolic reprogramming and significantly extended lifespan and healthspan, as assessed by improved intestinal barrier integrity and sustained muscle function during aging. KTM consumption also triggered age-dependent, tissue-specific mitochondrial remodeling. Using an HPLC-UV metabolomics platform, we found that KTM-fed animals were exposed to more acetic acid and glucuronic acid than animals fed KTM-M. Supplementation of *E. coli* with Kombucha cell-free supernatants was sufficient to induce mitochondrial remodeling, indicating that fermentation-derived metabolites drive these phenotypes. At the molecular level, KTM feeding activated AMPK, as indicated by phosphorylation of the catalytic subunit AAK-2. However, aak-2 null mutants retained responsiveness to KTM feeding, implicating the lesser-studied AMPK α isoform, AAK-1. Consistently, aak-1;aak-2 double mutants failed to exhibit improved muscle function during KTM consumption. Together, these data demonstrate that bacterial fermentation-derived metabolites modulate conserved metabolic and aging pathways through host-microbe interactions and highlight dietary fermentation products as a means to promote health during aging.

Identification of Genes Escaping X-Inactivation in Cardiomyocytes

Xingren Wang[1], Kayla Mason[1], Ryan Salcido[2], Mauro Calabrese[4,5], Frank Conlon[3,4]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2. Department of Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC 27599, USA
3. Department of Biology and McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
4. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
5. Department of Pharmacology, RNA Discovery Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

X-chromosome inactivation (XCI) is a fundamental mechanism that randomly silences one X chromosome in females and achieves dosage parity with males. Genes escape XCI through tissue-specific and temporally regulated processes.Our previous work demonstrates that key escapees are vital for cardiac physiology. Despite their importance, the comprehensive landscape of escape genes in the heart remains poorly defined. To bridge this gap, we utilized a C57BL6/J x CAST/EiJ F1 hybrid mouse model with skewed XCI to identify tissue-specific escape genes. Focusing on cardiomyocytes, we identified 45 X-linked genes that escape silencing. These escapees are widely distributed across the X chromosome rather than clustered at a single locus. We validated the escape status of candidate genes, such as *Ddx3x*, using restriction fragment length polymorphism. Functional enrichment analysis using Gene Ontology (GO) revealed enrichment for hypoxia response and metabolic pathways. This suggests that escape from XCI may critically contribute to cardiomyocyte adaptation to oxygen stress and to regulation of energy balance. Notably, escape genes are more likely to have escapee neighbours than inactive controls. These findings provide preliminary insights into the 3D chromatin structure of XCI, indicating that clustered escape genes may be epigenetically co-regulated. Future research will focus on identifying the specific regulators of these escape genes, potentially revealing novel therapeutic targets.

Establishing Spindle Positions in Oriented Cell Divisions

Lauren Griffith[1], Kendall Lough, Scott E. Williams[1,2]

1. Cell Biology and Physiology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
2. UNC Pathobiology and Translational Science Department, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Orientation of cell divisions is crucial for balancing differentiation, proliferation, and stem cell maintenance. In the epidermis, basal progenitor cells divide either parallel or perpendicular to the epithelial surface, which are symmetric/self-renewing or asymmetric/differentiative, respectively. Asymmetric cell divisions utilize a spindle orientation complex containing a key regulator of this process, LGN/Gpsm2, which localizes to the apical cortex during mitosis to promote perpendicular decisions. Centrosomes are linked to the apical cortex through interactions with factors of the spindle orientation complex and are thought to play an important role in fate determination, mitotic progression and cell polarity. Our lab has shown that LGN is required for perpendicular divisions and proper epidermal stratification, but how and when the mitotic spindle responds to this cue remains unclear. Here, we seek to investigate how earlier mitotic events, such as centrosomal migration (prophase), spindle assembly (prometaphase), and spindle rotation (metaphase) lead to specific cell division fates in oriented cell divisions. Typically, in stem cell division, one centrosome (the younger “daughter”) migrates while the other centrosome older “mother” retains microtubule organizing center (MTOC) activity and remains anchored in place. Daughter centrosome migration establishes the initial “default” axis of division, which can be altered by a 90°spindle rotation during metaphase. Surprisingly, we found laterally positioned centrosomes during prometaphase, suggesting that both centrosomes actively migrate in ~50% of divisions. Since half of divisions are planar, we hypothesized that dual centrosome migration may occur in cells lacking LGN, whereas apical LGN anchors one centrosome to promote an apical-basal axis. Consistent with this, wild-type cells, with an apical LGN crescent showed increased of apical-basal centrosome positioning compared to cells without. However, knockdown of LGN, or other factors related factors such as Par3 and downstream NuMA, had no obvious impact on centrosome positioning during prometaphase. Thus, while LGN is sufficient to influence centrosome positioning, it is not required. This suggests that LGN may function later in mitosis and may play a role in “docking” spindles in an apical-basal orientation as they rotate during metaphase.

Investigating the Roles of Actin-Binding Proteins in Apical Constriction

Bailey de Jesus[1,2,4], Pu Zhang[1,2,4], Taylor Medwig-Kinney[1], Bob Goldstein[1,3]

1. Biology Department, University of North Carolina, Chapel Hill, NC
2. Curriculum in Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC
3. Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC
4. The authors contributed equally to this work

During development, cells must actively change shape and reorganize to form complex structures and tissues. Apical constriction is a common cell shape change driven by myosin contracting along actin filaments coupled to cell-cell junctions. This causes shrinkage of a cell's apical surface and folding of epithelial sheets, such as during gastrulation or neural tube closure. Apical constriction relies on the proper spatiotemporal organization of the actin cytoskeleton, which is regulated by actin-binding proteins (ABPs) with diverse functions, including nucleating, bundling, or severing filaments. However, the specific ways in which ABPs modulate the actin architecture that contributes to apical constriction remain unclear. Here, using *C. elegans* gastrulation as a model, we determined the localization dynamics of nearly every ABP expressed in early embryos. By examining patterns in localization, we have identified potential subcellular hubs of actin-regulatory activity. We found that the Ena/VASP ortholog UNC-34 works with WAVE-Arp2/3 to prevent membrane blebbing. Furthermore, we found that simultaneous loss of membrane linkers ERM-1 and FRM-1 causes blebs specifically in the apically constricting cells. Together our results support a localization-based approach for identifying modules of actin-regulatory activity that drive cell shape change. This work will provide insight into how the actin network is dynamically organized during development to produce morphogenetic changes.

Mechanisms of Collective Cell Migration during Epicardial Formation

Ashlyn Laidman[1-2], Kathryn Scherrer[2], Yogeshwari S. Ambekar[3], Giuliana Scarcelli[3], Michael Bressan[1-2]

1. Department of Cell Biology and Physiology, The University of North Carolina at Chapel Hill, NC
2. McAllister Heart Institute, The University of North Carolina at Chapel Hill, NC
3. Fischell Department of Bioengineering, University of Maryland, College Park, MD

The outer cell layer of the heart, the epicardium, plays important roles in cardiac function, repair, and is critical for normal heart development. The formation of the epicardium requires a unique developmental process during which a group of multipotent progenitor cells known as the proepicardium extend across an acellular space, bind to the heart, and then undergo a stereotyped process of directed cell migration. Currently, the cues that guide proepicardial cell migration are poorly understood. Therefore, I have examined a series of potential chemical and mechanical guidance cues and uncovered that a form of collective cell migration that follows stiffness gradients, or durotaxis, likely plays a significant role in controlling epicardial development. I have discovered a relative stiffness gradient in the myocardium that aligns with the direction of epicardial migration using Brillouin microscopy. In support of this, I have also found that the absolute stiffness of the inner curvature of the myocardium, the site of initial PE binding, is softer than the outer curvature, the direction of PE spreading, using nanoindentation. Finally, I have shown that PE cells migrate further on stiffer substrates by plating PE explants on increasingly stiff polyacrylamide gels. These findings lay the foundation for a durotaxis-mediated collective cell migration hypothesis for PE formation during cardiac development. These data suggest that a mechanics-based system of guidance cues controls epicardial formation, which likely determines the number and fates of epicardial-derived cells that contribute to different regions of the developing heart.

The Influence of Background Genetics on Susceptibility to Alcohol-Induced Birth Defects

Rowland, V. L.1,2, Wray, S. L.2, Parnell, S. E.1,2

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill
2. Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill

Prenatal alcohol exposure can cause a variety of birth defects including Fetal Alcohol Syndrome (FAS), which affects brain and craniofacial development. We aimed to explore the genetic risk of prenatal alcohol exposure by comparing the highly susceptible C57BL/6J (B6J) strain to the NOD/ShiLtJ (NOD) strain, which had not been characterized in response to prenatal alcohol exposure. We exposed embryonic day 7 (E7) NOD embryos to two doses of 2.9 g/kg or 3.4 g/kg ethanol 4 hours apart. These doses were chosen, as NOD mice had a lower peak blood alcohol concentration (BAC), following the 2.9 g/kg dose (372 mg/dl), compared to B6J mice (410.5 mg/dl). We increased the EtOH dose in NOD mice to relatively equalize peak BAC between strains (3.4 g/kg in NOD 419 mg/dl). Embryos were analyzed for facial defects on E14. Eye size was measured using ImageJ and Z scores were calculated based on measurements of the control group. Eyes were considered small if they had a Z score of □-2. In NOD mice at 2.9 g/kg EtOH, 3.8% of left eyes and 8.9% of right eyes were small. At 3.4 g/kg EtOH, the incidence of small eyes increased to 17.8% and 20.5% for the left and right eyes, respectively. Even at the higher 3.4 g/kg dose, the NOD mice are not as susceptible to eye defects as compared to the B6J strain. No NOD fetuses with an abnormal philtrum were found in the 2.9 g/kg group, however, one (1.4%) of the 3.4 g/kg fetuses had a smooth philtrum. The B6J mice had a higher incidence (5.9%) of smooth philtrum. Interestingly, the NOD mice at the 3.4 g/kg dose had a rate of exencephaly of 4.1%, which is a defect very rarely seen in the B6J strain with a similar BAC. Overall, our work finds strain differences between NOD/ShiLtJ and C57BL/6J in response to prenatal alcohol exposure, with the NOD mice being less susceptible to FAS-like facial defects, even when peak BACs were equalized through an increase in dose. This supports the idea that genetic variability alters susceptibility to prenatal alcohol exposure.

