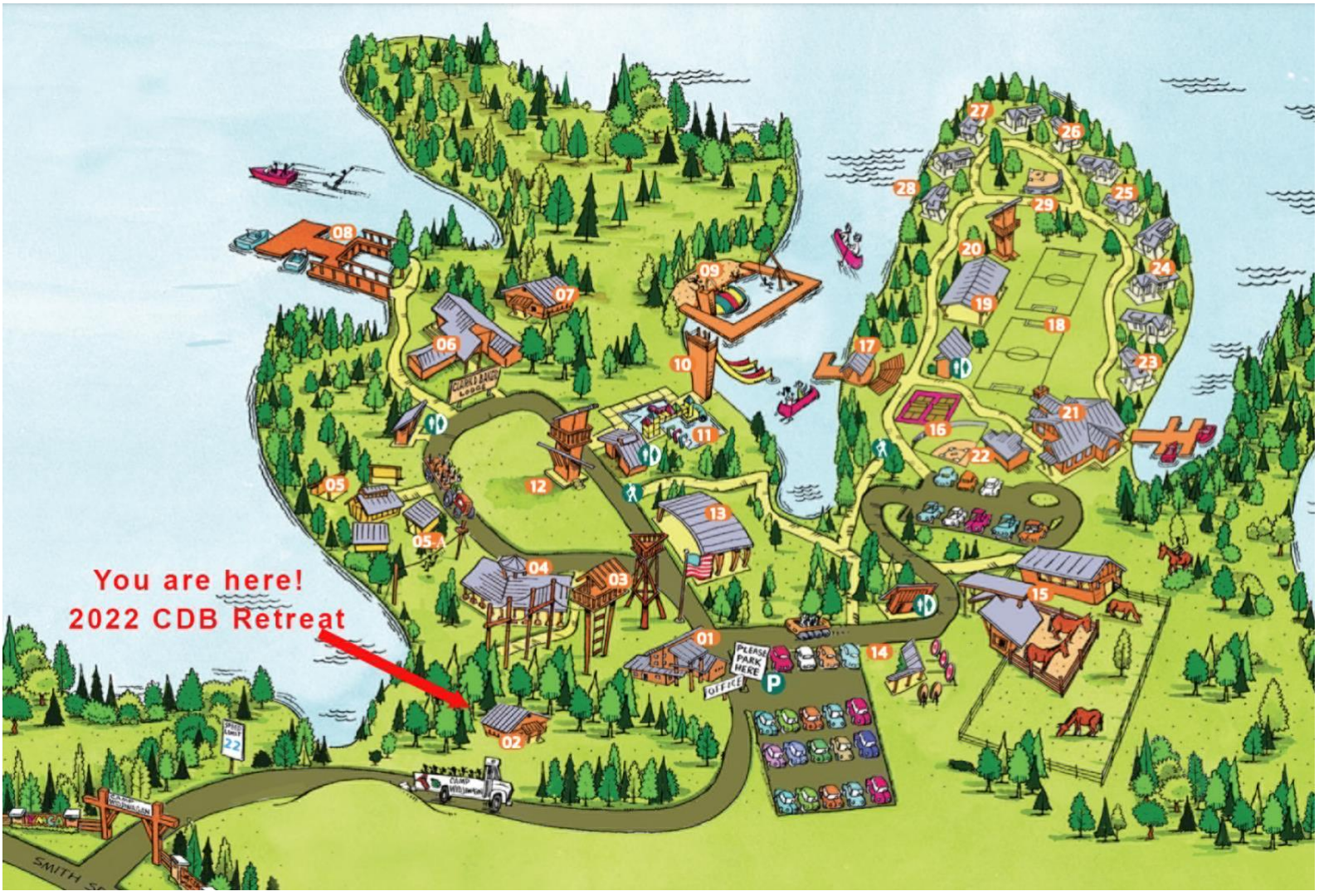
Department of Cell & Developmental Biology

Nineteenth Annual Retreat

May 27, 2022



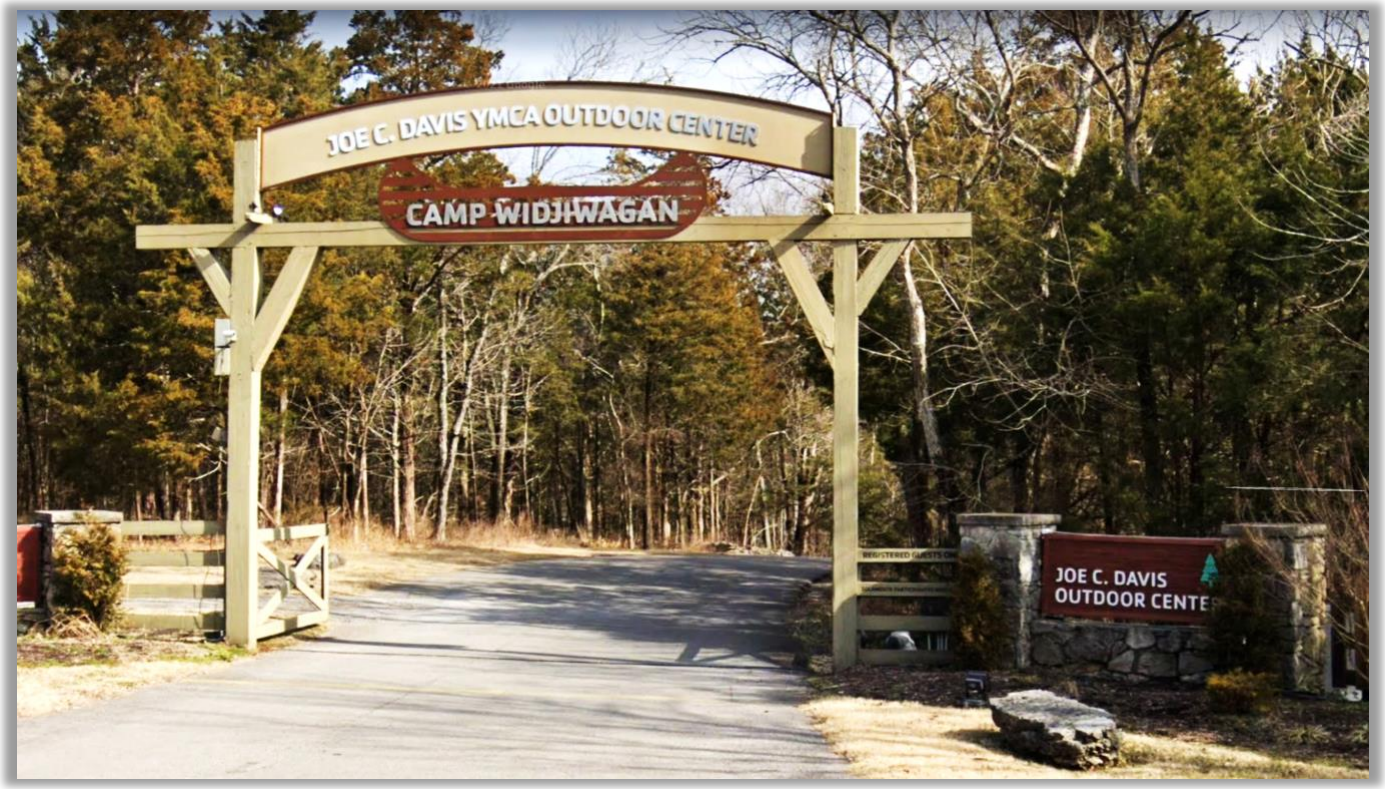
WELCOME BACK!

JOE C. DAVIS YMCA OUTDOOR CENTER
YMCA CAMP WIDJIWAGAN

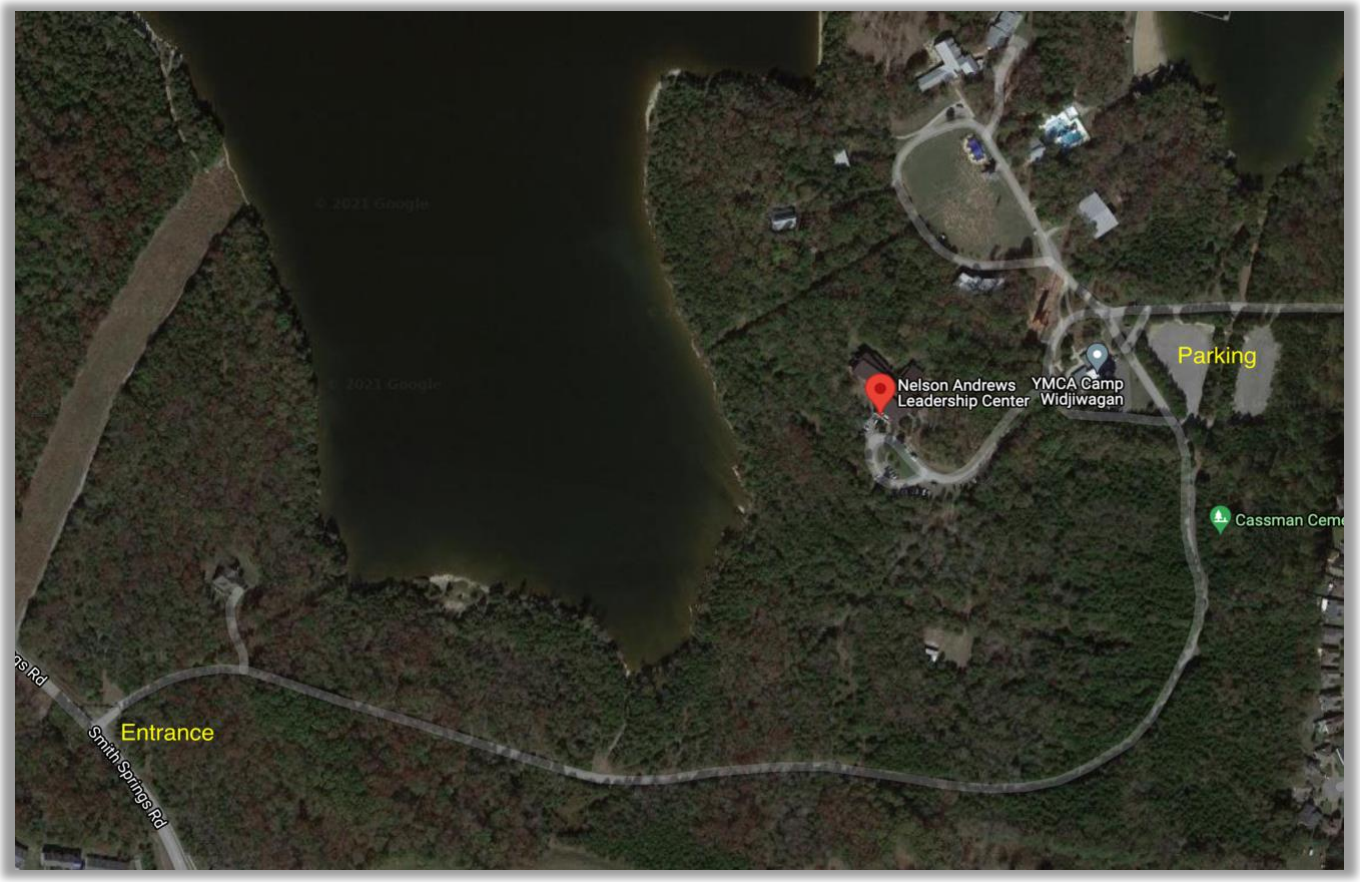
YMCA OF MIDDLE TENNESSEE
3088 Smith Springs Road, Nashville, TN 37013
P (615) 360-2267

https://www.campwidji.org/retreats-and-events/facilities/nelson-andrews-leadership-lodge-great-room

Street entrance to Camp Widjiwagan



Aerial view of Camp Widjiwagan and the Nelson Andrews Leadership Center



Front Entrance to the Nelson Andrews Leadership Center

Nineteenth Annual CDB Retreat
May 27, 2022
Nelson Andrews Leadership Lodge

8:00-8:30	**BREAKFAST AND POSTER SESSION I SET UP** (Morning speakers- please give your slide deck to Marc Wozniak)
8:30-9:05	**State of the Department Address by Ian Macara** **Image Awards -** Presented by Ian Macara **Staff Award Presentation -** Presented by Guoqiang Gu **DEI Award** - Presented by Irina Kaverina
9:05-9:45	**Graduate Student/Postdoc Award -** Presented by Andrea Page-McCaw Steve Hann Award Winner Outstanding Postdoc Award winner
9:50-11:05	**Poster Session I (ODD NUMBERS)** **Breakout Session I** (Students/Postdocs only) Moderated by Abigail Neininger-Castro Caroline Cencer (Tyska lab), Stephanie Medina (Irish lab), Zach Sanchez (Burnette lab), Avishkar Sawant (Kaverina lab), Aubrie Stricker (Page-McCaw lab)
11:10-12:10	**Second Session** – Moderated by Bill Tansey N. Heath Patterson (Spraggins lab) Alaina Willet (Gould lab) Linh Trinh (Magnuson lab) Abigail Neininger-Castro (Burnette lab)
12:10-1:45	**LUNCH** (Chang Noi Thai-Lao and Smokin' Buttz) **POSTER SESSION I TAKE DOWN AND SESSION II SETUP** (Afternoon speakers- please give your slide deck to Marc Wozniak) **ACTIVITIES ON THE YMCA CAMPUS AND FREE TIME**
1:45- 2:45	**DEI Session Ashley L. Brown, Ed.D. "Exploring Power and Privilege"** Director, Student Center for Social Justice and Identity Dean of Students | Vanderbilt University
2:50- 3:35	**Third Session**-Moderated by Ken Lau Angelo Morales (Tyska lab) Kai Bracey (Kaverina lab) Qin Zhang (Coffey lab)
3:40- 4:25	**Fourth Session**- Moderated by Anna Means Dylan Ritter (Knapik lab) Xinyu Dong (Zent lab) Mirazul Islam (Lau lab)
4:30-5:45	**Poster Session II (EVEN NUMBERS) - Open bar** **Breakout Session II** (Students/Postdocs only) Moderated by Eric Donahue Joseph Benthal (Southard-Smith lab), Deanna Bowman (Goldenring lab), Choudhary Dharmendra (Knapik lab), Gillian Fitz (Tyka lab)
5:45-8:30	**Reception** **Dream Events & Catering**

DEI Session: Exploring Power and Privilege

by

Dr. Ashley L. Brown

Using a U.S. centric framework, we will explore the social constructs of power and privilege from a historical lens. Next, we will foster understanding of how these constructs show up presently and how we can navigate them. Last, we will facilitate dialogue on how we, as community members, can envision a liberated society without the limitations of these oppressive systems.

Session 2 - Speaker 1

Multimodal data integration and analysis technologies for constructing multi-scale molecular tissue atlases

Nathan Heath Patterson[1,2], Elizabeth K. Neumann[1,2], Martin Dufresne[1,2], Jamie L. Allen[1], Maya Brewer[3], David M. Anderson[1,2], Mark P. deCaestecker[3], Richard M. Caprioli[1,2,4], Jeffrey M. Spraggins[1,2,4,5]

1. Department of Biochemistry, Vanderbilt University
2. Mass Spectrometry Research Center, Vanderbilt University
3. Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University Medical Center
4. Department of Chemistry, Vanderbilt University
5. Department of Cell and Developmental Biology, Vanderbilt University

Building a spatial atlas from mass spectrometry and microscopy imaging data poses challenges in both integrating disparate data sets and precisely linking molecular data with specific cell types and multicellular functional tissue units (FTUs). Challenges are further magnified when the analysis of large cohorts of samples is required to produce representative atlases, necessitating automated pipelines. Here, we develop technologies for mining matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) based on segmentation masks generated from co-registered label-free and antibody-based microscopy images. FTU-level MALDI IMS analysis of the human kidney spanning the nephron from the medulla through the cortex is driven using multi-channel widefield label-free autofluorescence microscopy. Segmentation models for autofluorescence microscopy are rapidly generated using complimentary multiplexed immunofluorescence microscopy collected on the same tissues. Segmentation training is achieved by developing models on the high-specificity multiplexed immunofluorescence data, then annotations are transferred to the co-registered autofluorescence images. Accurate whole slide microscopy segmentation of glomerulus, proximal tubule, descending thin limb, ascending thick limb, distal tubules, large vasculature, and collecting duct is achieved entirely on label-free microscopy and is immediately validated by corresponding specific immunofluorescence signals. These segmentations are registered with imaging mass spectrometry and can drive data analysis or acquisition. Recently, we have extended these workflows to move beyond FTU-level analysis to provide cell-type molecular analysis by directly linking MALDI IMS and single-cell segmentation masks. This is accomplished by performing MALDI IMS and antibody-based multiplexed immunofluorescence on the same tissues and then co-registering them. Together, these multimodal analytical technologies and advanced integrative computational workflows enable molecular mapping *in situ,* providing the tools necessary to create comprehensive molecular and cellular atlases of the human kidney.

Session 2 - Speaker 2

Conserved ankryin repeat proteins promote myosin-1 localization and function in fission and budding yeast

Alaina H. Willet[1], Jun-Song Chen[1], Liping Ren[1], and Kathleen L. Gould[1]

[1]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Endocytosis is an essential cellular process where extracellular contents such as nutrients are taken up by the cell. To do so in yeast, the cell forms pits in the plasma membrane with force generated by a branched actin network nucleated by the Arp2/3 complex. One component of endocytic patches is the myosin-1 family of motors. These are monomeric membrane tethering proteins with a N-terminal motor domain and C-terminal TH1, TH2, Sh3 and in yeast a CA domain that activates Arp2/3. To better understand the interactions and function of fission yeast Myo1, we performed a large-scale purification coupled with mass spectrometry analysis and identified an uncharacterized non-essential protein containing ankyrin repeats that we named Mai1 (myosin-1 ankyrin repeat interactor). Coimmunoprecipitation of a series of Myo1 C-terminal truncations showed that the Myo1 motor domain alone is sufficient for Mai1 interaction. Further, whereas Myo1-mNG normally localizes to endocytic actin patches, a dramatic relocalization of Myo1 to coat the entire plasma membrane occurred in cells lacking *mai1*. Additionally, in *mai1*Δ cells, the Myo1 light chains, Cam1 and Cam2, also redistributed along the plasma membrane, but other endocytic proteins (Fim1, Wsp1 and F-actin) did not. Preliminary in vitro experiments confirmed previous work showing that immunopurified Myo1 bundles F-actin in vitro. However, Myo1 immunopurified from *mai1*Δ cells does not although it still binds F-actin, presumably via the TH2 domain, as found in other myosin-Is. Myo1 membrane binding is thought to be mediated by the TH1 domain. Thus, we hypothesize that Mai1 promotes the interaction of the Myo1 motor domain with F-actin and in the absence of F-actin binding, Myo1 localization defaults to the membrane in a TH1 domain-dependent manner. We found that a Mai1 ortholog exists in *S. cerevisiae* and it regulates the localization of both *S. cerevisiae* myosin 1s. Thus, Mai1 represents a novel, conserved myosin-I regulator.

Session 2 - Speaker 3

Identification of a cis-regulatory region in *Sox17* that regulates hepato-pancreato-biliary system development

Linh T. Trinh[1,3,5], Anna B. Osipovich[2,3], Leesa Sampson[3], Jonathan Wong[4], Chris V.E. Wright[1,3,5], Mark A. Magnuson[1,2,3,5*]

[1]Department of Cell and Developmental Biology, [2]Department of Molecular Physiology and Biophysics, [3]Center for Stem Cell Biology, [4]College of Arts and Science, [5]Program in Developmental Biology, Vanderbilt University, Nashville, TN 37232, USA

The hepato-pancreato-biliary (HPB) system performs many essential functions and is subjected to a wide variety of diseases and congenital abnormalities. All organs and ducts within the HPB system arise as an outgrowth of the posterior foregut then undergo cell lineage segregations to form this complex and interconnected system. *Sox17* plays an essential role regulating the segregation of pre-HPB progenitor cells into organ- and duct-specific cell types but the mechanisms remain unknown.

To understand how *Sox17* contributes to HPB development we first sought to identify the proximal *cis*-regulatory elements responsible for *Sox17* expression in the posterior foregut. Using a multispecies sequence alignment approach, we identified two highly conserved, non-coding regions immediately upstream of two putative transcriptional start sites that produce long and short forms of *Sox17* mRNA. Using CRISPR mutagenesis in mice and cultured mouse embryonic stem cells, we observed that deletion of the upstream conserved region (CR1) causes a modest increase in lymphovasculogenesis due to reduced *Notch1* expression, whereas removal of the downstream region (CR2) results in failure of progenitor cell segregation in the HPB bud. However, our studies were complicated by the unexpected retention of an intron within the long form of *Sox17* mRNA in CR2-null embryos that impaired vascular development resulting in embryonic lethality, confounding our phenotypic analysis.

To circumvent the intron retention, and to begin to specifically determine the role of several predicted *cis*-regulatory elements, we again used CRISPR mutagenesis to remove a 50-base-pair (bp) region within CR2. Mice that are homozygous for the 50 bp deletion are viable but lack a gallbladder. Visual inspection of the 50 bp deleted region suggests it contains putative binding sites for both SOX and SP1 transcription factors and a gene regulatory network derived from single-cell RNA sequencing data predicts that a SOX protein may serve as an upstream regulator of *Sox17* expression in endoderm. These findings suggest a model in which a SOX protein, alone or in partner with SP1, bind to *cis*-regulatory elements within CR2 to drive *Sox17* transcription to a level sufficient for gallbladder formation. Identification of the transcription factors binding to this 50 bp element will provide important insights into the molecular mechanisms controlling HPB development, particularly the gallbladder.

Session 2 - Speaker 4

yoU-Net and sarcApp: User-friendly deep learning software for automatic image analysis of cardiac myocytes and beyond

Abigail C. Neininger-Castro[1,2], James B. Hayes Jr.[1], Zachary C. Sanchez[1,2], Nilay Taneja3, Aidan M. Fenix[4-6], Satish B. Moparthi[7], Stéphane P. Vassilopoulos[7], & Dylan T. Burnette[1,2].

1: Department of Cell and Developmental Biology, Vanderbilt University, Nashville, USA
2: Program in Developmental Biology, Vanderbilt University, Nashville, TN, USA.
3: Howard Hughes Medical Institute and Developmental Biology Program, Sloan Kettering Institute, New York, NY, USA
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5: Center for Cardiovascular Biology, University of Washington, 850 Republican Street, Brotman Building, Seattle, WA, 98109, USA.
6: Institute for Stem Cell and Regenerative Medicine, University of Washington, 850 Republican Street, Seattle, WA, 98109, USA.
7: Sorbonne Université, Institut National de la Santé et de la Recherche Médicale, Institut de Myologie, Centre de Recherche en Myologie, Paris, France.

The sarcomere is the fundamental unit of contraction in muscle cells. In cardiac myocytes, it is unknown how these sarcomeres form, and what machinery is required. The search for the mechanism for sarcomere formation has been limited by the lack of automated image analysis tools and quantification techniques. We recently presented a unifying mechanism for cardiac sarcomere formation in which Muscle Stress Fibers (MSFs) containing actinin2 puncta undergo retrograde flow and ‘stitch’ together to form Z-lines, the borders of sarcomeres. Here, I introduce sarcApp, an image analysis tool to quantify and analyze several components of the cardiac sarcomere and related structures, including actin, actinin2, paxillin, titin, and myomesin. This software package utilizes deep learning-based segmentation, as well as real-space quantification to analyze myofibril organization and orientation. We utilize sarcApp’s automatic and unbiased quantification scheme to show that while myosin II-based contractile force is required for the formation of actinin2-positive Z-lines, this force is not required for loading of more mature sarcomere markers including titin and myomesin. These findings contrast with the current model of sarcomere assembly in the field and presents an updated model, implicating unique roles of myosin II-based contractility in thin and thick filament formation.

Session 3 - Speaker 1

MISP is an actin bundler that selectively stabilizes the rootlets of epithelial microvilli

Morales EA[1], Arnaiz C[1], Krystofiak ES[2], Zanic M[1], Tyska MJ[1]

[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232, USA

[2]Cell Imaging Shared Resource, Vanderbilt University, Nashville, TN 37232, USA.

Microvilli are conserved actin-based surface protrusions that have been repurposed throughout evolution to fulfill diverse cell functions. In the case of transporting epithelial cells, microvilli are supported by a core of actin filaments bundled in parallel by villin, fimbrin, and espin. Remarkably, microvilli biogenesis persists in mice lacking all three of these factors, suggesting the existence of additional unknown bundlers. We identified Mitotic Spindle Positioning (MISP) as an actin binding factor that localizes specifically to the proximal end of the microvillar core bundle – the rootlet – in tissue and cell culture models. Gain/loss-of-function assays reveal that MISP facilitates rootlet elongation and stabilization, which is further is reinforce by fimbrin recruitment. Consistent with its ability to elongate rootlets in cells, purified MISP exhibits potent filament bundling activity *in vitro.* We also found that MISP confinement to the rootlet is partially enforced by ezrin, which prevents decoration of the membrane-wrapped distal end of the core bundle. Notably, in single molecule reconstitution assays, MISP preferentially binds to the pointed ends of actin filaments, which may further explain its rootlet specific targeting in epithelial cells. These discoveries reveal how epithelial cells optimize apical membrane surface area and offer insight on the remarkable robustness of microvilli biogenesis and maintenance.

Session 3 - Speaker 2

The Role of Kinesin-1 Mediated Microtubule Sliding in Regulation of Insulin Secretion

Kai Bracey[1], Hudson McKinney[1], Maggie Fye[1], William R. Holmes[2], Guoqiang Gu[1], Irina Kaverina[1]

Department of Cell and Developmental Biology[1] and Department of Physics and Astronomy[2], Vanderbilt University

Pancreatic beta cells have to secrete only a sub-population of available insulin granules to reduce blood sugar to normal levels but yet avoid hypoglycemia. This requires tight coordination between intracellular insulin storage and secretion. We have previously shown that cytoskeletal polymers microtubules (MTs) critically govern this coordination.
Molecular motors use MT tracks to transport and park insulin granules at specific cellular locations. In beta cells, MT network is complex, with interlocked, non-radial network in the cell interior, where secretory insulin granules are trapped by looped transport, and the sub-membrane MT bundle, which promotes insulin granule withdrawal from the secretion sites. Due to these features, MT network limits insulin secretion in healthy beta cells. Given a spike in glucose concentration the microtubule network is remodeled to allow for robust secretion. Here, we report that motor-dependent repositioning of existing MTs is an essential feature of glucose-triggered MT remodeling.

Using real-time imaging of microtubules labeled with single-molecule fiducial marks, we demonstrate that high levels of glucose rapidly induce MT movements. Kinesin-1, a molecular motor that is highly expressed in beta cells and activated downstream of glucose signaling, is capable of transporting MTs along MT tracks, a process known as MT sliding. We found that glucose-triggered MT movements are abolished by inactivation of kinesin-1 or introducing mutations preventing attachment of MTs as a cargo. We show that microtubule sliding is important for cellular distribution of MT minus ends and is influencing the overall MT directionality and architectural features. This means that kinesin-1 likely has a dual role in insulin secretion regulation: directly, via insulin granule transport, and indirectly, via remodeling MT network.
Ongoing work will utilize kinesin-1 mutant incapable of microtubule sliding but capable of insulin granule transport, combined with computational simulations, to reveal the specific contributions of MT sliding into regulation of insulin granule positioning and secretion.