Folate Receptor β Orchestrates Tumor-Associated Macrophage Immunosuppressive Functions

Matthew P. Zimmerman[1,2], Vasyl Zhabotynsky[2], Alex G. Bastian[2], Haiyang Wang[2,3], Emily K. Cox[2], Amy G. Reynolds[2,4], Anna Ebacher[2,3], Wan Lin Chong[2], Katherine Vietor[2], Sergey A. Krupenko[5], Susan Sumner[5], Blake Rushing[5], Jessica E. Thaxton[1,2], Stergios Moschos[6], Willy Hugo[6], Brian C. Miller[2,4,6,8]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514
2. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514
3. Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514
4. Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514
5. Department of Nutrition, University of North Carolina at Chapel Hill, UNC Nutrition Research Institute, Kannapolis, NC 28081
6. Department of Medicine, Division of Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514
7. Department of Medicine, Division of Dermatology, University of California, Los Angeles, Los Angeles, CA 90095
8. Department of Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

The tumor microenvironment potentiates the immunosuppressive functions of tumor-associated macrophages (TAMs), dampening the anti-tumor immune response and promoting resistance to immune-checkpoint blockade (ICB). The investigation and development of therapeutic strategies targeting TAMs is crucially important to broadly improve patient outcomes across different kinds of cancer. Previous studies have identified folate receptor β (FRβ) as a biomarker of activated TAMs associated with worse patient prognosis. FRβ (gene: Folr2) mediates the endocytic uptake of extracellular folate. Folate is a requisite coenzyme for one-carbon (1C) metabolism, a pathway critical for the maintenance of cellular redox balance, nucleotide synthesis, and global methylation reactions. The importance of FRβ-mediated folate uptake on TAM biology is unknown. Here we show FRβ-mediated 1C metabolism supports pro-tumor macrophage functions through the control of reactive oxygen species (ROS). To interrogate mechanistic questions regarding the function of FRβ in TAMs, we engineered a novel Folr2-/- mouse model on the C57BL/6J genetic background. Folr2-/- mice exhibit decreased melanoma tumor growth, increased cytotoxicity of tumor-infiltrating CD8+ T-cells, and elevated immunostimulatory gene expression in TAMs relative to wild-type mice. Folr2-/- bone-marrow derived macrophages (BMDMs) produced more pro-inflammatory cytokines and possessed a decreased capacity to suppress T-cell activation compared to wild-type BMDMs in vitro. Interestingly, Folr2-/- BMDMs also generated increased ROS, were enriched for oxidative stress gene signatures and possessed decreased reduced glutathione metabolite pools, indicating a dysregulation of redox homeostasis. Furthermore, Folr2-/- BMDMs upregulate NF□B signaling that can be positively regulated by ROS accumulation. Inhibiting ROS via antioxidant supplementation reversed the pro-inflammatory phenotypes observed in Folr2-/- BMDMs. Together, these data suggest FRβ

Tubulin acetylation governs organelle remodeling and lysosomal reformation during neuronal differentiation

Chih-Hsuan Hsu[1], Alexander Josiah Kinrade[1], Maria Clara Zanellati[1,2], Sarah Cohen[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
2. Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

A functional nervous system depends on neuronal morphology established during differentiation. The microtubule (MT) cytoskeleton supports neuronal differentiation by organizing organelle positioning and facilitating transport. The dynamics and properties of MTs are regulated by a variety of post-translational modifications (PTMs), with many organelle interactions occurring preferentially on modified MTs. Here we find that tubulin acetylation is enriched at specific subcellular locations during differentiation of human induced neurons. We apply a quantitative multispectral imaging pipeline to simultaneously analyze eight membrane-bound organelles and define how tubulin acetylation reshapes organelle architecture and interaction networks during neuronal differentiation. We find that loss of tubulin acetylation broadly alters organelle morphology, spatial distribution, and inter-organelle interactions, with lysosome-organelle interactions most affected. Loss of acetylated MTs leads to enlarged, highly acidified lysosomes, impaired lysosomal fission, and accumulation of autolysosomes, consistent with defective lysosomal reformation. Super-resolution microscopy further reveals that lysosome-endoplasmic reticulum (ER) contacts preferentially associate with acetylated MTs. Together, our data support a model in which tubulin acetylation coordinates lysosome-ER interactions to facilitate lysosome remodeling and turnover. pThis work establishes tubulin acetylation as a key cytoskeletal regulator that links organelle interactions to organelle homeostasis important for neuronal differentiation.

Nickase Cas9 as a Gene Therapy for Angelman Syndrome

Leo Blondel[1], Hannah Bazick[1,2], Hanqian Mao[2], Mark Zylka[1,2]

1. Department of Cell Biology and Physiology, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599.
2. Neuroscience Center, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599.

Angelman Syndrome (AS) is a rare genetic neurodevelopmental disorder resulting from loss of function of the maternal *UBE3A* ubiquitin ligase gene, for which there exists no cure. In neurons, the paternal *UBE3A* allele is silenced through genomic imprinting by an antisense transcript called the *UBE3A-ATS*. As such, loss of the maternal allele results in a functional lack of neuronal *UBE3A* expression. Previous research has shown unsilencing of paternal *UBE3A* and behavioral rescue in AS mice following treatment with an adeno-associated viral vector with Cas9 targeting the *ATS.* However, it has become clear that AAV genomes integrate at the site of Cas9-induced double stranded breaks (DSBs). These integrations contribute to unsilencing but are random, uncontrollable, and unacceptable for human therapies. Cas9 DSBs may also result in increased genotoxicity *in vivo*. To avoid AAV integration, we are investigating the use of Cas9 nickases, which only induce cleavage of a single DNA strand. Previous research in the lab indicates that Nme1 Cas9 nickases targeting the non-template strand of the Ube3a-ATS led to greater unsilencing of *Ube3a* in mouse cortical neurons than active Cas9 targeting the same sequence. We hypothesize that nickase-mediated unsilencing results from increased formation of RNA-DNA hybrids known as R-loops near the site of editing, which can sterically hinder RNA polymerase. We aim to confirm that nickase Cas9 targeting the *ATS* unsilences *UBE3A* in human iNeurons, and subsequently show that this unsilencing is sufficient for phenotypic rescue in humanized AS mice.

APOE interacts with COX-2 on lipid droplets to modulate inflammatory lipid signaling.

Alex E Powers[1], Malvika Nair[1], Kajal Kamble[1], Ian A Windham[1], Venkata R Chirasani[2], Laura Herring[3], Gregory Miner[1], and Sarah Cohen[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599
2. The R. L. Juliano Structural Bioinformatics Core, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599
3. Michael Hooker Metabolomics and Proteomics Core, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599

Alzheimer's disease (AD) is the most common neurodegenerative disease and the global leading cause of dementia. The strongest genetic risk factor for the development of late-onset AD is the *APOE4* allele, which encodes for a variant of the secreted protein apolipoprotein E (APOE). APOE is highly expressed in astrocytes, glial cells that play important roles in brain lipid metabolism. Astrocytes store lipids in spherical organelles called lipid droplets (LDs). Neutral lipids such as triglycerides and sterol esters are stockpiled in the LD core, which is enveloped in a monolayer of phospholipids decorated by an extensive network of proteins. We previously discovered that apolipoprotein E (APOE) can escape secretion and instead traffic to cytoplasmic lipid droplets under conditions of lipid synthesis. In this study, our objective was to identify the function of APOE on lipid droplets in astrocytes and explore how this function is affected by disease-associated APOE4 expression. Affinity-purification proteomics of lipid droplet-localized APOE identified the prostaglandin synthase cyclooxygenase-2 (COX-2) as the top interaction partner of APOE in both humanized *APOE3* and *APOE4* astrocytes. Immunofluorescence upon knockdown of APOE by siRNA demonstrated that APOE3 recruits COX-2 to lipid droplets, while APOE4 fails to recruit COX-2 above APOE-knockdown levels. Computational modeling of APOE interaction with COX-2 on lipid interfaces revealed differential binding properties between the variants, with APOE4 interacting more strongly with COX-2 at the enzyme's active site. A fluorescence-based COX-2 activity further assay revealed that enzyme activity is reduced in APOE4-expressing astrocytes. Similarly, targeted lipidomics of arachidonic acid metabolites revealed reduced production of prostaglandins downstream of COX-2 in *APOE4* astrocytes. In summary, we identified that APOE interacts with COX-2 and modulates enzyme activity on lipid droplets. APOE4 reduces COX-2 activity by blocking the active site of the enzyme, effectively reducing the enzyme's ability to produce inflammatory signaling lipids such as prostaglandins. Prostaglandins regulate astrocyte reactivity, microglial functions, neuronal functions, and synaptic plasticity. Thus, inhibition of COX-2 by APOE4 has important implications for Alzheimer's disease.

Branched Actin Regulation of Endomembrane Compartments

Michelle F Marchan[1,2], Mitchell T Butler[1,2], Max A Hockenberry[1], James E Bear[1,2]

1. Department of Cell Biology and Physiology, University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC 27599
2. University of North Carolina Lineberger Comprehensive Cancer Center, University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC

Cellular membrane trafficking is critical for cell function: regulating processes such as cargo sorting and molecular transport, degradation, secretion, and response to pathogens. Branched actin localizes to a wide variety of intracellular membrane surfaces, here referred to as endomembranes, where it operates as a cellular force generator and membrane insulator. We hypothesize that both polymerization and depolymerization of branched actin regulate endomembrane trafficking and maturation. To elucidate the role of actin branch assembly and disassembly at endomembranes, we first documented the colocalization of Arp2/3-branched actin with various endomembrane compartments. For these experiments, we are using a mouse dermal fibroblast cell line that has an endogenously-labeled essential subunit of the Arp2/3 complex, Arpc2, which can also be conditionally deleted. Using this system, we are also evaluating organelle morphology and distribution in the presence and absence of the Arp2/3 complex. Upon genetic depletion of Arpc2, we observe a reduction in F-actin puncta and enlargement and perinuclear accumulation of early endosomes. These results suggest that F-actin puncta are largely comprised of branched actin and support the involvement of branched actin in the sorting and scission of early endosomes. Additional roles of branched actin in membrane trafficking were evaluated by labeling additional compartments, such as lysosomes and mitochondria. Finally, the results of complete deletion of Arp2/3 will be compared to the phenotypes observed upon depleting Arp2/3 activator WASH. Understanding the roles and regulation of actin-dependent membrane trafficking within and between organelles is valuable for understanding homeostatic cell function and disease states with underlying alterations in membrane trafficking, such as tumor metastasis, viral infection, and protein processing disorders.

X-inactivation Escapee *Ddx3x* Enhances Translation Initiation of mRNAs Essential for Female Heart Function

Kayla K. Mason[1], Xingren Wang[1], Frank L. Conlon[1]

1. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC 27599, USA.

Sex differences in heart disease prevalence, severity, and outcomes are well established, yet underlying mechanisms remain unclear. These differences are often attributed exclusively to sex hormones. Through a series of genetic screens, we demonstrated that in addition to sex hormones, X-linked gene dosage can create cell-intrinsic effects on cardiac sex differences and function. While the long non-coding RNA (lncRNA) Xist typically inactivates one X chromosome (XCI) in female cells to balance gene dosage with male cells, we have identified the X-linked genes that escape XCI in cardiomyocytes (CMs). One such gene, the RNA helicase DEAD-box helicase 3, X-linked (*Ddx3x*), is a candidate regulator of sex chromosome-dependent differences in the mammalian heart. Consistently, heterozygous point mutations in *DDX3X* cause congenital heart disease (CHD) predominantly in females, highlighting its dosage-sensitivity in humans. To define DDX3X function in CMs, we generated a *Ddx3x* CM conditional allele in mice. Using Enhanced Crosslinking and Immunoprecipitation (eCLIP) in parallel with ribosome profiling, we found that DDX3X enhances translation initiation of mRNAs with structured 5’ UTRs in female CMs. Several of these targets encode proteins required for electrical signaling and contractility in CMs. While homozygous loss of *Ddx3x* in female CMs results in embryonic lethality, female heterozygous and male hemizygous mice are viable. Strikingly, heterozygous females exhibit reduced left ventricular contractile function compared to hemizygous males, indicating a sex-differential susceptibility to contractile dysfunction and heart failure. Collectively, these findings suggest that *Ddx3x* is highly dosage sensitive, and escape from XCI is sufficient to establish an RNA binding protein-mediated translational program underlying sex-specific cardiac phenotypes. These studies will provide insight into the basis for both cardiac sex differences and potential clinical relevance for DDX3X-associated CHD patients.