Overall, here we show a novel mechanism whereby glucose stimulation induces MT network remodeling, and, subsequently, MT-dependent control of insulin granule transport for secretion.

Session 3 - Speaker 3

Supermeres are functional extracellular nanoparticles replete with disease biomarkers and therapeutic targets

Qin Zhang[1], Dennis K. Jeppesen[1], James N. Higginbotham[1], Sarah E. Glass[2], Jeffrey L. Franklin[1,2], Qi Liu[3], Robert J. Coffey[1,2]

[1]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
[2]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA
[3]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, USA

Extracellular vesicles and exomere nanoparticles are under intense investigation as sources of clinically relevant cargo. We recently reported the discovery of a distinct extracellular nanoparticle, termed supermere (Zhang et al., Nature Cell Biology 23:1240-1254, 2021). Supermeres differ in morphology, cellular uptake, biodistribution *in vivo* from small extracellular vesicles (sEVs) and exomeres. The protein and microRNA compositions of supermeres are distinct from sEVs and exomeres. Supermeres are highly enriched with cargo involved in multiple cancers (glycolytic enzymes, TGFBI, miR-1246, MET, GPC1 and AGO2), Alzheimer's disease (APP) and cardiovascular disease (ACE2, ACE and PCSK9). Cancer-derived supermeres increase lactate secretion, transfer cetuximab resistance and decrease hepatic lipids and glycogen in vivo. Thus, we have identified a distinct functional nanoparticle replete with potential circulating biomarkers and therapeutic targets for a host of human diseases. I will present additional data that supports exomeres and supermeres being distinct nanoparticles, as well as future directions we are now pursuing.

Session 4 - Speaker 1

Rgp1 regulates collagen secretion and cell shape in zebrafish chondrocytes

Dylan J. Ritter, Gökhan Ünlü, and Ela W. Knapik

Department of Cell and Developmental Biology, Department of Medicine, and Vanderbilt Genetics Institute

Cells produce and secrete proteins to form an extracellular matrix (ECM) required for structural development. Normal ECM secretion is essential for the development of tissues including skin, bone, and cartilage. Defects in the ECM proteins themselves or their associated secretory machinery often manifest as clinical phenotypes. However, the role of secretory machinery in ECM secretion has remained largely unexplored. *In vitro* studies identified Rgp1 as a guanine nucleotide exchange factor (GEF) to activate Rab6a for intracellular trafficking. However, the function of Rgp1 in trafficking mechanisms *in vivo* is still largely unknown because some GEFs can activate multiple Rabs in different pathways. To study Rgp1 in vertebrate development, we used CRISPR/Cas9 to generate *rgp1*-null (*rgp1*$^{-/-}$) zebrafish. Since vertebrate Rgp1 sequences are highly conserved, our findings from zebrafish should be informative of Rgp1 function in humans.

We found that *rgp1*$^{-/-}$ zebrafish embryos present with abnormal craniofacial cartilage. Chondrocytes that compose the craniofacial cartilage have an abnormal cell shape and accumulate type II collagen intracellularly. Abnormal cell shape and collagen accumulations were not phenotypes previously identified *in vitro*, suggesting a novel *in vivo* role for Rgp1. In *rgp1*$^{-/-}$ chondrocytes, we have found that cell shape appears to be regulated by Rab11a, while Rab8a appears to regulate type II collagen trafficking. Interestingly, neither cell shape development nor collagen secretion appears to be regulated by Rab6a. Together, our findings suggest a novel, Rab6a-independent function for Rgp1 in cartilage development, collagen secretion, and chondrocyte cell shape development.

Session 4 - Speaker 2

α-Parvin regulates actin turnover to facilitate kidney ureteric bud development

Dong, X., Bock, F., Bulus N., Viquez, O., Mernaugh G., Zent, R.

Vanderbilt University Medical Center, Nashville, TN, USA

The kidney collecting duct system develops by iterative branching of the ureteric bud (UB), which requires the coordinated migration of epithelial cells. This process involves dynamic cell-extracellular matrix interactions mediated by integrins which tightly regulate the actin cytoskeleton. Integrin function is in part mediated by recruiting scaffold proteins like α-Parvin. We previously showed that a global knockout of α-Parvin led to kidney agenesis. We are currently investigating the role of α-Parvin in kidney branching morphogenesis by selectively deleting it in mice at the initiation of UB development. The mice exhibited severely dysmorphic kidneys and died within 2-3 months. The α-Parvin knockout kidneys showed a significant branching morphogenesis defect with abnormal tubular shape and excessive basal F-actin in the collecting ducts. Similarly, α-Parvin-null collecting duct (CD) cells showed excessive F-actin formation, suggesting a loss of actin turnover. There was a persistent increase in the phosphorylation of cofilin in the papilla of α-Parvin-knockout kidneys and in α-Parvin-null CD cells. Despite intact integrin signaling, α-Parvin-null CD cells had increased cell adhesion and spreading but impaired migration. Mechanistically, α-Parvin-null CD cells and kidneys demonstrated a profound increase in RhoA and Cdc42 activities. Inhibiting the RhoA and Cdc42 GTPases were sufficient to decrease the phosphorylation of cofilin, reverse the increased adhesion, spreading and revert the abnormal migration. Taken together, these finding suggest that α-Parvin is involved in cell adhesion and migration by regulating actin dynamics, which is required to facilitate kidney ureteric bud development.

Session 4 - Speaker 3

Direct sgRNA capture enables whole organism lineage tracking and single-cell CRISPR screening

Mirazul Islam, Yilin Yang, Vishal M. Shah, Alan J. Simmons, Yanwen Xu, Robert J. Coffey, and Ken S. Lau

Department of Cell and Developmental Biology, Vanderbilt University

Abstract: Recently, CRISPR-Cas9-based technologies have been developed to address a wide range of biological questions, including molecular recording of embryonic development and genetic screening at single-cell resolution. However, the use of these technologies is limited due to indirect capture of sgRNAs and no existing approach to capture sgRNAs without modifying plasmid libraries. Here, we design a platform called **N**ative **s**gRNA **C**apture and **seq**uencing (**NSC-seq**) to capture sgRNAs and mRNAs simultaneously at single-cell resolution to overcome these limitations. Applying this platform in a CRISPR-based transgenic mouse line (MARC1) allows us to reconstruct both embryonic and adult developmental lineages at single-cell resolution. In addition, we apply NSC-seq to assess a comprehensive gene expression phenotype for individual gene knockout at the single-cell level using existing whole-genome CRISPR knockout screening plasmid libraries. Thus, NSC-seq enables in vivo molecular recording of developmental processes and cells-of-origin at single-cell resolution. Furthermore, our approach makes the single-cell CRISPR screening simpler for the scientific community and facilitates the discovery of key genes that elicit a specific function.

Odd Posters

Poster Number	
1	Anthony, Christin
3	Arceneaux, Deronisha
5	Arpag, Goker
7	Baer, Brandon
9	Benthal, Joseph*
11	Bhattacharjee, Rahul
13	Bowman, Deanna*
15	Bryant, Jamal
17	Burman, Andreanna
19	Caplan, Leah
No poster	Cencer, Caroline*
21	Cephas, Amelia
23	Chalkley, Mary
25	Chen, Lei
27	Chen, Zhengyi
29	Choudhary, Dharmendra*
31	Colley, Madeline
33	Djambazova, Katerina
35	Donahue, Eric
37	Ebert, Adam
39	Farmer, Veronica
41	Farrow, Melissa
43	Feng, Gaomin
45	Fitz, Gillian*
47	Fort, Loïc
49	Gailey, Casey
51	Gil, Melanie
53	Glass, Sarah
55	Good, Christopher
57	Guenther, Alexis
59	Hayes, James
61	Hepowit, Nathaniel
63	Qualls-Histed, Susan

* Breakout Speaker

P1

Characterization of STK38, a Novel Nuclear Regulator of Wnt signaling

Christin C Anthony[1], Leif R Neitzel[1], Yashi Ahmed[2], and Ethan Lee[1]

[1]Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN 37232
[2]Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth College, Hanover, NH 03755

The canonical Wnt/β-catenin signaling pathway induces cellular responses such as proliferation and cell fate specification, and improper Wnt- pathway activation leads to diseases such as cancer. While much is known about the regulation of canonical Wnt/β-catenin signaling at the level of the receptor and β-catenin degradation, regulation downstream of the destruction complex is poorly understood. Additionally, Wnt signaling results in a wide variety of cellular responses, and how cells determine when to express specific genes also remains poorly understood. Our lab has identified a serine/threonine kinase, STK38, as a novel regulator of Wnt signaling. STK38 is a conserved positive regular, as shown by the effect of STK38 knockdown in *Drosophila*, zebrafish, and *Xenopus* resulting in a reduced Wnt signaling phenotype. Work in HEK293T cells show that knocking down STK38 results in reduced Wnt signal transduction, and that overexpression of STK38 results in overactive Wnt signaling. Additionally, manipulation of STK38 has no effect on β-catenin levels, suggesting it functions downstream of the destruction complex. Further studies are needed to identify the binding partners of STK38 in Wnt signaling and to determine if STK38 functions as a kinase in the Wnt pathway or as a scaffolding protein in the nucleus to regulate context dependent Wnt target gene transcription.

P3

Factors for High Quality scRNA-seq Data

Deronisha Arceneaux, Joey Simmons, Cody Heiser, Bob Chen, Ken Lau

Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN 37232

Single-cell RNA sequencing (scRNA-seq) is a transcriptomic approach that is useful for studying cellular responses, revealing rare cell populations, and tracking the trajectories of developing cell lineages. However, an ongoing challenge in single-cell analyses is distinguishing actual biological discoveries as opposed to artifacts from methodology. Previous work focuses on technical challenges in library preparation and on computational analysis pipelines to better address quality control, nevertheless, these tools all address post capsulation drawbacks, and thus don't combat artifacts introduced during single-cell encapsulation. In our work, we investigate optimizing single-cell dissociation and inDrop specific encapsulation, which has decreased the plethora of apoptotic cells and droplets containing doublets, which has led to a reduction in the abundance of artifacts previously associated with scRNA-seq datasets. We describe a series of experiments in which we optimize cell dissociation methods and methods of encapsulation and show that the alteration of dissociation and microfluidics in inDrop encapsulation decreases the amount of ambient RNA present in our datasets. We expect that these optimizations during the experimental setup will reduce the number of artifacts present, and hence a sounder biological interpretation.

P5

Determination of the tubulin GTP-hydrolysis rate through combination of image analysis and computational modeling

Arpag, G[1]; Farmer, V; Wang, S; Zanic, M[1, 2]

[1]Department of Cell and Developmental Biology
[2]Department of Chemical and Biomolecular Engineering
[3]Department of Biochemistry
Vanderbilt University, Nashville, TN

Microtubules are dynamic polymers essential for a variety of cellular processes. Microtubules polymerize by the addition of GTP-bound tubulin heterodimers to the ends of the microtubule polymer. Incorporation of GTP-tubulin is followed by GTP hydrolysis, forming a lattice composed of GDP-tubulin, with only a 'cap' of GTP-tubulin at the growing end. The GTP-cap is a stabilizing structure ensuring persistent microtubule growth; when the GTP- cap is lost, microtubules switch to depolymerization. The size of the GTP-tubulin cap is the result of the balance between the polymerization rate and the GTP-hydrolysis rate. As a result, determination of the GTP-hydrolysis rate is, in principle, possible by simultaneously measuring the microtubule polymerization rate and GTP-cap length. However, this method relies on accurate measurement of the GTP-cap length, which is challenging due to the unknown nature of the microtubule end structure. Current methods assume a blunt microtubule end structure, in which the protofilaments are all the same length and thus aligned at the same position at the microtubule end, resulting in a single exponential decay of GTP-tubulin population towards the microtubule lattice. However, high resolution imaging of microtubule ends revealed non-blunt microtubule ends, potentially resulting in inaccurate measurements for GTP-cap size, thus impacting estimations of the GTP hydrolysis rate. Standard methods to determine the GTP-cap size employ microtubule end-binding protein EB, whose comet-like localization marks the GTP-cap at the end of a growing microtubule. Here, we use a combination of computational simulations and EB-comet image analysis to improve the measurements of the GTP-cap size and determination of the GTP-hydrolysis rate. We address this problem in two ways: i) assuming a non-blunt microtubule end, and ii) measuring the hydrolysis rate directly by observing intensity decay in a given pixel in time-lapse images. Our findings bear direct relevance for the understanding of the molecular regulation of microtubule dynamics.

P7
Liraglutide as a Prophylactic Treatment for Sepsis-Induced Lung Inflammation and Edema

Brandon Baer, Nathan D. Putz, Dustin Gibson, Lorraine B. Ware, Shinji Toki, R. Stokes Peebles, Katherine N. Cahill, Julie A. Bastarache

Vanderbilt University Medical Center, Nashville, TN, USA

Background:
The severe lung dysfunction that characterizes acute respiratory distress syndrome is associated with excessive inflammation and edema. It occurs after an initiating insult to the lung, the most common of which being sepsis. As such, our research group has been investigating a glucagon-like peptide-1 (GLP-1) receptor agonist, liraglutide, that inhibits several pro-inflammatory pathways associated with sepsis including inactivation of the transcription factor NF-κB.

Hypothesis: prophylactic treatment with liraglutide will reduce the lung inflammation and edema associated with a septic insult through inhibition of NF-κB.