Hemidesmosomes and Notch signaling regulate epidermal differentiation during Embryogenesis

Juliet King[1,2], Kendall Lough, Ph.D[2]., Scott Williams, Ph.D[2]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
2. Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Epidermal stratification is achieved through careful regulation of basal keratinocyte differentiation and is critical to the skin's function as an environmental barrier. Basal epidermal progenitors adhere to the underlying basement membrane through adhesion complexes like the hemidesmosome, which links the keratin cytoskeleton to the basement membrane through integrin-α6β4 heterodimers. It has been proposed that high expression of integrins is a key component of basal residency, and thus progenitor capacity. However, there is limited evidence that integrins directly contribute to basal identity or play a role in differentiation programs. To address this, we use an *in utero* lentiviral knockdown to generate epidermal-specific loss of integrin-β4 and its ligand, laminin-α3β3γ2. We find that *Itgb4* and *Lama3* knockdown cells are biased towards a differentiative fate, while *Scramble* controls display equipotent clonal expansion. To determine how hemidesmosome loss biases progenitor cells towards differentiation, we evaluated the two differentiative mechanisms: 1) asymmetric cell division, where one daughter cell is displaced into the differentiated layer following mitosis, and 2) delamination, where basal keratinocytes lose contact with the basement membrane and upregulate a differentiative identity independent of division. Our results indicate that reduced basal adhesion predominantly drives differentiation by promoting delamination, with only mild effects on oriented cell divisions. Furthermore, whole epidermal overexpression of the pro-differentiative Notch1 pathway abrogates integrin-β4 expression, with a similar increase in delamination, while a reduction in Notch activation through knockdown of its effector protein, *Rbpj*, shows minimal delamination. These findings demonstrate that hemidesmosomes directly regulate epidermal differentiation, predominantly by promoting delamination, and suggest that canonical differentiative programs may suppress hemidesmosome adhesion as a natural part ofepidermal stratification.

Palmitoylation dynamics regulate mitochondrial function and redox homeostasis in embryonic cardiomyocytes

Matheus Sadovsky[1], Gregory Miner[1,2], Michael Forrester[3], Whitney Edwards[1,2]

1. 1Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2. McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
3. Division of Pulmonary, Allergy and Critical Care Medicine, Duke University School of Medicine, Durham, NC 27710

Congenital heart disease (CHD) remains the most prevalent birth defect in the United States and is a major contributor to infant mortality and healthcare costs, yet most causes of CHD are not known. Cardiac morphogenesis requires tightly coordinated mitochondrial maturation and mitochondrial dysfunction has been strongly implicated in CHD pathogenesis. However, the upstream regulatory mechanisms governing mitochondrial maturation during cardiac development remain unclear. Notably, studies have linked protein palmitoylation to mitochondrial dynamics and function. S-palmitoylation is characterized by the reversible attachment of palmitic acid to proteins and dynamically regulates protein localization, function, and membrane association. Proteomic analysis showed palmitoylating and de-palmitoylating enzymes that are directly involved in mitochondrial function are abundantly expressed and dynamically regulated during murine heart development. In this project, we aim to determine how palmitoylation dynamics regulate mitochondrial metabolic function and redox homeostasis in embryonic cardiomyocytes. To identify palmitoylated proteins in the developing heart, we performed acyl-resin assisted capture (acyl-RAC) coupled with LC-MS/MS on embryonic mouse hearts. We identified over 700 unique proteins, where 88 (12%) were mitochondrial. Proteomic analysis revealed significant enrichment of bioenergetic pathways, including components of the TCA cycle, respiratory chain, fatty acid oxidation, and metabolite transporters. Moreover, pharmacological inhibition of palmitoylation dynamics in embryonic cardiomyocytes resulted in impaired mitochondrial respiration and reactive oxygen species (ROS) accumulation. Together, these findings suggest that palmitoylation dynamics serve as a regulator of mitochondrial metabolism and redox homeostasis in embryonic cardiomyocytes. Ongoing studies aim to identify specific palmitoyl-transferases mediating these processes and define their role in cardiac development.

iPSC-derived human intestinal organoids to investigate gastrointestinal pathophysiology in cystic fibrosis

Hailey Dodson[1], Advika Sampathkumar1, Saanvi Gupte [1], Samara Williams [1], Sparsh Patel [1], Anne Marie Riedman [1], Deborah M. Cholon [2], Martina Gentzsch [2], Camille Ehre [2], David Hill [2], Heather A. McCauley[1]

1. Cell Biology and Physiology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, USA
2. Marsico Lung Institute, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, USA

People with cystic fibrosis (CF) can experience diverse gastrointestinal (GI) manifestations beginning at birth, including impaired nutrient absorption, intestinal obstruction and increased colon cancer risk. These complications are insufficiently improved by modulator therapies and understudied compared to pulmonary disease. Progress has been limited by the absence of models that accurately recapitulate the full diversity of intestinal epithelial cell types and their interactions with the mucus barrier. We use induced pluripotent stem cell (iPSC)-derived human intestinal organoids (HIOs) to address this gap and enable analysis of epithelial lineage dynamics, cell-cell interactions, and the epithelium-mucus interface.

Using iPSCs carrying CFTR mutations (F508del, G542X, and W1282X), we generated HIOs and transplanted them into immunocompromised mice for maturation. All transplanted organoids (tHIOs) developed the expected small intestine crypt-villus architecture. Single-cell RNA sequencing of tHIOs confirmed the presence of all major epithelial cell types but revealed shifts in the epithelial lineage proportions. Both F508del and W1282X mutants showed a reduction in the proportion of stem and proliferative cells accompanied by an expansion of secretory cells proportions. Histological quantification demonstrated increased goblet cell numbers in G542X and W1282X tHIOs, while F508del tHIOs exhibited more goblet cells containing acidic mucins. Immunofluorescence for MUC2, the dominant intestinal mucin, showed intracellular retention and reduced luminal secretion across all CF genotypes. WGA staining corroborated accumulation of glycoproteins along the crypt–villus axis. Luminal contents from CF tHIOs displayed elevated percent solids, indicating an increased mucus concentration.

Although CFTR dysfunction does not disrupt gross intestinal morphology, it profoundly alters epithelial proportions and mucus properties. Secretory lineage bias, goblet cell expansion, and abnormal mucin chemistry collectively alter the mucus barrier. These findings highlight epithelium-mucus interface abnormalities that may contribute to persistent GI complications in CF.

The Bifunctional Nature of Actin Capping Protein in Cell Division

Henry I. Uchenna[1,2,] Kelly Wan[2], Gerald A. Edwards[2], Michael E. Werner[2], Paul Maddox[2], Amy S. Maddox[1,2]

1. Curriculum in Cell biology and Physiology, Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC
2. Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC

Force generation by the actomyosin and microtubule-based cytoskeletons is crucial for proper cell division, tissue morphogenesis, and embryonic development. During mitosis, cortical actomyosin determines cell shape and drives shape changes; microtubules build and position the spindle and segregate the chromosomes. While the actomyosin and microtubule organize into distinct cellular structures, they are functionally linked via cross-talking regulation and structural coordination in ways that are not fully understood. To better understand how these force-generating systems are coordinated, we investigated the role of capping protein, a conserved actin-binding heterodimer that limit actin polymerization by capping the barbed end. Capping protein also contributes to the microtubule cytoskeleton as a component of the dynactin complex, where it stabilizes the Arp1 filament backbone and promotes dynein-mediated transport. The *Caenorhabditis elegans* zygote provides a powerful model to dissect the roles of conserved proteins in the actomyosin and dynein–microtubule cytoskeletons during successive mitotic events. We used RNAi to deplete both subunits of capping protein from the *C. elegans* zygote. Depletion of capping protein accelerated cytokinetic furrowing, consistent with its known role in shaping actomyosin-driven contractility and network architecture. In addition, capping protein depletion led to previously unreported defects in pronuclear migration, centrosome and bipolar spindle organization, and chromosome segregation, phenotypes reminiscent of dynein loss-of-function. Since dynein is essential for cortical pulling forces that drive spindle positioning and dynamics, our findings suggest that capping proteins influence dynein function, likely via its known structural role in the dynactin complex, a key dynein regulator. These results reveal that capping protein regulates both the actin and microtubule cytoskeletal systems during cell division. Future exploration of how the pool of capping protein heterodimers is distributed between actin and dynactin will reveal how contractility and microtubule dynamics are coordinated during cell division and development.

Genetic Regulation of Fetal Alcohol Syndrome: Evaluation of Phenotypic Variations Utilizing a Multigenerational Mouse Model System

Taraneh Sadritabrizi[1,2], Eric W. Fish[2], Olivia R. Iessi[2], Clayton R. Adams[2], Scott E. Parnell[1,2]

1. Department of Cell Biology and Physiology, UNC Chapel Hill
2. Bowles Center for Alcohol Studies, UNC Chapel Hill

Prenatal alcohol exposure (PAE) is one of the most common preventable causes of birth defects, and a known cause of fetal alcohol syndrome (FAS). PAE during the gastrulation stage of early pregnancy can result in impaired development of the brain, as well as severe craniofacial malformations. Key craniofacial dysmorphology of FAS includes small eyes, a smooth philtrum, and a thin upper lip. However, a wide range of phenotypic outcomes can occur because of PAE. Although there are underlying genetic mechanisms that account for this range, they are not well-characterized. Using an established gastrulation-stage PAE mouse model, we found that the 129S1/SvImJ (129S1) and C57BL/6J (B6J) collaborative cross founder inbred strains exhibit opposing outcomes to PAE. The B6J strain is susceptible to PAE and the 129S1 strain is completely resistant. We generated reciprocal matings between the two strains and then backcrossed their hybrid offspring back onto the B6J inbred strain over the course of six generations (N6), each with a progressive increase of B6J strain alleles. We have found thus far that fetuses derived from a B6J dam cross type are approaching similar levels of ocular defects (~37% in N5 and ~40% in N6) to that of the B6J inbred strain (~40%). From these data, we have also found that female fetuses are more often and more severely affected by PAE than males, a difference that is not observed in the B6J inbred strain. Female fetuses of the same B6J dam cross type are accruing an incidence of overall ocular malformations of ~60%, while males have an incidence of ~22%. Within the same cross, male fetuses of N5-N6 had incidences of severe ocular malformations of ~13% and ~20% respectively, while female fetuses had incidences of ~44% in N5 and ~39% in N6. Our findings indicate both a novel sex-effect and a pattern of genetic influence conferring susceptibility to PAE, which has aided us in narrowing down potential genes of interest contributing to phenotypic severity.

Characterizing a rodent, skeletal muscle-specific microexon in clathrin heavy chain (Cltc) during myogenesis.

R. Eric Blue, Adam J. Black, Sumaya A. Addish, Jessica L. Cote, Jimena Giudice

1. Cell Biology & Physiology Department, University of North Carolina at Chapel Hill, N.C.

Clathrin proteins assemble to coat vesicles and traffic cellular cargo between membrane bound compartments in eukaryotic cells. Humans express two genes encoding the clathrin heavy chain protein, *CLTC* and *CLTCL1*. While CLTC is ubiquitously expressed and heavily involved in intracellular trafficking, *CLTCL1* is skeletal muscle specific and primarily regulates glucose homeostasis through GLUT4-mediated transport. In mice and rats, *CLTCL1* is a non-functional pseudogene. The Giudice lab has previously shown that *Cltc* harbors an alternatively spliced microexon of 21 nucleotides (exon 31) that is striated muscle specific and developmentally regulated in mice and is evolutionarily conserved. Here, we introduce a previously unreported alternatively spliced microexon of 38 nucleotides (exon 33) within *Cltc*, specific to mouse and rat skeletal muscle. While humans have achieved functional diversity of clathrin heavy chain proteins through gene a gene duplication event, mice and rats have achieved this through tissue-specific alternative splicing. We hypothesize the peptides encoded by *Cltc* exon 33 in rodent skeletal muscle play a similar functional role as *CLTCL1* in human skeletal muscle.