Methods:
We pretreated male and female C57/Bl6J mice every 12 hours with liraglutide (0.1 mg/kg) or vehicle (PBS) for 36 hours prior to a two-hit model of acute lung injury (sepsis and hyperoxia). To induce sepsis, an intraperitoneal injection of either 5% dextrose (control) or cecal slurry (CS) (2.4 mg/g) was administered. Mice injected with CS were then exposed to hyperoxia (HO; FIO_2=0.95) or room air (RA; FiO_2=0.21). At 6-hours post-CS, mice were euthanized, and blood, bronchoalveolar lavage (BAL), as well as lung tissue were collected. Lung inflammation was measured by BAL cell counts as well as concentrations of IL-1b, TNF-a, KC/GRO, IFN-g, and IL-10. Lung edema was evaluated through wet-to-dry weight ratios and BAL protein. Non-pulmonary organ injury was assessed by serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)(liver injury), as well as urea nitrogen (BUN)(kidney injury).

Results:
Compared to PBS and liraglutide treated control mice, CS+HO increased markers of lung inflammation and edema. CS+HO mice pretreated with liraglutide showed a significant reduction in the number of BAL leukocytes (3.18 vs 5.06 x 10^4 cells/ml; p=0.0076) and IL-1b (13.52 vs 41.72 pg/ml; p=0.0041), but a significant increase in IL-10 (36.23 vs 11.60 pg/ml; p=0.0195) compared to vehicle. Pretreatment did not significantly affect BAL TNF-a, KC/GRO, or IFN-g in CS+HO mice. Although liraglutide pretreatment significantly reduced the wet-to-dry weight ratios (4.58 vs 4.92; p=0.028) it did not affect BAL protein for CS+HO mice compared to vehicle. Lastly, liraglutide pretreatment significantly reduced the serum levels of AST (1182.3 vs 1716.63 mg/dL; p=0.044), ALT (186.06 vs 392.95 mg/dL; p=0.0085), and BUN (136.24 vs 195.53 mg/dL; p=0.0321) in CS+HO mice compared to vehicle.

Discussion:
These results indicate that GLP-1 receptor agonism can attenuate CS+HO-induced increases in lung inflammation and edema. However, the cytokine data suggests that these effects are not elicited through NF-κB inhibition but instead may be associated with NLRP3/IL-1b inflammasome inhibition and IL-10 stimulation.

P9

DIFFERENCES IN CHROMATIN ACCESSIBILITY BETWEEN EMERGING ENTERIC NEURONS AND GLIA

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Mammalian enteric nervous system (ENS) development requires migration of neural crest (NC) cells into and along the developing foregut. During migration, enteric NC-derived cells undergo differentiation into neuronal and glial lineages. Differentiation towards mature cell fates requires remodeling of chromatin to stabilize subsequent gene expression patterns. While several single-cell RNA-sequencing (scRNA-seq) analyses of developing and adult ENS have been published, little is known about chromatin accessibility in the developing ENS. Through single-nucleus ATAC-sequencing (snATAC-seq), a method that assays transposase-accessible chromatin, the intermediate to differentiated cell states of the developing ENS can be characterized at single-cell resolution. In this project we aimed to define differences of chromatin accessibility among differentiating neurons and glia in the developing *Mus musculus* ENS using snATAC-seq. “Open chromatin” on a gene body can serve as a very good proxy for expression and in some cases proceeds expression. We hypothesize that chromatin accessibility as identified by snATAC-seq will reveal differential accessibility between enteric neuronal and glial progenitor states at regulatory elements or gene loci that are activated by known and novel transcription factors for ENS development. To accomplish our goal, we isolated enteric neuronal progenitors (ENP) at 15.5 days post coitus by fluorescence-activated cell sorting using a *Phox2b*-H2BCerulean transgene that labels both developing neurons and glia with differential expression (high in neurons; low in ENPs/glia; Corpening et al., 2008). To define cell states in our snATAC-seq data, we performed unsupervised clustering and compared chromatin accessibility between individual clusters. Our analysis has identified differentially accessible loci between chromatin state cluster populations of developing neurons, undifferentiated progenitors, and emerging glia. These are likely to reflect the transcriptional states previously reported in the literature by other groups. The positions of genes closest to these differentially accessible regions are being scanned for canonical binding sites of known transcription factors to identify novel regulatory factors that may be participating in hierarchical regulation of gene expression in the developing ENS. This approach will further expand the gene regulatory network that participates in ENS development and will complement prior scRNA-seq data sets.

P11

Multiple polarity kinases modulate F-BAR protein Cdc15 phosphostatus to inhibit cytokinetic ring assembly

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In *Schizosaccharomyces pombe,* cell polarity signaling is directly involved in cytokinesis by directing the proper positioning of the cytokinetic ring (CR) to the cell middle. The F-BAR protein Cdc15 is essential for cytokinesis and plays a key role in the attachment of the CR to the plasma membrane. Cdc15's ability to scaffold the CR is modulated by the phosphostate of its long intrinsically disordered region (IDR) with phosphorylation inhibiting its ability to bind the membrane and protein partners and also antagonizing the ability of its dimeric F-BAR domain to oligomerize. In this study, we investigated the importance of the major polarity kinases, DYRK kinase Pom1, MARK/PAR-1 kinase Kin1, protein kinase C Pck1 and p21activated kinase Shk1 in controlling Cdc15 phosphostate and inhibiting CR assembly. To understand the signal integration among this group of polarity kinases using Cdc15 as their common substrate, we identified the cohorts of Cdc15 sites phosphorylated by each kinase and determined that while all four kinases phosphorylate Cdc15 to impair its ability to bind the plasma membrane they do so to different extents. Coarse grain simulations indicated that the organization of the Cdc15 IDR relative to the F-BAR domain is impacted by phosphorylation, with a threshold of phosphorylation moving the IDRs apart and toward the F-BAR tips, where the overall IDR negative charge would prevent F-BAR domain oligomerization and membrane interaction. Simulations also suggested that dephosphorylation of Cdc15 would promote its phase separation. Indeed, we found that the dephosphorylated but not phosphorylated Cdc15 IDR undergoes liquid-liquid phase separation in physiological salt concentrations and forms micrometer sized dense and dynamic droplets. Our data suggest that polarity kinases collaborate to prevent Cdc15 condensation on the membrane to inhibit inappropriate CR placement and thereby promote medial cell division.

P13

Using MVID-Causing Mutations to Investigate MYO5B Motor Function

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Myosin Vb (MYO5B) is a nonconventional myosin motor. Mutations in MYO5B lead to the autosomal recessive disorder microvilli inclusion disease (MVID) that causes life-threatening diarrhea in neonates due to the mis-trafficking of apical transporters and proteins. There are over 50 different disease-causing mutations in MYO5B comprised of truncations or frameshifts and point mutations in specific domains of the motor. It is unknown how each mutation is manifested in the function of MYO5B to translocate along actin. Understanding how different MVID-causing mutations in MYO5B disrupt the function of the motor functionality is critical to learning how MYO5B operates within the apical recycling and delivery pathways. I hypothesized that point mutations in the MYO5B motor head lead to defects in motor functionality by impacting the ability to bind actin, translocate, or hydrolyze ATP. To address my hypothesis, I used a live-cell assay to examine the functionality of the MYO5B motor and mutants independently of the cargo domain. A truncated MYO5B (1-1015 A.A) motor construct with a triple citrine tag was created, and constructs containing select patient MYO5B motor mutations. The MYO5B motor domain constructs were co-expressed with mCherry-espin in protrusion forming cell line, LLC-PK-CL4 cells. The localization of the MYO5B-motor at the tips of microvilli indicated a functional motor, while a lack of MYO5B-motor at the tips of microvilli indicated a dysfunctional motor. A tip to cytoplasm ratio was used to quantify this change in distribution. In accordance with previous literature, the wild-type MYO5B motor localized to the tips of microvilli with a ratio of 4.087, while the P660L mutation localized to the bases of microvilli and had a tip ratio of 0.1000. Patient mutations I408F and R824C did not accumulate at the tips of microvilli with a tip ratio of 0.1380 and 0.4963, respectively. Surprisingly some MVID-causing MYO5B motor mutations did not fully impair MYO5B motor function. G519R had a tip ratio of 1.870, and D492G had a tip ratio of 1.660. These partially functional motors indicate that while the MYO5B motor function is not impacted by the mutation, the entire protein is affected by the mutation. Unfortunately for patients with MVID, the prognosis is generally poor, and there are few options for treatment with no curative treatment. Understanding the subtle differences in the phenotype of separate mutations could lead to a better understanding of the disease pathology and lead to personalized medicine for patients with MVID.

P15

Examining DYRK2 kinase as a novel positive regulator of canonical Wnt signaling

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Protein kinases regulate multiple steps of Wnt signal transduction. In an expression cloning screen for proteins that perturb development in *Xenopus* embryos, we identified a kinase, Dual Specificity Tyrosine Phosphorylation Regulatied Kinase 2 (DYRK2) as a Wnt pathway activator. DYRK2 overexpression in *Xenopus* embryos promotes axis duplication, whereas DYRK2 morpholino-mediated knockdown results in ventralized *Xenopus* embryos. In Drosophila DYRK2 is required to promote Wnt pathway activation, thereby demonstrating its conservation of function in the Wnt pathway. In human cells, DYRK2 overexpression enhances Wnt reporter activity, increases intracellular β-catenin levels, and enhances LRP6 phosphorylation. In contrast, knockdown of DYRK2 by RNAi in human cells inhibits Wnt pathway activation and prevents LRP6 phosphorylation. Preliminary *in vitro* kinase assays, using purified, recombinant protein, suggest that DYRK2 cooperates with GSK3 to phosphorylate LRP6. However, activation of human cells with Wnt3a does not shift the migration of DYRK2 on sucrose density gradients into fractions containing LRP6 signalosomes. We propose that DYRK2 has a role in regulating LRP6 receptor activation, but is not part of the active Wnt signaling complex. Future experiments will be performed to confirm the role of DYRK2 in regulating LRP6 activity at the plasma membrane in cells, to determine whether DYRK2 directly regulates GSK3 activity and recruitment towards LRP6, and to assess whether DYRK2 regulates intestinal growth in vivo and in an enteroid system.

P17

Generation of a Novel MVID Patient-Based MYO5B Mutation Mouse Model

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Background: Microvillus inclusion disease (MVID) is a congenital disorder characterized by severe diarrhea that presents within the first few days of life. Inactivating mutations of a motor protein, myosin Vb (MYO5B), have been identified as a cause of MVID. Recently, a patient with compound heterozygous MYO5B mutations, G519R point mutation on one allele and a de novo mutation that leads to early truncation on the other allele, was identified at Vanderbilt Monroe Carell Jr. Children's Hospital. This pediatric patient presented with severe diarrhea and the immunohistological assessment revealed that the patient's duodenum showed blunted villi and a lack of apical NHE3 and SGLT1 expression and basolateral GLUT2-expression in enterocytes. To better understand this novel MVID-inducing mutation in MYO5B, in coordination with the Vanderbilt Genome Editing Resource, we created a mouse carrying the patient-based mutation. Here, we established an MVID patient-mimicking mouse model that displays an MVID-like phenotype and could be used for exploring therapeutic strategies.
Methods: Homozygous MYO5B(G519R) mutations constructed in mice using CRISPR resulted in early lethality. An alternate genome editing technique was implemented to circumvent this early lethality, where chimeric pups for the G519R mutation were created by injecting a ribonucleoprotein targeted to the mutation site and a ssDNA donor carrying the MYO5B G519R mutation into only one cell of a 2-cell C59BL/6J embryo. One F0 founder was backcrossed to wildtype C57BL/6J. A sequence-verified N1 mouse was crossed with *VilCre^{ERT2}; Myo5$^{flox/flox}$* mice to produce progeny carrying a tamoxifen-inducible intestinal epithelial-directed Cre recombinase (VilCre) along with one floxed MYO5B allele and one G519R MYO5B mutant allele. Mice with the desired patient-mimicking genotype (*VilCre^{ERT2}; MYO5B$^{flox/G519R}$*) were induced with 2 mg tamoxifen at 8-9 weeks of age and their GI tissues were collected for immunohistochemical studies.
Results: Heterozygous MYO5B(G519R) mice, recapitulating the patient mother's genotype, showed healthy phenotype. *VilCre^{ERT2};MYO5B$^{flox/G519R}$* mice lost approximately 16% of their original body weight because of watery diarrhea 4 days after the induction of heterozygous MYO5B loss. F-actin staining revealed the appearance of numerous inclusions within the enterocytes. Compared to control (*VilCre^{ERT2}; MYO5B$^{flox/WT}$*) littermates, *VilCre^{ERT2}; MYO5B$^{flox/G519R}$* mice have abnormal accumulation of PAS positive vesicles within enterocytes, thinner brush borders, and blunted villi with an elongated crypt, similar to MYO5B KO mice as well as the patient biopsies. The elongated crypts of these mice correspond to a longer PCNA-stained proliferative zone. MYO5B in enterocytes accumulated in large subapical vesicles and several nutrient transporters were mis-localized away from the brush borders in *VilCre^{ERT2}; MYO5B$^{flox/G519R}$* intestine. These results suggest that the mouse model phenocopies the pathology in the MVID patient.
Conclusions: The two-cell stage mutagenesis provides a highly successful approach to generate heterozygous mice carrying deadly mutations within 4 months. This strategy is a useful tool to understand personalized pathobiology and to seek therapeutic approaches for treatment of congenital disorders, such as MVID.

P19

Enteroendocrine cell formation is an early event in pancreatic tumorigenesis

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Background & Aims:
Pancreatic acinar-to-ductal metaplasia (ADM) is an early response to tissue injury that recapitulates many processes identified in pancreas development. Expression of oncogenic $Kras^{G12D}$ induces ADM, which can progress to pancreatic intraepithelial neoplasia (PanIN) and, eventually, pancreatic ductal adenocarcinoma (PDAC). We have discovered that injury-induced ADM results in substantial enteroendocrine cell (EEC) subtype heterogeneity. ADM-derived EEC formation has been shown in PanIN resulting from expression of oncogenic $Kras^{G12D}$ by lineage tracing and expression of the marker synaptophysin. To examine the presence and proportion of EECs in PanIN we conducted histological analyses in multiple genetic mouse models of PDAC.