Alzheimer's Associated Primary Cilia Changes In Hippocampal Interneurons

[1,2]Kavya Balasubramanian, [3]Guillermina Rentsch, [1]Shahzad Khan

1. Department of Neurology, UNC-Chapel Hill, Chapel Hill, NC 27599
2. Cell Biology and Physiology Graduate Curriculum, UNC-Chapel Hill, Chapel Hill, NC 27599
3. International Graduate Program Medical Neurosciences, Berlin, GER

The primary cilium (PC) is a microtubule-based organelle emanating from the neuronal cell surface that senses environmental cues and transduces key intracellular signaling pathways important for neuronal homeostasis. Importantly, neuronal PC are dynamic, changing in length or GPCR composition in response to pathological or physiological stimuli. Mouse models of Alzheimer's disease (AD) demonstrate altered PC structure and composition in hippocampal neurons. However, these changes have not been mapped to neuron subtypes that are uniquely vulnerable to AD pathology, such as hippocampal GAD1+ inhibitory interneurons. To address this gap, we analyzed hippocampal GAD1+ interneuron ciliary length and percent ciliation in 5xFAD mice that harbor three mutations in the amyloid precursor protein (APP) and two mutations in presenilin 1 (PSEN1). We specifically assessed GAD1+ interneurons in the CA1 hippocampal subregion, as this is an early site of AD pathology. In 5xFAD mice, GAD1+ interneurons in the CA1 exhibit significant PC shortening without a change in percent ciliation at 6 months, a timepoint when AD-associated learning and memory deficits and early pathology emerge. To determine disease relevance, our future work will examine whether GAD1+ interneuron cilia display dynamic changes across disease progression in 5xFAD mice. Structural alterations in PC may represent changes in ciliary protein composition. To begin assessing the functional mechanisms of cilia shortening, we will also characterize changes in GAD1+ neuron G-protein coupled receptors that uniquely localize to neuronal primary cilia.

Mechanical stretch induces changes in CLK1 and SR protein functions in myotubes

Gabrielle B Bais[1,2,3], Emma R Hinkle[2,4], R Eric Blue[2], Matthew Combs[5], Joan M Taylor[5,6], Jimena Giudice[2,4,6]

1. Curriculum in Cell Biology and Physiology, University of North Carolina at Chapel Hill (UNC-CH).
2. Department of Cell Biology and Physiology, UNC-CH.
3. Integrative Vascular Biology Training Program, UNC-CH.
4. Curriculum in Genetics and Molecular Biology, UNC-CH.
5. Department of Pathology and Laboratory Medicine, UNC-CH.
6. McAllister Heart Institute, UNC-CH.

Alternative splicing is an RNA processing mechanism that allows single genes to produce multiple mRNA transcripts. Global changes in alternative splicing are associated with tissue development, cellular stress, and disease state. Mechanotransduction refers to the ability of cells to sense and respond to mechanical stimuli from their physical environment through signaling pathways and changes in gene expression—which is particularly important in myocytes because they are constantly generating and responding to mechanical forces. Recently, our group and others have shown that cellular exposure to mechanical stimuli can induce changes in alternative splicing and phosphorylation of splicing factors. More narrowly, our lab demonstrated that cyclic mechanical stretch of skeletal muscle cells induces global changes in transcription and alternative splicing. Several differentially expressed and spliced transcripts identified in our deep RNA-sequencing study were targets of the serine/arginine rich splicing factor 4 (SRSF4). CDC2-like kinase 1 (CLK1) regulates the subcellular localization of serine/arginine-rich (SR) proteins and their splicing regulatory functions via phosphorylation. Interestingly, Clk1 pre-mRNA is itself a splicing target of SR proteins. Therefore, here, we investigate the interplay between CLK1 kinase production and activity, SR protein phosphorylation, and mechanical stretch in muscle cells. We found that mechanical stretching of muscle cells led to an increased production of functional Clk1 transcripts and increased SRSF4 phosphorylation. Inhibition of CLK1 kinase activity (TG003) resulted in an increase in CLK1 mRNA and protein levels and a decrease in SRSF4 phosphorylation. These findings suggest that CLK1 phosphorylation of SRSF4 regulates the production of functional CLK1 protein via a feedback loop that connects mechanical stretching, with CLK1 splicing regulation by phosphorylated SRSF4. The function of SRSF4 as a mechanically sensitive RNA-binding protein that is regulated by CLK1 expands the current understanding of the intricate interplay between mechanotransduction and alternative splicing.

Mechanism of Intracellular ANGPTL3 and ANGPTL8 Trafficking

Christina So[1], Mary Bray Mann[2], Uriel Jean-Baptiste[2], Simcha Singh[2], Saskia Neher[2]

1. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC
2. Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC

Lipids circulate in the blood in lipoproteins including chylomicrons and very low-density lipoproteins (VLDLs). Lipoprotein Lipase (LPL) is the main enzyme that hydrolyzes the triglycerides from circulating lipoproteins into free fatty acids that can be taken up by cells. Without LPL, dangerously high levels of lipoproteins circulate in the blood, which can lead to cardiovascular disease. LPL inhibitors, known as angiopoietin-like (ANGPTL) proteins, have key roles in the regulation of lipid metabolism. ANGPTL3 is a potent inhibitor of LPL. ANGPTL8 can form a complex with ANGPTL3 which results in more efficient secretion of ANGPTL8 and greater inhibition of LPL than ANGPTL3 alone. Based on immunofluorescence staining of LAMP1 (lysosomal-associated membrane protein 1), I have found that ANGPTL8 when expressed alone, gets trafficked toward lysosomes. However, when ANGPTL8 is co-expressed with ANGPTL3, both ANGPTL3 and ANGPTL8 are effectively trafficked out of the cell. Using the Retention Using Selective Hooks (RUSH) assay, I confirmed that a population of ANGPTL8, when expressed alone, gets trafficked to lysosomes. I have also found that ANGPTL3 and ANGPTL8 are synthesized and form a complex in the endoplasmic reticulum (ER) and get trafficked out of the cell together. Thus, ANGPTL3 diverts a pool of ANGPTL8 from lysosomal degradation. Future goals include understanding why ANGPTL8 is unstable when it is not in complex with ANGPTL3, as well as visualizing the trafficking dynamics of the ANGPTL3/8 complex.

Characterizing the Role of the Vinculin Isoform, Metavinculin in Force Transmission

Jocelyn V Alvarado[1], James Bear, Sharon Campbell[1,2]

1. Dept. of Cell Biology and Physiology
2. Dept. of Biochemistry and Biophysics

Vinculin (Vcn) and its larger splice isoform, Metavinculin (MVcn), are core adhesion proteins that link the actin cytoskeleton to the cell periphery to modulate adhesion formation, strength, and motility. Vcn, a well-characterized mechanosensory protein, tightly links filamentous actin (F-actin) into bundles or stress fibers, which are key to maintaining adhesion strength and force transmission. MVcn is limited to smooth muscle and cardiac tissue and is expressed sub-stoichiometrically compared to Vcn. Yet MVcn's role in force transmission, cell morphology, and adhesion formation is understudied. Notably, patients with MVcn-specific mutations show defects in actin organization, cell morphology, and development of the often-fatal group of cardiac diseases, cardiomyopathies (CM). While both Vcn and MVcn bind F-actin, MVcn lacks F-actin bundling capabilities. When increasing amounts of the actin-binding tail domain of MVcn are mixed with Vcn *in vitro,* actin-bundling progressively diminishes. These findings suggest that MVcn may negatively regulate F-actin bundling when co-expressed with Vcn. Moreover, MVcn CM mutations promote higher-order yet more disordered F-actin assembles. **Thus, I hypothesize that MVcn acts as a negative regulator of Vcn to fine-tune cell morphology and force transmission**. To understand how the stoichiometric relationship between the isoforms affects actin-associated functions, I will utilize murine embryonic fibroblasts that conditionally express mScarlet tagged MVcn. Under differing doxycycline concentrations, I can mimic the endogenous sub-stoichiometric expression of MVcn to test differences in actin organization, motility, and focal adhesion characteristics by subjecting cells to fluorescent confocal microscopy, random motility assays and 2D traction force experiments. Additionally, I will repeat these experiments in fibroblasts expressing the R975W MVcn CM mutant that has been previously shown perturbation to higher order actin structures. Results will be shown.

TRIM9 switches melanoma cell morphological phenotypes

Kimberly Lukasik[1], Aneri B. Shah[1], Chris T. Ho[1], Matthew Li[1], G. Becky Patrick[1], Jordan Brooks[1], Simon Rothenfusser[2], James E. Bear[1,3], Stephanie L. Gupton[1,3,4]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2. Institute of Clinical Pharmacology, LMU University Hospital, LMU Munich, Munich, Germany
3. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
4. Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Melanoma is a highly plastic cancer characterized by distinct cellular phenotypes associated with broadly unique gene expression profiles. TRIM9 is a brain-enriched E3 ubiquitin ligase detected in melanoma, but how TRIM9 expression regulates melanoma phenotype is unknown. Using two metastatic human melanoma cell lines and a mouse melanoma model, we found that TRIM9 promoted melanoma proliferation and altered cell morphology. In cell lines, TRIM9 promoted cellular blebbing and negatively regulated adhesion, secretion, and mesenchymal motility. TRIM9 interacted with VASP in melanoma cells, altering VASP modification, localization, and dynamics. In the absence of TRIM9, cells had an altered actin organization and more focal adhesions, where VASP accumulated and exhibited rapid turnover. We find the alterations in actin architecture and adhesion associated with TRIM9 deletion were coincident with increased motile and contractile mesenchymal behavior in vitro. In vivo loss of TRIM9 in melanoma slowed tumor growth and altered metastasis frequency, size, and destination. Our findings indicate TRIM9 alters the proliferative and morphological phenotypes of metastatic melanoma cells to influence disease progression.

Calcium regulated plasma membrane expansion and remodeling during neuronal morphogenesis

Samantha E. Ryken[1], Ellen C. O'Shaughnessy[1], Nandan Kiran[1], David Adalsteinsson[2], Stephanie L. Gupton[1]

1. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC 27599
2. Department of Mathematics, University of North Carolina, Chapel Hill, NC 27599

The plasma membrane (PM) of a developing neuron undergoes dramatic expansion and remodeling to establish the complex, elongated morphology necessary for neural network connectivity and function. New membrane material is inserted into the expanding PM via SNARE-mediated exocytosis. Two SNARE proteins, vesicle associated membrane protein (VAMP) 2 and VAMP7 are enriched in the embryonic brain and associated with distinct vesicle populations. Both are required for proper neuronal morphogenesis, but the mechanisms regulating when and where these vesicle pools fuse during development are not known. Here I utilize TIRF microscopy and automated image analysis to investigate PM expansion and remodeling in developing neurons. I show that VAMP2 and VAMP7 mediate non-synaptic exocytic events that spatially cluster in distinct areas of the developing neuron, suggesting regulatory pathways dictating their distribution and fusion. I found that VAMP2-mediated exocytosis is sensitive to Ca^{2+} chelation, whereas VAMP7-mediated exocytosis is not, mirroring their differential Ca^{2+} sensitivity at the synapse in mature neurons. This suggests a potential role for Ca^{2+} signaling in the regulation of VAMP2-mediated exocytosis during development. To investigate the source of Ca^{2+} signaling, I examined Endoplasmic reticulum (ER)-PM membrane contact sites, key regulators of Ca^{2+} dynamics. These close interfaces between the ER and the PM are uniquely positioned to be potential regulators of exocytosis through Ca^{2+} signaling and could also influence PM remodeling through non-vesicular lipid transfer. I found that ER-PM contacts in developing neurons are dynamic and form in the soma, neurites, and growth cone. ER-PM contacts are spatially clustered in the soma and colocalize with regions of elevated cytosolic Ca^{2+}. Additionally, VAMP2-mediated exocytic events occur significantly closer to ER-PM contact sites than spatially randomized simulations, suggesting that VAMP2-mediated exocytosis is spatially coordinated with ER-PM contacts. Taken together, these results suggest that VAMP2-mediated exocytosis is regulated by local Ca^{2+} transients and that ER-PM contact sites act as signaling hubs and spatial organizers of VAMP2-mediated membrane fusion during neuronal development.

Interrogating the role of vascular smooth muscle cells in guiding kidney innervation and development

Jake Roetcisoender, Pierre N'Guetta, Samuel Honeycutt, Lori O'Brien

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC

End-stage renal disease has only two treatment options: dialysis or transplant, highlighting a critical need for alternative therapies. This necessitates a thorough understanding of the developing vasculature and how it interacts with both the nerves and the kidney during development for alternative regenerative therapeutic strategies. Despite the importance of innervation and vascularization for proper kidney development, there is still little known about how signaling between the nerves and the vasculature influences kidney organogenesis. To interrogate this, we use iDISCO+ tissue clearing with light-sheet microscopy to elucidate the mechanisms of neurovascular development in the kidney. Developing nerves are tightly associated with vascular smooth muscle cells (VSMCs) throughout development. Our lab also found that loss of nerves decreases kidney glomerular number and increases tubule diameter, indicative of impaired nephron development. Taken together, we hypothesize that VSMCs play a critical role in guiding nerves to their appropriate location which is required for sufficient nephrogenesis. We have used a SMC-specific and SMC-progenitor Cre mouse models paired with a diphtheria toxin (DTA) allele to assess the impact of ablating VSMCs on kidney development. Our preliminary results suggest that VSMC ablation after the establishment of innervation alters nerve patterning in the kidney, and ablation before the start of innervation may impair early innervation. This suggests a key role for VSMCs in guiding proper innervation of the kidney throughout development. Future studies will continue using this VSMC ablation model to their contribution to normal kidney development and innervation.

The ARP2/3 Complex Regulation of Clathrin Dynamics is Induced through ACK

Mark A. Hazelbaker[1], Dennis Michalak[2], Jordan R. Beach[3], Justin W. Taraska[2], James E. Bear[1]

1. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC
2. National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD
3. Department of Cell and Molecular Physiology, Stritch School of Medicine, Loyola University, Chicago, IL

Clathrin-mediated endocytosis (CME) is an important cellular process that plays a role in cell-cell communication, signaling, and migration. In addition to the canonical clathrin coated pits, clathrin can form flat lattice structures on the membrane. Previously, Arp2/3 has been shown to be involved in the breakdown of large flat clathrin lattices which had formed while branched actin formation was transiently inhibited. Consistent with this finding, we can induce accumulation of large, long-lived clathrin structures in mouse fibroblasts (JR20 cells) via conditional knockout of *Arpc2*. However, platinum replica transmission electron microscopy images of unroofed Arpc2 null cells reveal that these structures are a heterogenous network of large flat clathrin lattices and arrested curved clathrin structures not observed in control cells. Identifying factors involved in the regulation of clathrin dynamics is an area of ongoing research. Our JR20 cells present an interesting model to study this pathway because *Arpc2* KO cells form large, highly stable clathrin structures, which we refer to as Large Arrested Clathrin Lattices (LACLs), which parental JR20 cells do not readily maintain under typical experimental conditions. Phospho-proteomic data comparing *Arpc2* KO and control JR20 cells revealed that many CME-related proteins have increased levels of tyrosine-phosphorylation upon *Arpc2* deletion. Immunofluorescence shows that LACLs co-stain with a pan-phospho-tyrosine antibody. Alternatively, LACLs which form naturally in ITGB1 null GD25 cells do not costain for phospho-tyrosine. This suggests the tyrosine phosphorylation drives ARP2/3 branched actin LACL resolution. Based on the phospho-proteomic data, we have identified activated-Cdc42 kinase (ACK), a non-receptor tyrosine kinase, as a potential LACL regulator upstream of Arp2/3 activation. Exogenously expressed mScarlet-tagged WT ACK shows preferential accumulation on LACLs over pits when imaged via TIRF microscopy. Additionally, ACK KO, small molecule inhibition, or overexpression of a kinase dead mutant results in increased LACL accumulation in JR20 cells. Together, these data provide an interesting potential pathway wherein ACK within LACLs, if activated, stimulates Arp2/3 branched actin activity and LACL resolution via its kinase activity.

Investigating neuroimmune signatures of peripheral neurodegeneration

Mady Chlebowski[1,2], Ethan Bedsole[1], Jinglun Wang[1], Ed Hirata[4], Allyson Ryan[4], Talon Chandler[4], Shalin Mehta[4], and Celia E. Shiau[1,2,3]

1. Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
2. Department of Cell Biology & Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
3. Department of Microbiology & Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
4. Chan Zuckerburg Biohub San Fransico, San Fransico, CA 94158

Lysosomal dysfunction is a central feature of neurodegenerative diseases, yet its role has largely been focused on neurons, overlooking contributions from the surrounding tissue microenvironment. Here, we leverage a novel loss-of-function mutation in *atp6ap2*, a lysosomal vacuolar ATPase accessory protein, to investigate how impaired lysosomal acidification impacts neurons and interacting cell types in vivo. Using the zebrafish posterior lateral line (PLL) – a tractable, conserved mechanosensory system that enables high-resolution imaging across scales – we model peripheral neurodegeneration in a living vertebrate. Because of its superficial location and axons that span almost the entire length of the zebrafish body, the PLL is highly accessible for imaging intra-neuron dynamics in detail. We show that *atp6ap2* mutants exhibit progressive degeneration of axons, cell bodies, and axonal terminals. Notably, macrophage accumulation around PLL ganglia precedes overt structural defects, suggesting early neuroimmune crosstalk. Moreover, neurons in *atp6p2* mutants display defective lysosomal acidification and impaired lysosomal transport. Degeneration is further accompanied by loss of neuromast hair cell organization, highlighting downstream effects on neuronal targets. To dissect cell-type-specific contributions, we are developing multiplex CRISPR approaches for targeted *atp6ap2* perturbation alongside cell-specific rescue strategies. In parallel, we are implementing machine learning–based, label-free imaging pipelines for large-scale phenotyping of neuromasts, the innervation targets of PLL, as an effective means to delineate the impact of cell-type-specific manipulations on PLL degeneration. Together, this work establishes a systems-level framework to study lysosomal dysfunction in peripheral neurodegeneration, with particular emphasis on early macrophage–neuron interactions that may serve as indicators for detection and intervention of neurodegenerative disease.

Adrenomedullin enhances endometrial receptivity: insights into cytoskeletal reorganization and pinopode formation

Anna L.S. Beeson[1], Margeaux W. Marbrey[2], Meredith F. Gillis[1], Kathleen M. Caron[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.
2. Department of Obstetrics and Gynecology, Duke University, Durham, NC 27710.

Failure of a healthy blastocyst to implant in a receptive endometrium accounts for a significant portion of early pregnancy losses. Despite this barrier to fertility therapeutics, mechanisms governing endometrial receptivity remain poorly understood. We previously found that in mice, intrauterine administration of adrenomedullin (AM) before blastocyst transfer improved implantation rates and increased pinopode size and uterine surface coverage. Maternal haploinsufficiency for AM led to decreased uterine receptivity, evident in subfertility phenotypes and reduced epithelial pinopode coverage. To identify genes differentially expressed upon AM treatment, we treated mice expressing uterine epithelium-specific RiboTag with either AM or vehicle and compared the ribosome-associated mRNA profiles. Among the top 35 up- and down-regulated genes ($p<0.05$, 1-way ANOVA Bonferroni correction), we observed significant changes in many genes with well-documented roles in cytoskeletal reorganization and actin-myosin dynamics such as *Fermt1*, *Ank3*, *Tubb3*, and *Arpc1a*. Preliminary results show that treating human endometrial epithelial-like Ishikawa cells with AM trends towards peripheral F-actin arrangement ($p=0.06$, 1-way ANOVA Tukey correction), consistent with AM-induced cytoskeletal rearrangement. We are now using murine endometrial organoids to study AM-induced pinopode formation in vitro. Scanning electron microscopy reveals that endometrial organoids treated with 17β-estradiol and medroxyprogesterone acetate display many features seen in mouse endometrial tissue biopsies, including microvilli, cilia, distinct cell borders, and pinopodes. Importantly, mouse endometrial organoids treated with AM display increase pinopode number, size, and epithelial surface coverage, similarly to our in vivo findings. Further studies will use this powerful in vitro technique to explore the cellular mechanisms by which AM induces pinopode formation, focusing on cytoskeletal regulators.

Sublethal DNA Damage in Neurons: Is the Apoptotic Pathway Even Activated?

Nicole Hondrogiannis[1], Mohanish Deshmukh[1,2]

1. Cell Biology and Physiology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC
2. Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

During the lifetime of an organism, cells experience many stressors which result in the accumulation of DNA damage. Once DNA damage has been detected, most cell types will either repair the damage or undergo apoptosis. In mitotic cells, the removal of cells that accumulate DNA damage is critical as their replication may lead to cancer, cellular dysfunction, or a loss of homeostasis. Neurons however, are an irreplaceable post mitotic cell type whose prolonged survival is essential. Therefore, neurons have developed strategies to restrict cell death, even in the presence of sublethal stressors.
Strikingly, post-mitotic neurons have been shown to accumulate and sustain DNA Double-Stranded Breaks (DSB) with age in the absence of cell death. Currently, it remains unknown if neurons engage apoptotic machinery in response to sustained sublethal DNA damage. Furthermore, the molecular mechanisms that enable their long-term survival in these conditions remain unknown. In this work, we investigated if neurons activate the apoptotic pathway in response to sustained sublethal DNA DSB. Excitingly, our work shows that neurons indeed engage apoptotic machinery but below a lethal threshold and restrict the pathway prior to caspase activation. Importantly, we have uncovered a crucial function of Bcl-xL in enabling the long-term survival of neurons that sustain sublethal DNA DSB.

NFκB governs CD8 T cell fate during exhaustion

Amber Gomez[1,2], William D Green[2], Albert S Baldwin[2], J Justin Milner[2]

1. Department of Cell Biology and Physiology, University of North Carolina Chapel Hill
2. Lineberger Comprehensive Cancer Center, University of North Carolina Chapel Hill

Harnessing CD8 T cell antitumor activity has become the cornerstone of cancer immunotherapy, yet the challenge of acquired T cell dysfunction, or exhaustion, remains a critical barrier to durable responses. Resolving the molecular regulators of exhaustion fate decisions offers a path toward engineering T cells that resist or reverse dysfunction in the tumor microenvironment. While the transcription factor family NFκB has been implicated in T cell activation and survival, its role in regulating exhaustion fate decisions remains poorly defined. Here, we combine high dimensional spectral flow cytometry, new molecular tools, and single-cell RNA sequencing to systematically dissect the consequences of NFκB perturbation in exhausted CD8 T cells. Using an inducible adoptive transfer system, we individually and combinatorially targeted NFκB family members (RelA, RelB, cRel, NFKB1) to define their distinct contributions to exhaustion fate specification. Using these tools, we resolved how NFκB family members regulate discrete paths of exhaustion differentiation, and found that manipulation of the NFκB pathway can be leveraged to reprogram T cell exhaustion in infection and cancer. Together, these findings establish NFκB signaling as a critical regulator of exhaustion fate progression and represent a potential target for enhancing T cell responses in cancer.