Methods:
Pancreata from $Kras^{G12D};Ptf1a^{Cre/+}$ (*KC*) were collected at 6 and 12 months of age. Pancreata were analyzed from $Kras^{G12D};Trp53^{R172H};Pdx1\text{-}Cre$ (*KPC*) mice with or without PDAC. Immunohistochemistry (IHC) was performed for general EEC marker synaptophysin, as well as for EEC subtypes: enterochromaffin cells (serotonin, 5-HT), delta cells (somatostatin, SST), epsilon cells (ghrelin), and gamma cells (PPY). Approximately 60 ADM and pancreatic intraepithelial neoplasia (PanIN) lesions were graded per mouse, from 12 KC and 20 KPC mice. Co-immunofluorescence (co-IF) was performed to examine hormone co-expression. EEC subtype marker expression was examined in human PanIN and PDAC.

Results:
Frequency of all EEC subtypes decreased with increasing lesion grade in all genetic mouse models analyzed. ADM and PanIN1a lesions contain the highest percentage of EECs. Co-IF for EEC subtype hormones demonstrates co-expression of specific hormone combinations. Similar expression patterns were identified in human PanIN and PDAC.

Conclusions:
In mouse models and human PDAC, EECs are most abundant in ADM and low-grade PanIN, consistent with EEC formation as an early event in disease progression. EEC subtypes differ in abundance with disease progression, suggesting differing function(s) throughout tumorigenesis.

No Poster

A novel adhesion-based mechanism stabilizes microvilli on the surface of transporting epithelial cells and supports cell-cell junctions

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The evolution of actin-supported cell surface protrusions, known as microvilli, has allowed transporting epithelia to modify their apical cell surface structure to optimize their function. Such organization is a central theme to how tissues such as the kidney and small intestine expand surface area available for solute transport. Microvilli found on the surface of these epithelia exhibit a well-organized 'brush border' made up of thousands of protrusions connected to their neighbors via a tip-localized adhesion complex composed of cadherins CDHR2 and CDHR5. While it is known that the intermicrovillar adhesion complex (IMAC) plays a role in the final organization and maintenance of the brush border, how nascent microvilli accumulate on the apical surface during differentiation and the role of the IMAC in this process remain unclear. Here we show that, at time points early in differentiation, epithelial cells present two general populations of microvilli: (1) a marginal population at the edges of cells, characterized by high protrusion density, and (2) a medial population characterized by much lower protrusion density. Strikingly, marginal microvilli extend across cell-cell junctions and make direct physical contact with protrusions on neighboring cells. Based on these static observations, we set out to test the idea that the edges of cells provide a point of capture and stabilization for nascent microvilli. Fluorescence recovery after photobleaching (FRAP) and microvilli tracking experiments reveals that transcellular adhesion complexes, formed between marginal microvilli of neighboring cells, are more stable than those bridging medial clusters of microvilli. Furthermore, tracking analysis on live CL4 cells shows that medial microvilli are more motile than marginal microvilli, suggesting that transcellular adhesion serves as an anchoring point for motile microvilli. Given the presence of stabilizing transcellular adhesion complexes, we predicted that cell-cell junction assembly may be influenced by apical CDHR2/CDHR5 cell-cell contacts. Indeed, in a CDHR2 KO mouse model and in CDHR2 KO CL4 cells, tight junction protein ZO-1 is significantly reduced. As a result, KO CL4 cells exhibit increased cell motility, leading to stretched and disorganized cell monolayers. Overall, these findings suggest a new, adhesion-based mechanism for the stabilization of microvilli and support of cell-cell junctions, changing our understanding of how transporting epithelial cells utilize cell-cell contacts to create optimal tissue structure.

P21

Pancreatitis-induced Epithelial Plasticity Enhances the Susceptibility of Multiple Exocrine Cell Types to Oncogenic Transformation

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Background and Aims: Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths in the United States. *KRAS* mutations are found in >90% of sequenced PDACs and pancreatitis is a major risk factor of PDAC. Both pancreatic acinar and ductal cells can seed PDAC, however histological studies suggest that ductal cells are the dominant cell of origin in humans. In mice, acinar cells are readily transformed by mutant $Kras^{G12D}$, but ductal cells are more resistant and require additional tumor suppressor mutations. Recently, we showed that pancreatitis-induced ductal cells derived from acinar to ductal metaplasia (ADM) are susceptible to transformation by $Kras^{G12D}$ expression. Interestingly, we also found that pancreatitis preceding $Kras^{G12D}$ expression in ductal cells results in transformation without additional tumor suppressor mutations. The goals of this study are to 1) explore how $Kras^{G12D}$ mutations occurring in the setting of pancreatitis affect tumorigenesis and 2) identify pancreatitis-induced changes to ductal cells and how this enhances their susceptibility to transformation.

Methods: To induce pancreatitis, mice were injected with the cholecystokinin ortholog, caerulein, for 4 weeks. Subsequently, we expressed mutant $Kras^{G12D}$ in both ADM and pancreatic ductal tissue by employing two tamoxifen inducible cre recombinase mouse models, *HNF1β* and *CK19*. Mice were allowed to recover for 1 to 4 months post-$Kras^{G12D}$ induction and tumorigenesis was analyzed by several histological stains (Trichrome, Alcian Blue, Hematoxylin and Eosin and Immunohistochemistry). To identify pancreatitis-induced changes in ductal cells, single cell RNA sequencing data was mined, differentially expressed genes between normal and pancreatitis-associated ductal cells were identified, and results were confirmed using immunohistochemistry.

Results: Oncogenic *KRAS* expression in pancreatic ducts in the setting of pancreatitis led to pancreatic intraepithelial neoplasia (PanIN) lesions within normal ducts (ductal cell of origin) and ADM tissue in both $HNF1\beta Cre^{ERT/+}$ and $CK19Cre^{ERT/+}$ mice. Comparison of gene expression between normal and pancreatitis-associated ductal cells identified significant changes, including expression of GKN3 and MMP7, which was confirmed histologically.

Conclusions and Future Directions: Pancreatitis alters the pancreatic ductal epithelium and enhances susceptibility to transformation by KRAS. Some of these injury-induced changes were identified, however further studies are required to thoroughly identify how pancreatitis changes ductal cells at the gene and epigenetic levels. Our studies suggest that oncogenic KRAS expression occurring in the context of pancreatitis may more accurately model disease progression as it occurs in humans, providing us with better tools to understand and target this disease.

P23

Early Neurodevelopment and Cytoarchitecture is Altered in Tuberous Sclerosis

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Tuberous Sclerosis Complex (TSC) is a debilitating developmental disorder characterized by a variety of clinical manifestations. While benign tumors in the heart, eyes, lungs, kidney, skin, and brain are all hallmarks of the disease, often the most severe symptoms of TSC are neurological, including seizures, autism, psychiatric disorders, and intellectual disabilities. TSC is caused by a loss of function mutation in the *TSC1* or *TSC2* genes, which encode the hamartin/tuberin proteins respectively. Patients are heterozygous for mutations, but the most widely accepted model is that second hit mutations in *TSC1/2* occur in tumorigenesis. Hamartin/tuberin function as a heterodimer that negatively regulates mechanistic Target of Rapamycin Complex 1 (mTORC1). While TSC neurological phenotypes are well-documented, it is not yet known how early in neural development *TSC1/2*-mutant cells diverge from the typical developmental trajectory, and whether such phenotypes are seen in the heterozygous-mutant populations that comprise the majority of cells in patients.

To examine early neurodevelopmental phenotypes in a cell-based model of TSC, we utilized TSC patient-derived induced pluripotent stem cells (iPSCs) that harbor a heterozygous microdeletion mutation in *TSC2*. To model this state, CRISPR was used to create a similar deletion mutation in the other *TSC2* allele, producing a homozygous mutant line. A TALEN system was also used to correct the heterozygous mutant to wild type, creating a set of isogenic lines. This isogenic series was then compared to a second allelic series in which *TSC2* was deleted using CRISPR editing. Using immunofluorescent microscopy, immunoblotting, and flow cytometry, we observed aberrant early neurodevelopment in both sets of *TSC2* mutant iPSCs. Homozygous mutant neural progenitors exhibit altered behavior as *in vitro* differentiation proceeds, including changes in multicellular structures within the first 10 days with misexpression of key transcription factors associated with lineage commitment. As expected, mutant cells have more active mTORC1, with increased phosphorylation of ribosomal S6 protein, than heterozygous and wild-type cell lines. Collectively, these data suggest that mutation or loss of *TSC2* has early effects on proper neural development. Understanding precisely when development is disrupted *in TSC1/*2- mutant brain will be essential to tailoring treatment and determining whether prenatal diagnosis or treatment should be pursued.

P25

Sin3a epigenetically regulates differentiation and cell fate of cerebellar granule cell precursors

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Granule cells, the most abundant neurons in the central nervous system, are the principal source of excitatory neurons in the cerebellum. Their function is essential for motor coordination and motor learning. Granule cells (GCs) originate from granule cell precursors (GCPs) that undergo rapid and transient proliferation in the external granule layer of the cerebellar cortex. A master intrinsic regulator of GCP development is the Atoh1 transcription factor essential for the formation of GCP lineage. Overexpression of *Atoh1* inhibits, whereas postnatal depletion of *Atoh1* promotes, GCP differentiation. Despite the central role of Atoh1 in regulating GCP development, it is still unclear how *Atoh1* expression is terminated to ensure timely differentiation of GCPs. Dynamic changes in gene expression are generally associated with widespread alterations of the epigenetic landscape of the chromatin. Although epigenetic regulators of histone proteins are essential for cerebellar development, whether or how they control cerebellar cell fates is unknown. In this study, we show that Sin3a, a component of the Histone deacetylase (Hdac) complex, is essential for GC lineage progression. In the absence of Sin3a, GCPs fail to differentiate and exhibit significantly reduced viability. Accordingly, cerebellar size and foliation are severely compromised. ChIP-seq and transcriptome analysis reveals that *Sin3a*/Hdac1 complex acts as an H3K27 deacetylation factor on many loci to repress genes involved in cell fate determination and specification as well as other cellular processes. Notably, the expression of *Atoh1* is repressed by Sin3a/Hdac1 complex. Mechanistically, we show that *Insm,* a novel transcriptional repressor, recruits Sin3a/Hdac1 complex to the 3' *Atoh1* enhancer, thereby inhibiting the transcriptional activity of Atoh1. In summary, our results reveal a novel epigenetic mechanism by which GC lineage progression is achieved during cerebellar development.

P27

Alternative Serrated Origins of Microsatellite Stable Colorectal Cancers

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Molecular profiling of colorectal cancers (CRC) has established the classification of tumors into clinically relevant subtypes, such as the Microsatellite Stable (MSS) and Microsatellite Instability-Hight (MSI-H) classes of CRC. These molecular profiles are performed on established cancers, the endpoints of complex evolutionary processes, and thus, the resulting tumor subtyping may not capture the heterogeneity of tumorigenesis routes. The prevalent model in the field is that adenomas mainly progress to MSS tumors, whereas serrated polyps mainly give rise to MSI-H tumors. However, given the mixed expression profiles seen in bulk transcriptomics data, we hypothesize that a subset of MSS tumors can have a serrated or metaplastic origin. To investigate this unconventional path from serrated polyps to MSS tumors, single-cell transcriptomic analyses of 7 precancerous (9,598 cells) and 60 (60,665 cells) cancerous samples were performed. Serrated-specific gene signatures were identified to be highly expressed in a subset of MSS patient samples. Further examination of the mutational profiles of the MSS samples with high serrated-specific signatures may reveal underlying molecular pathways. Since MSS and MSI-H CRCs have different prognoses, metastatic rates, and therapeutic strategies, our study of human colonic tumor progression paths can provide new insights to facilitate precision treatment of CRC.

P29

Loss of PLOD3 facilitated pro-collagen processing results in autophagy-driven craniofacial defects

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The function and stability of collagen depend on the precise triple-helix configuration of three separate polypeptide chains. Disruption of this triple-helical structure can result in connective-tissue related disorders. Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3 (PLOD3) is a post-translational modification enzyme that catalyzes the hydroxylation of lysine residues and O-glycosylation of hydroxylysine residue on the procollagen molecules. These modifications are necessary for collagen cross-linking, secretion, and structural stability in the extracellular matrix (ECM). It is known that pathogenic variants in PLOD3 result in clinical phenotypes such as joint contractures, scoliosis, reduced palmar creases, low bone mineral density, bone fractures, and sense organ-related abnormalities. Our phenotypic expansion analysis using human genetic data also includes further associated clinical phenotypes such as circulatory and vascular system abnormalities. However, the biological mechanisms linking PLOD3 function to these disease traits are unknown. In this study, we investigated human fibroblast cells and a mutant *plod3*$^{-/-}$ vertebrate zebrafish model to better understand the functional relevance of PLOD3 to its associated pathophysiology.

Our mutant *plod3*$^{-/-}$ zebrafish model displays several clinically relevant phenotypes, such as defects in craniofacial development, contractures in the body, and malformations in sensory and muscle tissue. Human *PLOD3* mRNA is able to partially rescue our zebrafish model, showing that PLOD3 has a highly conserved biological function. We demonstrate that human fibroblast cells from subjects carrying variants in *PLOD3* are defective in collagen processing, leading to collagen accumulation within the cells. Through ultra-structural and molecular studies in our zebrafish model, we show that defects in collagen crosslinking also lead to endoplasmic reticulum (ER) stress as proteins accumulate within the ER. A functional ER is essential during embryonic development, as cells produce a large amount of secreted ECM proteins necessary to build and maintain cartilage and other tissues. Prolonged ER stress disrupts internal secretory pathways and causes trafficking issues which eventually trigger autophagy signals, making the cells more vulnerable to apoptosis. Together, our data suggest that impaired function of Plod3 results in protein accumulation in the ER, triggering the ER stress and leading to cellular autophagy-driven apoptosis. These mechanisms are the likely cause of the clinical phenotypes associated with aberrant PLOD3 function in humans and will help discover new treatment options for novel drug candidates.