High-Resolution Single-Particle Imaging of Actin Dynamics at the Leading Edge

Max Hockenberry[1], Jason Haugh[2], Wesley Legant[3,4], James Bear[1,5]

1. Department of Cell Biology and Physiology, UNC Chapel Hill, Chapel Hill, NC 27599
2. Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695
3. Lampe Joint Department of Biomedical Engineering, UNC Chapel Hill and North Carolina State University, Chapel Hill, NC 27599
4. Department of Pharmacology, UNC Chapel Hill, Chapel Hill, NC 27599
5. UNC Lineberger Comprehensive Cancer Center, UNC Chapel Hill, Chapel Hill, NC 27599

Actin is a highly conserved protein that polymerizes into a dynamic cytoskeletal network essential for cell migration, division, and intracellular trafficking. Assembly and disassembly of actin filaments are tightly regulated by a large and diverse set of actin-binding proteins, and disruptions to actin dynamics lead to pathological phenotypes. Although actin biochemistry and kinetics have been extensively characterized through *in-vitro* reconstitution assays, the inability to directly observe actin dynamics with single-molecule spatial and temporal resolution in living cells has limited experimental testing of many proposed models. To bridge this gap, we developed a single-particle microscopy platform enabling direct visualization of individual actin molecules in vivo. Using sub-saturating HaloTag labeling of actin and highly inclined laminated optical sheet (HILO) microscopy, we resolve both freely diffusing and stably incorporated actin populations. We apply this approach to the leading edge of fibroblasts, an ideal model system due to its flat geometry and well-characterized actin network dynamics and develop computational tools to quantitatively analyze particle trajectories. Beyond the canonical globular (G) and filamentous (F) actin states, we identify a substantial population of intermediate diffusive trajectories and detect state transitions consistent with actin assembly and disassembly at the leading edge, recapitulating prior spatial trends measured by ensemble approaches. A dual-camera configuration further allows correlation of actin kinetics with subcellular features such as focal adhesions and dynamic behaviors including membrane protrusion and retraction. Finally, we demonstrate the extensibility of this platform by generating a cell line stably expressing Halo-Arpc2, a component of the Arp2/3 complex, revealing diffusive state distributions distinct from actin, including shifts in freely diffusing, intermediate, and bound populations. Together, this platform represents a technological advance for directly quantifying molecular-scale actin kinetics in living cells, establishing a versatile framework for testing in vitro–derived models and extending single-particle measurements to diverse cytoskeletal regulators and dynamic cellular behaviors.

A biphasic model for the morphological development of astrocytes in white matter

Kathleen Holmes, Mumu Fang, and Katherine Baldwin

Astrocytes are a morphologically complex and heterogeneous glial cell type whose elaborate processes enable extensive interactions with neurons, vasculature, and other glia. These interactions support their essential roles in brain homeostasis, synapse development, and neurovascular coupling. In gray matter (GM), astrocyte morphological maturation occurs in concert with synaptogenesis. Current evidence suggests that astrocytes and synapses engage in a continuous, reciprocal feedback loop, through which each shapes the development and specialization of the other. White matter (WM) astrocytes, however, develop in a microenvironment largely devoid of synapses, and as a result support distinct functions including myelination and axonal signal propagation.
Despite WM comprising approximately half of the human brain and containing the highest glia-to-neuron ratio of any brain region, the developmental programs shaping WM astrocyte morphology remain poorly defined. As most studies of astrocyte development focus on gray matter, it remains unclear how WM astrocytes acquire their flattened territories, elongated processes, and spindly morphology without synaptic cues. To start addressing this gap, we used an adeno-associated viral labeling strategy to sparsely label astrocytes in the mouse cortex and corpus callosum, enabling high-resolution analysis of individual astrocyte morphology and tiling across four timepoints in postnatal mouse development. We identified two distinct phases of WM astrocyte maturation. During an early window (postnatal days P8–P14), WM astrocytes increase in morphological complexity without a corresponding expansion in territorial volume. In contrast, during a later phase (P21–P70), WM astrocytes exhibited a striking increase in volume which is accompanied by reduced complexity. This developmental trajectory indicates that WM astrocytes mature in a protracted, biphasic manner, continuing to refine their morphology into adulthood, whereas GM astrocytes develop linearly and reach maturity by approximately P21. Additionally, mature GM and WM astrocytes reach a similar maximum surface area, suggesting a conserved upper limit on astrocytic membrane expansion despite differences in territorial volume. We propose that early WM astrocyte maturation is driven by astrocyte–axon interactions within the corpus callosum, while later maturation reflects emerging astrocyte–myelin interactions. Together, these findings establish a developmental framework for understanding how WM astrocyte morphology is shaped by non-synaptic cues and provide new insight into astrocyte heterogeneity across brain regions.

The Role of TNIK and tPA in Tumor-Stroma Crosstalk, ECM Degradation and Metastasis in Pancreatic Cancer

Michelle LaBella MD[1,2,4]; Hannah Trembath MD[4]; Matthew Flick PhD[1,3]; Jen Jen Yeh MD[1,2,4]

1. Lineberger Comprehensive Cancer Center, Chapel Hill, NC.
2. Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC.
3. Department of Pathology, University of North Carolina, Chapel Hill, NC.
4. Department of Surgery, University of North Carolina, Chapel Hill, NC

5-year survival in Pancreatic Ductal Adenocarcinoma (PDAC) is 11-13% and is predicted to be the second leading cause of cancer related death by 2030. Our lab has previously identified two transcriptomic subtypes of PDAC, classical and basal-like, based on RNA expression that hold clinical relevance. Classical PDAC has improved response to chemotherapy, and longer overall survival, though more work is needed to develop therapeutics leveraging subtype differences.

Through a comprehensive unbiased proteomic approach that enriches for kinases, multiplex inhibitor bead mass spectrometry, we compared kinase expression between classical and basal-like patient derived xenografts (PDX). Traf2 and Nck interacting kinase (TNIK) was identified as the top differentially expressed classical kinase in PDAC. TNIK is a germinal center serine/threonine kinase, a known regulator of the beta-catenin/TCF4 – Wnt signaling pathway, and its inhibition has been studied in colorectal cancer. In contrast to TNIK's role in colorectal cancer, our lab has observed that TNIK may be required to maintain PDAC differentiation, organization and restrain invasion. We observed an increase in PDX cell invasion across a transwell with shRNA mediated knockdown (KD) of TNIK. Classical TNIK knockout organoids displayed increased invasive and migratory potential when plated in collagen. Transcriptomic analysis of control and TNIK knockout organoids showed an increase of basal-associated genes, as well as a significant increase in tissue plasminogen activator (tPA) expression.

tPA is a secreted serine protease that converts plasminogen to plasmin, which drives fibrin degradation. This is highly relevant in PDAC where fibrin is abundant in the tumor microenvironment (TME). Notably, urokinase plasminogen activator (uPA) is well studied in PDAC, and is consistently associated with worse overall survival. Given our transcriptomic finding of increased tPA with TNIK loss, we investigated the TNIK–tPA axis as a potenti

Multi-gene biomarkers reveal spatial organization and subpopulation-specific damage response in intrahepatic cholangiocytes

Kendall G Kanakanui[1,2], Fransky Hantelys[3], Hannah R Hrncir[3,4], Sergei Bombin[3], Adam D Gracz[2]

1. Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA USA
2. Department of Cell Biology & Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC USA
3. Department of Medicine, Division of Digestive Diseases, Emory University. Atlanta, GA USA
4. Graduate Program in Biochemistry, Cell and Developmental Biology, Emory University, Atlanta, GA USA

Background & Aims: Intrahepatic cholangiocyte (biliary epithelial cell; BEC) heterogeneity remains challenging to define. Here, we sought to identify BEC subpopulations and biomarkers in mouse liver.
Methods: We performed scRNA-seq on Sox9EGFP+ liver epithelium from mice subjected to bile duct ligation (BDL) and sham controls. A machine learning algorithm, NSForest, identified minimal, multi-gene signatures for BEC subpopulations. These "metagenes" were validated using hybridization chain reaction (HCR) FISH in tissue sections from wild-type mice and on primary BECs expanded in vitro. Metagenes were used to match BDL subpopulations to their corresponding sham subpopulations for differential gene expression (DGE) analysis.
Results: We identified 4 BEC subpopulations in sham controls, each associated with 1-2 gene metagenes. Spatial localization of metagene-defined BEC subpopulations by HCR FISH revealed heterogeneous cellular composition of intrahepatic bile ducts. BECs belonging to a given subpopulation were most likely to have neighbors of the same identity, forming homogenous cellular compartments within ducts. BDL downregulated subpopulation-specific genes and upregulated a damage-associated gene set. BDL samples also included a proliferative subpopulation not found in sham controls, which contained populations enriched for three of the four metagenes. All BEC subpopulations were also found in monolayers in vitro, where they clustered spatially with BECs of the same subtype.
Conclusions: Novel metagene biomarkers of BEC subpopulations facilitated spatial localization of BECs in situ, identified subpopulation-specific injury responses, and confirmed that BEC heterogeneity is preserved in vitro. The presence of locally homogenous BEC "neighborhoods" in vitro suggests some degree of BEC organization may be epithelial-autonomous.

A high-throughput approach to measuring environmental and behavioral changes in mouse home cages

Jovan Tormes-Vaquerano[1,2], Raymond S. Carpenter III[1,2], Eloise Kuijer[3,4], Shokhjahon Berdiyorov[1,2], Kameron Thomas[1,2], Krisha Mehta[2], Isabel Castro Rivera[2], Sophia Dato[2], Sawyer Grizzard[1,2], Hugo Tejada[3], Nicolas C. Pégard[1,6,7,8], and Jose Rodriguez-Romaguera[1,2,6,7,9,10]

1. Department of Applied Physical Sciences,
2. Department of Psychiatry,
3. National Institute on Alcohol Abuse and Alcoholism,
4. National Institute of Mental Health,
5. Neuroscience Curriculum,
6. UNC Neuroscience Center,
7. Carolina Stress Initiative,
8. Department of Biomedical Engineering,
9. Carolina Institute for Developmental Disorders,
10. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill

Standard behavioral assays have finely tuned controls that allow researchers to take specific measurements related to development and innate conditions. However, behaviors measured are time-bound and may not be generalizable outside the assays themselves. Continuous environmental monitoring of home cages can provide researchers with insight into external factors which may influence rodent behavior across development. To enable this, we developed an integrated device that can continuously record mouse behavior and local environmental changes. We have integrated a thermal imaging camera to track animal movement as well as 3-axis accelerometers and gyroscopes to monitor cage motion. Environmental changes are recorded with multiple sensors to track C02 level, air quality, sound level, temperature, humidity, and ambient light. This approach allows us to align changes in behavioral activity to multiple environmental variables. This approach allows us to understand how mice themselves change their environment through their repeated, periodic behaviors. The specificity of each sensor has provided us insights on changes associated with the light and dark cycle across multiple stages of mouse development. Our device is tailored for high-throughput animal research applications and provides turn-key monitoring capabilities that would otherwise require multiple independent synchronized devices that could potentially disrupt experiments. Continuous monitoring of animal behavior and environments can provide insights into the relationship between both and provide a systematic approach to improve the reliability of experimental results. Studies into how stubble environmental differences induce disruptions in behavior of mouse models may also provide insights into psychiatric and neurodevelopmental disorders such as anxiety and autism.

Identifying small GTPase binding sites on the exocyst tethering complex

Gabrielle Puller[1,2], Guendalina Rossi[2], Rick Baker[3], and Patrick Brennwald[2]

1. Curriculum in Genetics and Molecular Biology, UNC Chapel Hill, Chapel Hill, NC 27599
2. Department of Cell Biology and Physiology, UNC Chapel Hill, Chapel Hill, NC 27599
3. Department of Biochemistry and Biophysics, UNC Chapel Hill, Chapel Hill, NC 27599

During polarized exocytosis, the exocyst tethering complex must recognize and respond to a variety of spatial queues to properly tether post-Golgi vesicles to specific places on the plasma membrane. These queues include small GTPases of the Rab and Rho families, which reside on the vesicle and plasma membrane respectively. Although small GTPase interaction with the exocyst is long-established, the precise sites where these GTPases bind to the exocyst remain a mystery. In this work, we utilized AlphaFold3 (AF3) to model *S. cerevisiae* Rab and Rho GTPase interactions with all eight subunits of the exocyst and ranked predicted models by the AF3 interface predicted template modeling (ipTM) score. To validate the accuracy and specificity of predictions in AF3, we selected two known GTPase:exocyst interactions with high ipTM scores for further study. The first interaction, between the Rho3 GTPase and the Exo70 subunit of the exocyst, has been the subject of controversy for many years due to a disputed binding site. The second, between the Sec4 Rab GTPase and the Sec15 subunit of the exocyst, has had no binding site discovered to date. To investigate our AF3-predicted binding sites, we constructed mutants in yeast of *EXO70* and *SEC15* that would be likely to disrupt interaction with Rho3 and Sec4 respectively, based on the AF3 models. In testing these mutants as the sole source of *EXO70* or *SEC15* in the cell, we identified three *EXO70* mutants and four *SEC15* mutants that had clear loss-of-function phenotypes (lethal or cold sensitive), strongly supporting our AF3-predicted binding sites. Excitingly, through both structure-based sequence alignment and AF3 modeling with mammalian homologues of Sec4 and Sec15, we also discovered that our novel Sec4 binding site on Sec15 is likely conserved in mammals. This work highlights the power of yeast genetics as an efficient readout to corroborate AF3 predictions.

A humanized mouse model of Giant Axonal Neuropathy with early functional phenotypes

Audrey Smith[1], Xi Yang[1], Jonathan Nagel[2], Virginia Godfrey[2], Sheryl Moy[3], Maryam Faridounnia[1], Diane Armao[4] and Natasha Snider[1]

1. Departments of Cell Biology & Physiology,
2. Pathology and Laboratory Medicine,
3. Psychology and
4. Radiology, UNC-Chapel Hill

Giant axonal neuropathy (GAN) is a hereditary neurodegenerative disease characterized by progressive axonal degeneration affecting the peripheral and the central nervous system. Our goal was to develop a clinically relevant mouse model to advance mechanisms and enable preclinical therapeutic testing for GAN. Specifically, we sought a model to capture the early-onset and progressive disease course observed in GAN patients carrying loss-of-function mutations in the *KLHL16* (*GAN*) gene. Previously we discovered uniquely human features of the 3'UTR region within the *KLHL16* mRNA. Therefore, we replaced the 1.2kb mouse *Klhl16* 3′UTR with the 13kb human *KLHL16 3'UTR*. This resulted in the generation of phenotypically normal control humanized mice (hGAN mice). *KLHL16* encodes gigaxonin, a ubiquitin ligase adaptor that promotes the clearance of intermediate filament (IF) proteins, which accumulate in GAN cells. Most pathogenic variants are gigaxonin missense mutations. Therefore, we also introduced a known GAN-causing missense mutation (G332R) together with the human 3′UTR (hGAN-G332R mice). We then compared 3-12 month-old hGAN and hGAN-G332R mice using functional, histologic and biochemical assessments. The hGAN-G332R mice showed complete gigaxonin loss compared to hGAN and wild-type C57BL/6 control mice, similar to GAN patient neurons with the G332R mutation. The hGAN-G332R mice had increased IF protein levels and displayed giant swollen axons with densely packed IFs. At 3 months of age, the hGAN-G332R mice had significantly decreased activity in an open field test ($p=0.0203$), decreased center time ($p=<0.0001$) and decreased vertical rears ($p=0.0003$). Grip strength was normal at 3 months but significantly decreased at 12 months in hGAN-G332R mice ($*p<0.05$). Ongoing studies are aimed at translating targeted therapies from cell-based models to *in vivo*. In conclusion, the hGAN-G332R mice model the progressive motor deficits seen in GAN patients and will help clarify the involvement of the gigaxonin mRNA and IF accumulation in disease onset and progression.

Lymphatic-specific protein interactome of RAMP1 in migraine

Madison Kalk[1], Nathan Nelson-Maney[2], Stephen Serafin[2], Kathleen Caron[2]

1. Pharmacology, University of North Carolina Chapel Hill, Chapel Hill, NC 27599
2. Cellular Biology and Physiology, University of North Carolina Chapel Hill, Chapel Hill, NC 27599

Migraines are a debilitating neurological condition afflicting ~15% of the global population. While causes of migraine remain unclear, the most well-established mediator is calcitonin gene-related peptide (CGRP), a neuropeptide elevated in the plasma and cerebral spinal fluid during migraines. CGRP primarily acts upon its canonical receptor, a heterodimer composed of the G-protein coupled receptor calcitonin-receptor like receptor (CLR), and its allosteric regulator, receptor activity-modifying protein 1 (RAMP1). The CGRP receptor (RAMP1-CLR) is expressed in various cell types involved in migraine pathophysiology, including those within the central and peripheral nervous systems and the blood vasculature. Recently, our laboratory was the first to show that CGRP-mediated signaling in meningeal lymphatic vessels significantly contributes to migraine pain. CGRP tightens cell-cell junctions of lymphatic endothelial cells (LECs) that line these vessels, which attenuates permeability and impairs their drainage function. Currently, it is unknown whether RAMP1 modulates CLR activity uniformly across all cell types or whether its regulation is shaped by both internal cellular mechanisms and external cues. To investigate whether RAMP1 regulates CLR signaling in a cell-dependent manner, we leveraged proximity labeling technology to compare the proximal protein network of RAMP1 in LECs to Schwann cells, a glial cell in the peripheral nervous system implicated in migraine pain. Preliminary results suggest that while LECs and Schwann cells both recruit endosomal proteins upon CGRP treatment, they also interact with various unique proteins. Ongoing experiments aim to delineate the impact of the differentially enriched proteins on receptor activation and downstream signaling profiles in each cell type.

Protein Geranylgeranylation Regulates Cardiac Morphology and Contractility During Embryonic Development

David Cully[1,2,3], Gregory Miner[2,3], Harinarayanan Kottala[4], Shenice Harrison[2,3], Mark Distefano[4], Whitney Edwards[1,2,3]

1. Curriculum in Genetics and Molecular Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
2. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
3. McAllister Heart Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
4. Department of Chemistry, University of Minnesota-Twin Cities, Minneapolis, Minnesota 55455, United States

Protein prenylation is a post-translational modification (PTM) that involves the covalent attachment of isoprenoid lipids to proteins. Prenylation promotes localization of target proteins to the plasma membrane or other organelle membranes. Emerging studies, including ours, implicate geranylgeranylation, a type of prenylation, as a critical regulator of heart development. Despite its essential role, we lack a mechanistic understanding of how this PTM influences heart formation and function. In particular, the specific geranylgeranylated proteins involved and the cellular/molecular processes they regulate are unknown. In this project, we aimed to determine the role of geranylgeranylation in cardiomyocyte function and myocardial development. We found that inhibition of geranylgeranylation in isolated embryonic cardiomyocytes results in reduced sarcomere protein abundance, impaired sarcomere organization, and compromised cardiomyocyte contractility. We also generated a cardiomyocyte-specific knockout mouse model of PGGT1B, a critical enzyme that mediates protein geranylgeranylation. We found that loss of PGGT1B results in perinatal lethality due to congenital heart defects, including severe ventricular noncompaction. Moreover, in support of our in vitro findings, PGGT1B knockout hearts exhibit disrupted sarcomere organization and proteomic profiling revealed a significant downregulation of sarcomere components. Finally, we leveraged a quantitative chemical proteomics approach to identify and quantify the unique subset of prenylated proteins in cardiomyocytes. Using this method, we have identified candidate prenylated proteins that may serve as a mechanistic link between protein geranylgeranylation and cardiomyocyte sarcomere formation and function. Overall, our findings demonstrate that geranylgeranylation is a critical cellular process in the regulation of cardiac development and cardiomyocyte function.

The scramblase RFT1/RFTH-1 maintains metabolic homeostasis via mTORC2-specific signaling

Kylie R. VanDerMolen[1], Yunjing Gao[2], Robert H. Dowen[1, 2, 3]

1. Department of Genetics, UNC-Chapel Hill, Chapel Hill, NC. 27510
2. Department of Biology, UNC-Chapel Hill, Chapel Hill, NC. 27510
3. Department of Cell Biology and Physiology, UNC-Chapel Hill, Chapel Hill, NC. 27510

Proper regulation of metabolic homeostasis by multiple conserved signaling pathways licenses growth, development, and reproduction. Organisms integrate developmental and environmental cues that appropriately allocate resources to maintain fitness. We have previously shown that Hedgehog signaling, an essential developmental pathway, regulates lipid homeostasis via mTORC2 signaling. How these two pathways communicate remains unclear. To further investigate this signaling axis, we carried out a forward genetic screen to isolate genetic suppressors of the *grd-3; grd-4* Hedgehog mutant, uncovering six unique gain-of-function (GOF) mutations in *rfth-1*, the *C. elegans* ortholog of *RFT1*. RFT1 is an endoplasmic reticulum transmembrane scramblase with a putative role in N-glycan biosynthesis. The *rfth-1(gof)* alleles suppress the metabolic defects conferred by loss of *grd-3/4* in a mTORC2-specific manner, independently of both p38 MAPK and insulin signaling. Moreover, we found that RFTH-1(GOF) upregulates a subset of genes that are downregulated in Hedgehog mutants, and that this subset is highly enriched for neuronal expression. As RFT1 is relatively under-studied, we generated a fluorescent and AID-tagged RFTH-1 to define RFTH-1 tissue localization and the effect of RFTH-1 depletion for the first time in a multicellular model organism. We find that RFTH-1 is strongly expressed in the germline and intestine, and whole-body depletion causes severe developmental delays and metabolic defects, including growth and vitellogenesis. Human RFT1 loss-of-function mutations result in severe and poorly understood congenital disorders of glycosylation (CDG) that are characterized by multi-organ defects in growth and development. We generated a *C. elegans* RFT1-CDG model by introducing a pathogenic mutation at a conserved residue, which resulted in a severe loss in viability. Our findings reveal that RFTH-1 regulates metabolic control via the Hedgehog-mTORC2 signaling axis and has a highly conserved, essential role in development and growth. This may contribute to the pathophysiology of RFT1 mutations in humans, and as such, this work may help better understand and improve RFT1-CDG patient outcomes.