P31

A Comprehensive and Spatial Lipidomic Library of the Human Kidney

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Introduction/Background: Lipidomic analyses are frequently targeted and the data is used for a single cohort of samples. More recently, custom LC-MS/MS libraries have been generated to create a semi-targeted environment tailored to individual disease states, organ systems, and populations. The benefit is shorter analysis time, improved statistics and higher confidence in metabolic assignments. However, these libraries are generated without the consideration of tissue morphology or localization. To enhance this, we use a new technology, PASEF IMS, to generate a spatially targeted library in which species are identified by performing MS/MS on tissue. By pairing custom LC-MS/MS databases with collision cross-section aware spatial lipidomic libraries, we generate a comprehensive and qualitative understanding of a biological system within a visual framework.

Methods: Normal human kidney portions from renal cancer nephrectomies were studied for both spatial and bulk lipidomic analysis. Lipidomic analysis was performed by reversed-phase LC-MS/MS with a timsTOF Flex mass spectrometer and a Waters Acquity Premier liquid chromatography system with a 100 mm CSH C18 Waters Premier column. Initial identifications were made with MS-DIAL software using the lipidmaps total lipid library and confirmed manually. These species were then integrated into the BiblioSpec 2.0 sqlite3 custom database format and manipulated with Microsoft Visual Studio SQL Server Data Tools. In parallel, PASEF imaging mass spectrometry is utilized to perform MS/MS on tissue to confirm lipid identity in spatial context. An annotation for the spatially confirmed lipids are added to the RefSpectra table in the “otherKeys” column. All annotations from tissue and LC-MS/MS are also linked with their unique center $1/k_0$ value. Further evaluation of subsequent datasets are searched in Skyline using the custom database and the software utilizes Bruker’s C++ library to convert $1/k_0$ to CCS using their proprietary calculations.

Preliminary Data: Samples from 5 patients were analyzed separated and a pooled reference standard was made from each. We obtained an unfiltered list of > 3000 features from untargeted database searching and narrowed down to 308 unique features in negative ion mode with matched MS/MS spectra and 690 unique features in positive ion mode with matched MS/MS spectra. Each of these species were submitted to the database using LipidCreator to annotate the fragment ions and add them to the database. The library was then populated with each species’ $1/k_0$ value with the Visual Studio SQL editor. To add to this semi-targeted library, spatial MS/MS was obtained with PASEF imaging mass spectrometry on the timsTOF Flex mass spectrometer of two patients. A total of 80 unique annotations have been made which have a spatial context. Furthermore, MALDI ionizes different species than what can be obtained through standard reversed-phase LC-MS/MS, so many phosphorylated and sugar containing lipid species were annotated through on-tissue MS/MS alone. Thus we have created the beginnings of an iterative, living library of lipids from the human kidney which can be used across a multitude of platforms.

P33

Resolving Ganglioside Heterogeneity within Murine *Staphylococcus aureus* Soft Tissue Abscesses using MALDI TIMS IMS

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Gangliosides are sialic acid-containing glycosphingolipids, known to play a role in the immune system. The high degree of structural heterogeneity results in significant variability in ganglioside expression patterns and immune system modulation – processes that are not completely understood. Structural characterization at the site of infection is essential in elucidating host ganglioside function in response to invading pathogens, such as *Staphylococcus aureus* (*S. aureus*). *S. aureus* causes soft tissue abscesses, where infiltrating bacteria form staphylococcal abscess communities and recruited immune cells eventually form a lesion around the pathogen. Imaging mass spectrometry (IMS) allows for untargeted spatial investigation of biomolecules within complex biological systems, such as the mature abscess. Coupled with trapped ion mobility mass spectrometry (TIMS), comprehensive structural and spatial analysis of ganglioside diversity within the abscesses can be achieved.

Preliminary on-tissue matrix-assisted laser desorption/ionization (MALDI) TIMS IMS data were collected from a 10-day post-infection mouse kidney. The data indicate the presence of numerous ganglioside species, whose heterogeneity was categorized based on ceramide and oligosaccharide chain composition, as well as sialic acid composition and position. Specifically, twelve distinct ceramide chains, varying in degree of saturation and number of hydroxyl groups were identified. In terms of the glycan headgroup, seven ganglioside classes, including monosialylated: GM3, GM2, GM1, GalNac-GM1b, extended GalNAc-GM1b and polysialylated: GD1 and GT1 were detected. Two sialic acids – Neu5Ac and Neu5Gc, differing in the presence of a hydroxyl group, were detected. When considering the TIMS-resolved species, isomeric gangliosides, including a- and o- series GM1 species, that differ in the position of a single sialic acid along the chain, were also detected. Preliminary data show that ganglioside distributions vary by ceramide chain composition, within ganglioside classes (GM3 vs GM1), and within isomeric species (a- vs o-series). Currently, MALDI TIMS IMS, supervised and unsupervised machine learning strategies are being developed to help decipher the complexity of the *S. aureus* soft tissue abscess using unique ganglioside molecular signatures.

P35

Endoplasmic reticulum remodeling drives mitochondrial fragmentation in aging

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The world population is aging, and by the mid-2030s, senior adults will outnumber children for the first time in US history. Chronic, age-dependent diseases including cancers, heart failure, and dementias are the leading causes of disability and mortality, and this demographic shift continues to increase their prevalence. The growing public health burden of these diseases highlights a need to prevent or mitigate their progression, allowing patients to live healthier lives as older adults. Declines in cellular processes such as protein homeostasis and mitochondrial bioenergetics are thought to drive age-onset deterioration in organisms ranging from yeast to primates. However, we lack insight on the sequence of events by which these processes fail, and the field has traditionally studied organelles in isolation. A growing body of work implicates the endoplasmic reticulum (ER) as a central regulator of inter-organelle communication, but this role remains under-studied in aging biology. The ER is traditionally associated with the proteostatic role of its sheet subdomains, yet its tubule subdomains have been recently shown to regulate metabolism at inter-organelle contact sites, which are thought to be dysregulated across age-related diseases. These insights link ER form and function to multiple hallmarks of aging, which leads to our overarching hypothesis that alterations in functionally specialized ER subdomains drive cellular dysfunction in aging. To understand the dynamics of ER subdomains in aging, we first used CRISPR/Cas9 to tag the genetic loci of subdomain-enriched proteins in *C. elegans*, a rapidly aging, transparent invertebrate. *In vivo* imaging of multiple markers reveals a striking, age-related remodeling of the ER, including an apparent loss of ER sheets across tissues. Furthermore, the ER tubule network appears to spread through cells early in the aging process. Given the recent discovery that ER tubules direct mitochondrial dynamics at ER-mitochondrial contact sites, we then hypothesized that ER tubules play a role in the mitochondrial fragmentation commonly observed in contexts of aging and age-related disease. By genetically manipulating ER morphology, we show that suppressing ER tubulation is sufficient to preserve mitochondrial morphology with age. Together, these studies demonstrate that the ER is remodeled with age and establish ER tubules as an upstream driver of age-dependent mitochondrial fragmentation. Future studies will investigate the molecular drivers of age-related ER remodeling and mechanisms whereby ER tubules promote mitochondrial fragmentation in aging, thus establishing ER form and function as therapeutic targets in the treatment of age-related disease.

P37

Myriocin’s effect upon Sphingolipid biosynthesis and their role in cellular aging

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Myriocin is a potent inhibitor of sphingolipid biosynthesis that increases lifespan in a variety of model organisms, but how slowing sphingolipid (SL) biosynthesis promotes longevity remains unknown. We recently reported that myriocin treatment of yeast results in a dramatic decrease in intracellular amino acid availability, leading to a state of starvation. Our data suggest that this outcome is driven by decreased activity of amino acid transporters and broad remodeling of protein composition at the plasma membrane (PM), indicating that myriocin effectively uncouples extracellular nutrient availability from cellular metabolism to extend lifespan. In addition to addressing these acute responses to sphingolipid depletion, we have characterized how myriocin preserves translational capacity and mitochondrial function in aging cells. Taken together this work reveals how cells adapt and respond to the inhibition of sphingolipid biosynthesis in a way that promotes cellular longevity.

P39

Investigating the GTP-cap size in cells

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Dynamic instability of microtubules, the stochastic switching between phases of microtubule growth and shrinkage, enables the microtubule network to remodel throughout the cell cycle. A growing microtubule end maintains a cap of GTP-bound tubulin, thought to protect it against transitions from growth to shrinkage, known as 'microtubule catastrophe'. This 'GTP-cap' is hypothesized to increase in size as the microtubule growth rate gets faster. Indeed, in vitro reconstitution studies have demonstrated that the GTP-cap size, marked by the tip-tracking protein EB1, increases as the microtubule growth rate increases. Although EB1 has been used as a probe to track growing microtubule ends in cells for decades, whether EB1 localization, and thus GTP-cap size, scales with microtubule growth rate in cells is not known. Here, we image EB1-GFP in stably-expressing LLC-PK1 cells with high spatiotemporal resolution using iSIM to determine the relationship between microtubule growth rate and GTP-cap size in cells. We directly compare the cellular measurements to those obtained using reconstitution approaches with purified protein components in vitro using the same imaging modality. Furthermore, to determine if the established changes in microtubule dynamics throughout the cell cycle impact the relationship between EB1-GFP comet size and microtubule growth, we analyze cells in both interphase and mitosis. Overall, understanding the relationship between microtubule growth rate and GTP-cap size in cells provides a direct test for the models of microtubule dynamics developed using in vitro reconstitution approaches.

P41

From a cellular and molecular atlas to a biological roadmap of tissue structure and function

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The goal of the Human BioMolecular Atlas Program is to provide a molecular and cellular map of human tissues. Given the number of cells in the human body, high resolution, spatial measurements of cells and molecules are needed to begin to fully grasp the relationship amongst cells and how those relationships impact tissue integrity and function. Our efforts are directed at specifically identifying and interrogating the molecules and cell types that comprise the human kidney. Beyond cataloging and profiling these molecules, true insight is gained by investigating the composition of the tissue on both the molecular and cellular levels and integrating that information into a comprehensive tissue map with high spatial resolution. By interrogating these molecules in biological space, we can generate hypotheses about the connection between spatial location and tissue function of specific cell types as well as the many molecules identified within those cells. Fully integrating the high spatial resolution of immunofluorescence modalities with the rich output of multiomic measurements, and combining these with custom data analysis tools, our team can begin to synthesize the vast array of chemical and cellular data into biological knowledge of human tissue function.

P43

The InsP3R coordinates mitochondrial behaviors to promote longevity

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Mitochondrial function plays a central role in aging and age-onset diseases. While interventions that mildly inhibit mitochondrial function can extend lifespan from yeast to mammals, too severe a disruption in mitochondrial function is toxic. Therefore, in order to therapeutically leverage the beneficial effects of mitochondrial reprogramming in contexts of age or mitochondrial disease, we must understand how cells control this delicate balance between mitochondrial protection and dysfunction. Using a *C. elegans* model, we now reveal that lifespan extension via mild inhibition of electron transport chain (ETC) requires the inositol triphosphate receptor (InsP3R), a conserved calcium release channel on the endoplasmic reticulum (ER) surface. As a key component of inter-organelle communication between ER and mitochondria, the InsP3R mediates calcium flux into the mitochondrial matrix where calcium activates multiple bioenergetic enzymes. We therefore hypothesized that the InsP3R acts as a crutch to support mitochondrial bioenergetics and longevity under conditions of ETC deficiency. Surprisingly, however, while the InsP3R is required for bioenergetic tone in both normal and ETC mutant contexts, mitochondrial calcium import is not required for longevity. This finding suggested matrix-independent pathways by which the InsP3R supports cells undergoing mitochondrial stress. We reveal multiple novel routes by which InsP3R coordinates mitochondrial health and longevity. First, we have identified that the InsP3R also plays novel roles in regulating retrograde transcriptional responses and gene expression. Secondly, the InsP3R regulates mitochondrial fission/fusion dynamics in *C. elegans*. InsP3R mutants possess hyperfused mitochondria, and reversing mitochondrial morphology towards a more fragmented state can rescue ETC longevity. Given the links between mitochondrial fragmentation and mitophagy, we are currently investigating a novel role for the InsP3R in promoting mitochondrial quality control through this pathway. Collectively, these results suggest that the InsP3R plays a central role in reprogramming mitochondrial functions and promoting longevity when ETC function is impaired.

P45

Protrusion growth driven by myosin-generated force

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Actin-based surface protrusions are defining cell features that support a range of biological activities. Long-standing models of protrusion growth suggest that actin filament assembly provides the mechanical force for bending the plasma membrane outward. However, membrane-associated myosin motors are abundant in protrusions, though their potential for contributing growth-promoting force remains unexplored. We developed an inducible system that docks myosin motor domains to membrane binding modules with temporal control. Using this system, we found that application of myosin-generated force to the plasma membrane is sufficient for driving robust elongation of protrusions. Protrusion growth scaled with motor accumulation, required active, barbed end-directed force, and was independent of cargo delivery or the recruitment of canonical barbed-end elongation factors. Application of growth-promoting force was supported by several membrane binding modules and myosin motor domains. Our results reveal that force generated by membrane-bound myosins can directly drive protrusion growth and suggest that this mechanism is applicable in diverse biological contexts.