Spatiotemporal characterization of endothelial proliferation in liver regeneration post-CCl4 injury

Won Yoon[1], Aashita Rajput[1], Kanesha Travis[1], Chris Glontz, D. Berfin Azizoglu[1]

1. Department of Cell Biology and Physiology & McAllister Heart Institute, UNC Chapel Hill, Chapel Hill, NC 27599

The mammalian liver has the unique capability to fully regenerate upon injury. CCl4-induced acute chemical injury in mice mimics acetaminophen overdose and kills the primary liver cell type, hepatocytes. Liver vasculature and endothelial cells are known to contribute to post CCl4 injury regeneration by inducing hepatocyte proliferation.
However, how the vasculature itself is altered upon injury remains poorly characterized. Curiously, recent studies demonstrated an increase in endothelial cell proliferation post-CCl4 injury. Which endothelial subset proliferate and how this is spatially coordinated with hepatocyte proliferation remains unknown.
This study aims to characterize the spatial and temporal dynamics of endothelial cell proliferation during liver regeneration post-CCl4 injury. To effectively identify endothelial proliferation in its native spatial context within the liver, we optimized antigen retrieval-mediated immunostaining on volumetric, floating liver sections followed by automated quantification. This workflow allowed us to visualize the pan-endothelial nuclear marker ERG alongside the cell cycle marker Ki67 in their native spatial organization in volumetric samples, Our preliminary data suggests a significant increase in endothelial cell proliferation 3 days post-CCl4 injury and we are currently analyzing the spatial distribution of endothelial cell proliferation in these samples. Future studies focused on endothelial alterations following injury to the liver will help advance our understanding of how endothelial cells contribute to the liver regeneration process.

Vascular Plasticity in the Regenerating Liver

Aashita Rajput[1], Won Yoon[1], Chris Glontz[1], Kanesha Travis[1], D. Berfin Azizoglu[1]

1. Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC 27599.

The liver has a unique capacity to regenerate, unlike other mammalian visceral organs which rapidly undergo fibrosis and scarring upon injury. The current cannon of research on liver regeneration has largely focused on epithelial hepatocyte proliferation. Yet, hepatocyte proliferation occurs slowly and fails to explain how rapid fibroblast activation is evaded. There must be precursory mechanisms in place to evade fibrosis, likely involving cell types other than hepatocytes. Indeed, hepatocytes do not build the liver alone; they are integrated with vasculature and arranged into repeated lobules.
Here, we visualize lobule structure and vascular changes during liver regeneration in mice. Following paracetamol-like injury causing hepatocyte death at the lobule center, we unexpectedly observe rapid vascular reorganization on the opposite end of the lobule. Subsequently, an ectopic vascular cell signature emerges within the damage zone at the lobule center, possibly as a cytoprotective measure. Concurrently, lobules shrink in size by one fourth, closing in on the injury. These changes precede hepatocyte proliferation. Our findings demonstrate drastic vascular reorganization in response to liver injury, suggesting a novel form of liver lobule plasticity. We propose that this lobule plasticity safeguards vascular structures and obviates scar formation during liver regeneration. We are currently testing this idea using genetic lineage tracing and loss-of-function tools.

Disturbed flow impairs ATGL-dependent lipid droplet clearance in endothelial cells

Harold Heatherly[1], Qingye Zeng[1], Schuyler Bowers[1], and Boa Kim[1,2]

1. McAllister Heart Institute,
2. Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

Atherosclerosis develops preferentially at vascular regions exposed to disturbed flow, where endothelial cells adopt a proinflammatory and dysfunctional phenotype. Although disturbed flow-induced transcriptional programs have been extensively characterized, how hemodynamic forces regulate endothelial lipid metabolism remains poorly understood. Here, we identify endothelial lipid droplet (LD) accumulation as a metabolically regulated response to disturbed flow. We show that endothelial cells located at atheroprone vascular regions in vivo, as well as ECs exposed to disturbed flow in vitro, exhibit markedly increased LD abundance compared with laminar flow-exposed counterparts. This accumulation is not driven by increased fatty acid uptake or enhanced triglyceride synthesis, but instead reflects impaired triglyceride hydrolysis and defective clearance of pre-existing LDs. Mechanistically, we find that disturbed flow suppresses expression of adipose triglyceride lipase (ATGL), the rate-limiting enzyme for triglyceride hydrolysis in ECs. Restoring ATGL activity or normalizing LD abundance through independent metabolic interventions attenuates inflammatory gene expression induced by disturbed flow. Together, these findings identify disturbed flow as a regulator of endothelial LD metabolism and reveal suppressed triglyceride hydrolysis as a key mechanism linking atheroprone hemodynamics to endothelial inflammatory remodeling.

Tubulin polyglutamylation modulates Golgi morphodynamics and neurite branching during neuronal morphogenesis

Sherry Hsu[1], Alexander Kinrade[1], and Sarah Cohen[1]

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill 27599.

Morphological remodeling is crucial for establishing functional networks within neurons during differentiation. Microtubule polyglutamylation is a key post-translational modification that is highly enriched during neurogenesis and plays an important role in differentiation. However, how the remodeling of organelle features, such as morphology, distribution, and interactions, rely on tubulin polyglutamylation during neuronal differentiation remain unclear. To address this, we employed multispectral imaging paired with quantitative 3D analysis to profile eight organelles simultaneously in human induced pluripotent stem cell-derived neurons. Depletion of tubulin polyglutamylation resulted in pronounced alterations in somatic Golgi morphology, distribution, and polarity. Additionally, we found alterations in the morphology and dynamics of Golgi-derived compartments in proximal neurites including decreased retrograde directionality. These changes were accompanied by an increased neurite branching, tortuosity, and decreased neurite width. Overall, these findings point to an uncharacterized role of tubulin polyglutamylation in coordinating organelle organization and neurite architecture, providing a mechanistic link between the tubulin post-translational modification, Golgi morphology, dynamics, and neuronal morphogenesis.

Enteroendocrine Cells and Intestinal Barrier Function in IL-10$^{-/-}$ Inflammatory Bowel Disease

Samara Williams[1], Avery Staub1, Anne Marie Riedman2, Heather McCauley, PhD1

1. Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill

Current therapies for inflammatory bowel disease (IBD) do not target the integrity of the epithelial barrier. Given persistent intestinal permeability that may limit the effectiveness of existing anti-inflammatory treatments, there is a need to investigate potential therapies that strengthen the barrier and modulate mucosal immune responses. We propose a novel hypothesis that enteroendocrine cells (EECs) play an important role in maintaining intestinal barrier integrity and regulating immune responses in the gut. EECs are rare epithelial cells that sense nutrients, microbes, and other stimuli. EECs secrete more than 20 products, including hormones, neurotransmitters, cytokines, and metabolites, to coordinate local and systemic responses to the gut environment. Our recent work identifies EECs as essential for promoting a strong epithelial barrier in enteroid monolayer cultures. In mouse models, preliminary data further support the role of EECs in limiting intestinal permeability. In a pilot study, we found loss of EECs exacerbated DSS-induced colitis, suggesting that EECs contribute to the pathophysiology of IBD. We propose investigating the role of EECs in the IL-10$^{-/-}$ mouse model, which models key features of Crohn's disease, including spontaneous disease onset, genetic susceptibility, and small intestinal involvement. Defining the role of EECs in barrier maintenance and immune regulation may uncover new strategies to promote gut health and improve disease outcomes.

Investigating the mechanisms by which Kombucha Tea-associated microbes induce host autophagy

Sophia Blatchley[1], Annalee Schmidt[2], Dr. Rachel DuMez Kornegay[2], Peter Breen, Dr. Robert Dowen[1,2]

1. The University of North Carolina at Chapel Hill, Department of Biology
2. The University of North Carolina at Chapel Hill, Department of Cell Biology and Physiology

Research into preventative measures for metabolic disease remains relevant, with one avenue of investigation being the impact of the gut microbiome on metabolic health. Our lab uses *C. elegans* to study the probiotic potential of metabolite-producing microbes in Kombucha Tea, a Chinese fermented beverage with long-purported health benefits. We found that animals fed Kombucha Tea-associated microbes (KTMs) experience decreased lipid accumulation that is partially driven by increased lipophagy—the degradation of lipids via autophagy. The molecular mechanisms through which microbial-derived metabolites govern this response are unclear. A key autophagy regulator is TFEB/HLH-30, a transcription factor that, upon activation, translocates to the nucleus from the cytoplasm to promote autophagy-related gene expression. Using a strain that co-expresses an intestinal nuclear blue fluorescent protein along with GFP-tagged HLH-30, we found increased nuclear localization of HLH-30 in KTM-fed animals compared to animals fed the standard *E. coli* laboratory diet, indicating that KTMs may act through HLH-30 to induce autophagy. Upstream nutrient sensing pathways, including AMPK/mTOR, are known to regulate HLH-30 translocation in mammals and may play a role in modulating the KTM-consumption response. Therefore, we measured HLH-30 nuclear localization in mutants lacking one or both AMPKα isoforms. We found that KTM-induced HLH-30 nuclear localization decreases only upon loss of both AMPK isoforms, indicating that AMPK mediates this response. Additionally, our previous data demonstrate that KTM-consumption has no effect on *hlh-30* mRNA expression, suggesting that KTM-induced nuclear localization is due to increased activation of HLH-30 as opposed to increased expression of the *hlh-30* gene.

Unbiased genetics identifies a glutamatergic signaling network as a mediator of daily sleep patterns

Alicia L. Ravens, Sawyer Grizzard, Julia S. Lord, Yajing Hai, Wei Tang, Sung Hyun Lee, Sarah Jo Sleiman, Kathryn K. Joice, Keven D. Donohue, Bruce F. O'Hara, Fei Zou, Tal Kafri, Graham H. Diering

1. Neuroscience Center, University of North Carolina at Chapel Hill, NC;
   Department of Cell Biology and Physiology, University of North Carolina Chapel Hill, NC;
   Carolina Institute for Developmental Disabilities, University of North Carolina Chapel Hill, NC

Sleep is a fundamental, evolutionarily conserved behavior essential for survival across animal species. Sleep behavior is controlled by a poorly understood interaction between the circadian rhythm (CR), which promotes sleep at ecologically appropriate times, and a homeostatic sleep drive that accumulates with time spent awake. While the CR is driven by molecular oscillations of "clock genes" in nearly all cells, the cellular mechanisms underlying the homeostatic regulation of sleep remain poorly understood. Emerging evidence suggests neuronal synapses as key loci for the accumulation and resolution of sleep need, supporting a cellular basis of sleep need. Indeed, previous efforts to identify genes mediating sleep need identified the synaptic gene Homer1a. To advance mechanistic understanding linking synaptic function to sleep regulation, we measured daily sleep patterns in genetically diverse strains of mice from the Collaborative Cross. Strains with 1) highly consolidated light-phase sleep, or 2) fragmented, arrhythmic sleep, were identified for genetic analysis using quantitative trait loci (QTL) mapping. Excitingly, in F2 hybrids, 19 of 32 metrics of sleep and circadian behavior mapped to a narrow QTL containing GRM5, a postsynaptic glutamate receptor and binding partner of Homer1a, and GCPII, an astrocytic enzyme that regulates NAAG, a peptide agonist for the presynaptic/astrocytic glutamate receptor GRM3. Collectively, these genes form a coordinated glutamatergic signaling network across the tripartite synapse. Pharmacology targeting GRM5, GCPII, and GRM3 strongly modulated sleep, functionally validating them as sleep-regulating genes. Our findings support a model in which synapses act as a cellular site for the integration of circadian and homeostatic sleep signals.