P47

Stem cell conversion to the cardiac lineage requires nucleotide signaling from apoptosing cells

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Pluripotent stem cells can be driven by manipulation of Wnt signaling through a series of states similar to those that occur during early embryonic development, transitioning from an epithelial phenotype into the cardiogenic mesoderm lineage and ultimately into functional cardiomyocytes. Strikingly, we observed that initiation of differentiation in induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) triggers widespread apoptosis, followed by a synchronous epithelial-mesenchymal transition (EMT). Apoptosis is caused by absence of bFGF from the differentiation medium. EMT requires induction of transcription factors SNAI1/SNAI2 downstream of MESP1 expression, and double knock-out of SNAI1/2, or loss of MESP1 in iPSCs blocks EMT and prevents cardiac differentiation. Remarkably, blockade of early apoptosis chemically or by ablation of pro-apoptotic genes also completely prevents the EMT, suppressing even the earliest events in mesoderm conversion, including BRA/T, TBX6, and MESP1 induction. Conditioned medium from WNT-activated WT iPSCs overcomes the block to EMT by cells incapable of apoptosis (Apop-), suggesting involvement of soluble factors from apoptotic cells in mesoderm conversion. Knockout of the PANX1 channel blocked EMT, while treatment with a purinergic P2 receptor inhibitor or addition of apyrase demonstrated a requirement for nucleotide triphosphate signaling. ATP and/or UTP was sufficient to induce a partial EMT in Apop- cells treated with WNT activator. Notably, knockout of the ATP/UTP-specific P2Y2 receptor blocked EMT and mesoderm induction. We conclude that nucleotides, in addition to acting as chemo-attractants for clearance of apoptotic cells can function as essential paracrine signals that, with WNT signaling, create a logical AND gate for mesoderm specification.

P49

Identifying Regulators of Presynaptic Remodeling in *C. elegans* D-type GABAergic Motor Neurons

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Neural circuits are actively restructured during development as synapses are dismantled in some locations and assembled in others. Despite the importance of synaptic remodeling to circuit function, the underlying molecular mechanisms are largely unknown. To investigate this question, we are exploiting the DD-type GABAergic motor neurons in *C. elegans* which undergo synaptic remodeling during early larval development. In the newly hatched larva, DD presynaptic boutons are initially positioned on ventral body muscles but are then relocated over a ~5 hr period to connect with dorsal muscles. DD remodeling is regulated by the Iroquois-type homeodomain protein IRX-1 which suggests that the DD remodeling program is transcriptionally regulated. To identify potential downstream effectors of this pathway, we used single cell RNA-Seq (scRNA-Seq) to profile DD neurons at periodic intervals that span the remodeling period. Differential expression analysis identified 93 genes that are transiently expressed in remodeling DD neurons. An RNAi screen of these candidates detected a necessary role for the neural cell adhesion protein, NCAM-1, in DD synaptic remodeling. This finding is intriguing because NCAM functions as a key regulator of synaptic plasticity in developing mammalian circuits. NIH Funding: 5T32HD007502 (CG), R01NS10695 (DMM).

P51

MCL-1 as a modulator of mitochondrial dynamics during oligodendrogenesis

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Oligodendrogenesis, the production of myelin-forming oligodendrocytes, is a fundamental process during brain development as alterations may trigger white matter dysfunction leading to neurological disorders. MCL-1 (myeloid cell leukemia-1) is a well characterized anti-apoptotic protein, known to inhibit mitochondrial-mediated cell death. The Gama laboratory investigates the impact of mitochondrial biology and function during brain development using human pluripotent stem cells (hPSCs) model systems. We have shown that MCL-1 regulates mitochondrial morphology and dynamics of hPSCs and neural precursor cells (NPCs), independent of its role in apoptosis. This function of MCL-1 is critical for maintaining the bioenergetic needs of cells during differentiation. However, little is known about how MCL-1 impacts the development and maturation of oligodendrocytes. Our studies show that knocking out MCL-1 in murine neural progenitor cells leads to a decrease in myelinating oligodendrocytes. Thus, our overarching hypothesis is that MCL-1 modulates oligodendrogenesis through the regulation of mitochondrial morphology and metabolism of oligodendrocyte progenitor cells (OPCs). Interestingly, MCL-1 is required for survival of hPSCs, but not NPCs. Thus, novel tools are required to study the role of MCL-1 during neural and glial cell fate transitions. We will silence expression of MCL-1 using CRISPRoff, a programmable epigenetic memory writer, in OPCs. We engineered this system to be Doxycycline-inducible. Our preliminary data demonstrate that CRISPRoff effectively silences the expression of MCL-1 in an inducible manner in HEK293T cells. We are currently generating a CRISPRoff human embryonic stem cell line that will be differentiated into OPCs. We will then use these tools to uncover the molecular mechanisms by which MCL-1 modulates survival and integration of OPCs during critical periods of maturation. Generation of these tools will enable the development of a platform to test therapeutics aimed to alleviate metabolic dysfunctions in white matter diseases such as leukodystrophies.

P53

Evaluation of dipeptidase-1 as an extracellular vesicle-bound marker for colorectal cancer with informative localization patterns

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Colorectal cancer is still the second leading cause of cancer death in the US, highlighting the need for early detection strategies. One promising marker is dipeptidase-1, DPEP1, as it is one of top upregulated genes in both adenomas and colorectal cancers, is not present in normal colonic epithelium, and has been shown to have roles in invasion, proliferation, and metastasis. We sought to characterize DPEP1's release in colorectal cancer for the purpose of developing an informative liquid biopsy modality. We identified DPEP1 as the most abundant protein in extracellular vesicles released from colorectal cancer cell lines by mass spectrometry and determined that it is present in a subset of EGFR+/CD81+ exosomes along with other clinically relevant biomarkers by FAVS analysis. Furthermore, DPEP1+ extracellular vesicle numbers were increased in the plasma from three colorectal cancer patients in comparison to normal controls, underscoring the clinical translatability of our findings. By immunofluorescence, colorectal cancer and adenoma organoids cultured in 3D display one of two distinct localization patterns: apical and luminal or diffuse cytoplasmic staining that is associated with a worse progression free and overall survival for colorectal cancer patients. These two localization patterns also correlate with two different forms of DPEP1 as seen by immunoblot, one of which has been previously uncharacterized. While future studies will involve defining this unexplored form of DPEP1 in terms of secretion and cancer progression, our current results suggest that DPEP1 is an informative marker of extracellular vesicles released from colorectal cancers that could be further developed for diagnostic assessments.

P55

Investigating molecular alterations in fresh-frozen bone tissue as a result of *Staphylococcus aureus* osteomyelitis using multimodal imaging mass spectrometry

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Osteomyelitis is an invasive infection of bone that induces necrosis of skeletal structures and peripheral soft tissues. This debilitating condition is most commonly caused by *Staphylococcus aureus* and carries significant patient morbidity due to the high rate of treatment failure. *S. aureus* gain access to the bone microenvironment and seed inflammatory tissue lesions known as abscesses, which have a segmented cellular architecture defined by layers of leukocytes that surround a central staphylococcal abscess community (SAC). *S. aureus* virulence factors and host immune defenses yield dynamic molecular interactions at the host-pathogen interface, and it is important to understand how these spatially-defined interactions drive the progression of *S. aureus* osteomyelitis. Technologies such as matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) can be employed to facilitate the discovery of spatially resolved chemical information in biological tissue samples to help elucidate the complex molecular processes underlying pathology. Traditionally, preparation of osseous tissue for MALDI IMS has been difficult due to its mineralized composition and heterogeneous morphology. Here, sample preparation methods were advanced to enable multimodal MALDI IMS of undecalcified, fresh-frozen murine femurs allowing the distribution of endogenous lipids to be linked to tissue structures and cell types. The molecular imaging workflow was then utilized to define the lipid and metabolite composition of bone marrow abscesses including the SACs. Host ether lipids and sphingolipids were observed to be abundant in abscesses, and preliminary evidence suggest their involvement in antibacterial immunity and leukocyte signaling. Furthermore, different SACs within the same femur possessed molecular heterogeneity depending on their access to necrotic bone fragments.

P57

SOX9 IS EXPRESSED IN SPEM CELLS AND TROP2+ CELLS DURING GASTRIC CARCINOGENESIS

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Background
Intestinal-type gastric cancer proceeds through a cascade of metaplastic and dysplastic precancerous lesions before becoming adenocarcinoma. While some aspects of this carcinogenesis are well understood, others remain elusive, such as the molecular drivers of the carcinogenic progression. We investigate a candidate protein, transcription factor Sox9, as a driver of gastric carcinogenesis. One of the earliest events in the carcinogenic cascade is the transdifferentiation of gastric chief cells into spasmolytic polypeptide-expressing metaplasia (SPEM) cells. We therefore hypothesize that Sox9 has key roles as a driver of cell transdifferentiation in gastric carcinogenesis. Here, we characterize the expression of Sox9 in all cell types during the carcinogenic cascade, and find that Sox9 is expressed in all SPEM cells throughout the carcinogenic cascade in mouse and human models, and is expressed in a small population of Trop2 positive cells in dysplastic lesions.

Methods
Gastric corpus tissues were obtained from a murine acute injury model of SPEM using drug L635, a parietal cell protonophore, and from chief cell specific Kras-induced mice for each of the major carcinogenic phases: SPEM, intestinal metaplasia (IM), and dysplasia. Additionally, human gastric corpus samples displaying SPEM, IM and dysplasia were examined. All samples were immunostained with a panel of antibodies for Sox9, CD44v9 and AQP5 (SPEM cells), TFF3 (IM cells), Trop2 (dysplastic cells), PCNA (proliferative cells), and MUC5AC (gland surface cells).

Results
$Sox9^{+}/CD44v9^{-}$ cells were observed in the base of the glands in the acute injury model before the onset of SPEM which indicates that Sox9 is upregulated at the beginning of chief cell transdifferentiation into SPEM. In the Kras-induced model, Sox9 was highly expressed in nearly all SPEM cells positive for CD44v9 and AQP5. Sox9 was not observed in cells positive for TFF3 (IM cells) or MUC5AC (surface cells), but was observed in a small population of Trop2-positive dysplastic cells. These results were found in both mouse and human samples. In addition, $Sox9^{+}/CD44v9^{+}/AQP5^{+}$ SPEM cells in the Kras-induced model were largely PCNA (proliferation) negative, but some glands show a region of $Sox9^{+}/CD44v9^{-}/PCNA^{+}$ cells just above the SPEM cell-dominated base which indicates a possible proliferative transition state between SPEM cells and further differentiated cell types.

Conclusions
We conclude that Sox9 is expressed CD44v9+ and AQP5+ SPEM cells, and some Trop2+ dysplastic cells, but is not expressed in TFF3+ IM cells or MUC5AC+ surface cells in mouse or human samples of metaplasia and dysplasia. Therefore, our studies suggest that Sox9 may confer a degree of stemness to chief cells that allows them to transdifferentiate into SPEM.

P59

A role for “non-muscle” alpha-actinins in cardiac myocyte sarcomerogenesis

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The protein alpha-actinin is a dimeric actin-crosslinking protein with multiple paralogs in vertebrates. In general, each specific alpha-actinin paralog is believed to function primarily in either a “muscle-specific” context or in a more general, “non-muscle” context. I present data showing that the traditionally deemed “non-muscle” alpha-actinins, alpha-actinin-1 and alpha-actinin-4, may have unexpectedly important – and opposing – roles during the formation of the cardiac sarcomere, a muscle-specific structure that is fundamental in driving the mammalian heartbeat. In a sarcomerogenesis assay, human cardiac myocytes depleted of alpha-actinin-1 assemble fewer sarcomeres than controls, while those depleted of alpha-actinin-4 are hypertrophic and assemble more sarcomeres. Restoring the levels of alpha-actinin-1 and -4, respectively, with overexpression restores control phenotypes. Furthermore, alpha-actinin-1-depleted myocytes have smaller and fewer adhesions, which normally anchor tension-producing sarcomere precursors to the extracellular matrix as they mature into sarcomeres. Meanwhile, adhesions in alpha-actinin-4-depleted myocytes are also smaller, but more abundant. Taken together, these data indicate alpha-actinin-1 promotes adhesion formation and maturation, while alpha-actinin-4 promotes adhesion turnover. Recently, I have shown >90% knockdown of alpha-actinin-4 in zebrafish embryos using morpholinos, alongside phalloidin staining of zebrafish heart muscle actin 3 days post-fertilization, which I will use to quantify sarcomeres in the zebrafish developing heart in vivo. My overall goal is to use in vitro studies to define the mechanistic underpinnings of actinin-1 and -4-dependent influence on sarcomerogenesis at the level of the individual cell and, subsequently, to establish what magnitude of influence, if any, that mechanistic phenomena uncovered at the cell level have in the context of a developing organ in vivo.

P61

Understanding the role of RBFOX1 in Alzheimer's Disease and tau aggregation

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Background: RBFOX1 was recently identified as a genetic risk factor for AD, with lower RBFOX1 expression correlating with worse cognition in AD patients. How RBFOX1 functions to protect against cognitive decline in AD patients remains unclear, but our preliminary data indicates that it associates with ubiquitin in the context of AD, and that it may have a role in autophagy. Here, we provide an update on our efforts to dissect the cellular function of RBFOX1 and to better understand its role in late-onset AD. Understanding how RBFOX1 protects against cognitive decline in AD patients has strong potential to fuel novel therapeutic strategies for improving outcomes in AD patients.

Methods: We are using a combination of approaches, including ***(i)*** proximity ligation assays to analyze association of RBFOX1 with ubiquitin in brain tissues, ***(ii)*** affinity purification of RBFOX1, followed by analysis of post-translational modifications by enzymatic assays and quantitative mass spectrometry, ***(iii)*** analysis of the LC3-interaction network in brain tissues, and ***(iv)*** analysis of RBFOX1 isoform abundance in AD brain tissues by quantitative mass spectrometry.

Results: Although experiments are ongoing, our preliminary data indicates that specific isoforms of RBFOX1 may function as autophagy receptors, and that RBFOX1 association with ubiquitin occurs specifically in AD brain tissues. We also present recent results to identify differences in RBFOX1 that occur in the AD context, including changes in isoform abundance, alterations to post-translational modifications, and changes to the RBFOX1 protein interaction network.

Conclusions: Based on our preliminary data, we hypothesize that AD-specific isoforms of RBFOX1 function as autophagy receptors, perhaps to promote the turnover of aggregated tau. Ongoing and future experiments will include structure-function analysis to map the features of RBFOX1 that are required for its association with tau aggregates.

P63

TXNIP and ubiquitin sequentially regulate GLUT1 clearance during adaptation to excess glucose

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Glucose transporters are principal gatekeepers of cellular glucose metabolism. Understanding mechanisms that regulate their activity, trafficking, and degradation can provide insight into physiological regulation of glucose homeostasis and diseases that arise from dysregulation of glucose transport. Glucose-stimulated endocytosis and lysosomal trafficking of the human glucose transporter GLUT1 is mediated by the arrestin-like protein TXNIP, which is known to interact with E3 ubiquitin ligases. Here, we report that TXNIP variants unable to interact with E3 ubiquitin ligases are impaired for glucose-stimulated GLUT1 lysosomal trafficking. We also find that glucose availability regulates GLUT1 ubiquitylation, which in turn promotes GLUT1 endocytic clearance and lysosomal trafficking. However, our results do not support a simple E3 adaptor function for TXNIP. Instead, our results suggest excess glucose first stimulates TXNIP-mediated endocytosis of GLUT1 and, subsequently, ubiquitylation to inhibit endosomal recycling and promote lysosomal trafficking. Our findings underscore how complex coordination of multiple regulators is required for fine tuning of GLUT1 stability at the cell surface.

Even Posters

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6	Jimenez, Lizandra
8	Jung, Youn Jae
10	Jyotsana, Nidhi
12	Kassel, Sara
14	Lee, Su-Hyung
16	McAtee, Caitlin
18	Medina, Stephanie*
20	Meegan, Jamie
22	Mulligan, Alexandra
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*** Breakout Speaker**

P2

The microtubule minus end-binding protein CAMSAP2 promotes insulin vesicle biogenesis via facilitating Golgi-ER trafficking, independent of microtubule dynamics

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Abstract

Glucose stimulation induces the remodeling of microtubules in islet β-cells to potentiate insulin secretion. CAMSAP2 is a microtubule minus-end binding protein and is reported to stabilize and position microtubules in several cultured non-β-cells. We found that CAMSAP2 specifically binds to microtubule minus ends in immortalized insulinoma MIN6 cells. Surprisingly, CAMSAP2 is localized to the Golgi apparatus in primary islet β-cells. This novel localization is specific to primary β- but not α-cells in islets and it is independent of microtubule-binding. Henceforth, depletion of CAMSAP2 by shRNA impairs Golgi-ER trafficking, reduces total insulin content, and attenuates GSIS without affecting microtubule dynamics or releasability of insulin vesicles in islet β-cells. Corresponding to these results, we found that primary islets and MIN6 cells express different CAMSAP2 isoforms. We propose that primary islet β cells use a novel CAMSAP2 isoform for a microtubule-independent non-canonical function, which promotes Golgi-ER trafficking that supports efficient production of insulin secretory vesicles.

P4

Epigenetic variation in embryonic islet progenitors can pre-dispose their descendant beta cells to different levels of proliferation and function

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The endocrine islets in vertebrates contain several cell types, including beta cells that produce and secrete insulin to regulate blood glucose homeostasis. Building sufficient functional β-cell mass, the product of mass and function, is a prerequisite for preventing diabetes. Intriguingly, past studies have shown that islet beta cells are a mix of subpopulations with distinct gene expression profiles, proliferation rate, metabolic profile, Ca2+ activities, and levels of function (insulin secretion per cell). Thus, understanding how beta-cell subpopulations are made has important implications on how to improve functional beta-cell mass to reduce the risk of diabetes. We have recently reported that the endocrine progenitor cells (transiently express transcription factor Ngn3) can be divided into subsets based on DNA methylation status on some gene enhancers and their co-expression of protein markers (e.g., Myt1). Henceforth, we detected the co-presence of Myt1+Ngn3+ and Myt1-Ngn3+ cells, with the former expressing higher levels of DNMT1 (a gene required for DNA methylation) and biased toward beta-cell fates. Because DNA enhancer methylation has been suggested to direct beta-cell function, we postulated different endocrine progenitors with different DNA methylation status can give rise to beta-cell subsets with different properties. Using irreversible combinatorial lineage tracing, we now show that the subset of β cells derived from $Myt1^{+}Ngn3^{+}$progenitors have higher proliferation rate and glucose-stimulated insulin secretion compared to those from $Myt1^{-}Ngn3^{+}$ progenitors. Corresponding to these functional differences, we found several differentially expressed genes and differentially methylated gene regulatory regions between the two beta-cell subpopulations. These findings suggest that the beta-cell heterogeneity can arise due to the differential epigenetic modifications (including DNA methylation) that happen before endocrine commitment. Future studies will examine the causal relations between these modifications and beta-cell proliferation and function.

P6

Extracellular vesicles from non-neuroendocrine SCLC cells promote adhesion and growth of neuroendocrine SCLC cells

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Small Cell Lung Cancer (SCLC) tumors are made up of distinct cell subpopulations, including neuroendocrine (NE) and non-NE cells. While secreted factors from non-NE SCLC cells have been shown to support the growth of the NE SCLC cells, the underlying molecular factors are not well understood. In this study, we found that conditioned media and small extracellular vesicles (EVs) secreted from non-NE SCLC cells promote adhesion and growth of NE SCLC cells. NE SCLC cells serially grown in non-NE conditioned media maintained the adhesive morphology for up to six passages. We observed that this NE SCLC morphology change is not permanent, as we observed that the NE SCLC cells reverted to their suspension appearance in regular growth media. A TMT proteomic analysis of purified small EVs revealed that extracellular matrix (ECM) proteins and integrins are highly enriched in small EVs of non-NE SCLC cells, whereas ribosomal proteins are abundant in small EVs purified from NE SCLC cells. Addition of purified ECM proteins, particularly fibronectin, laminin 411, and laminin 511, were able to substitute for the role of non-NE-derived small EVs in promoting adhesion and survival of NE SCLC cells. These data suggest that non-NE SCLC cells play a key role in supporting SCLC tumor growth and survival through secretion of ECM-carrying small EVs.

P8

Efficient extracellular vesicle production in hollow fiber bioreactors for RNA delivery

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Extracellular vesicles (EVs) have emerged as a promising strategy to deliver effector molecules for intercellular signaling. Current approaches for EV production typically rely on 2D cell culture due to lack of a scalable biomanufacturing platform. However, the low EV production yield from 2D cell culture remains a challenge. In this study, we used hollow fiber bioreactors, which allows cells to grow to high density under 3D-like conditions to produce EV-enriched conditioned media without serum contamination. We investigated the production yield and the characteristics of small EVs (sEVs) purified from DKO-1 cells and hTERT-MSCs via hollow fiber bioreactors. sEVs were collected by iodixanol gradient ultracentrifugation and characterized by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and Western blot analysis. The number of sEVs purified from bioreactors was increased approximately 20-fold compared with that purified from an equivalent volume of conditioned medium from cells cultured in 2D. DKO-1 and hTERT-MSC derived sEVs from holl-ow fiber bioreactor displayed the expected round shape and size (50-200 nm in diameter) along with EV marker proteins, including Flotillin-1 and CD63. In addition, we evaluated the expression levels of miRNAs in sEVs by RT-qPCR. Small ncRNAs in sEVs collected from bioreactors, including U6, miR-100, miR-125b and let-7a, were well expressed and protected from RNase. The ability of miR-100 transfer to recipient cells via sEVs was also evaluated. Our results demonstrate that hollow fiber bioreactors can enhance the production of RNA-containing EVs from cells while also preserving the integrity.

P10

Lipid nanoparticle mediated targeting of genetic drivers in pancreatic cancer and its precursors.

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Background & Aims:
Nearly all cases of pancreatic ductal adenocarcinoma (PDAC) are caused by mutation of the KRAS gene. Intraductal papillary mucinous neoplasms (IPMNs) are pre-malignant precursors of PDAC. IPMNs harbor *KRAS* (~80%) and/or *GNAS* (~66%) driver mutations. Loss of tumor suppressor *SMAD4* occurs in 13% of IPMNs and 50% of PDAC and is associated with disease progression in humans and mouse models. Emerging evidence suggests that KRAS-mutant cancers are particularly vulnerable to inhibition of cystine import via SLC7A11 (Solute Carrier Family 7 Member 11). Despite much research, oncogenic drivers such as *KRAS* and *GNAS* have proven difficult to target and there are currently no therapies designed to restore tumor suppressor function. SLC7A11 inhibitors in clinical trials are associated with significant off-target toxicity. To address these issues, we combined the latest lipid nanoparticle (LNP) delivery systems with siRNAs targeting SLC7A11, mutant-KRAS, and/or mutant-GNAS or with mRNA to restore SMAD4 expression as a novel and effective therapeutic strategy against PDAC.

Results:
LNP-RNA formulations are efficiently and stably taken up by human PDAC and murine IPMN cells. MSLN antibody- conjugated LNPs showed enhanced uptake in PDAC cells. Target specificity of LNP-mutant-KRAS, LNP-mutant-GNAS, and LNP-SLC7A11 was confirmed in various cell lines. LNP-SMAD4 RNA treatment resulted in restoration of SMAD4 levels and its target genes. PDAC cell lines show differential dependency on cystine for their survival and upregulate SLC7A11 expression under conditions of cystine starvation. Co-targeting of mutant-KRAS and SLC7A11 decreased total live cell count in various PDAC cell lines and significantly increased cell death in MIA Paca-2 cells under low cystine conditions. *In vivo* pilot studies suggest that LNP uptake is enhanced in pancreatic cancer precursor lesions as compared to the normal pancreas.

Conclusions:
LNP-mediated delivery of RNAs can efficiently silence mutant-KRAS and mutant-GNAS drivers and restore levels of tumor suppressor SMAD4 in IPMN and PDAC cell lines. LNP-siRNA mediated co-targeting of mutant-KRAS and SLC7A11 synergistically inhibit PDAC cell growth *in vitro*. LNPs are efficiently taken up by pancreatic cancer precursor lesions *in vivo*. Based on these results, we will use this LNP-RNA targeting/co-targeting approach to 1) understand the role of common genetic drivers in IPMNs/PDAC and to 2) effectively target IPMNs/PDAC *in vivo*.

P12

USP47 deubiquitinates Groucho/TLE to Promote Wnt-β-catenin Signaling

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The Wnt pathway is essential for embryonic development and adult tissue homeostasis. We previously showed that Wnt signaling converts T cell factor (TCF), also known as lymphocyte enhancer factor (Lef), from a transcriptional repressor to an activator in a process facilitated by the E3 ligase, X-linked inhibitor of apoptosis (XIAP). XIAP monoubiquitinates the transcriptional repressor Groucho (also known as transducing-like enhancer protein, TLE), decreasing its affinity for TCF/Lef. In a genome-scale screen in *Drosophila melanogaster* S2 cells, we identified the deubiquitinase USP47 as a positive regulator of Wnt signaling. We found that USP47 was required for Wnt signaling in *Drosophila, Xenopus laevis,* and human cells, indicating evolutionary conservation. Knockdown of USP47 inhibited Wnt reporter activity and USP47 acted downstream of the b-catenin destruction complex. USP47 interacted with TLE3 and XIAP but did not alter their amounts; however, knockdown of USP47 enhanced XIAP-mediated ubiquitination of TLE3 in cultured human cells. Finally, we showed that USP47 inhibited ubiquitination of TLE3 by XIAP in vitro in a dose-dependent manner. Our data suggest a mechanism by which regulated ubiquitination and deubiquitination of Groucho/TLE enhances the ability of b-catenin to cycle on and off TCF, and thereby promotes the b-catenin-dependent regulation of Wnt target genes.

P14
DISCRETE FIBROBLAST SUBSETS NURTURE GASTRIC CARCINOGENESIS

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Introduction: In gastric carcinogenesis, stomach epithelium undergoes several sequential stages, including atrophic gastritis, metaplasia and dysplasia. Chronic inflammation with alterations in a variety of immune cells and fibroblasts promotes metaplasia progression. However, it remains unclear how diverse fibroblast populations contribute to gastric cancer (GC) development.

Methods: Stomach tissues from 5 GC patients were used for single cell-RNA sequencing (scRNA-seq) to evaluate cellular heterogeneity and classify each type of cell. We performed histopathology and immunofluorescence on gastric tissues for geographical analysis of fibroblasts. Isolated gastroid and fibroblast lines were used for co-culture experiments.

Results: From scRNA-seq, we obtained 2,709 visible fibroblast transcriptomes that can be divided into four subsets based on the differential gene expression of four genes; *ACTA2*, *PDGFRB*, *PDGFRA* and *FBLN2*. *ACTA2* was prominently expressed in myofibroblasts which were further divided into two subsets; myofibroblast A and B (MfA and MfB), with MfB also expressing *PDGFRB*. Two fibroblast A and B (FbA and FbB) populations were defined by expression of *PDGFRA* or *FBLN2*, respectively. We examined the distribution of these four different subsets. FbA were located in the isthmus in normal corpus, but expanded throughout te entire metaplastic gland in close proximity with the metaplastic lineages. This subset in dysplastic or cancerous tissues also surrounded the epithelial compartment, but showed a more disorganized pattern. Expansion of FbB appeared at the base of inflamed or metaplastic mucosa associated with lymphoid aggregation. FbB cells were interspersed between metaplastic glands, but were set back from the metaplastic lineages. MfB were dramatically increased only in dysplastic or cancerous tissues, with some association with cancerous lineages. There was no significant change in MfA in most samples. Based on this observation, we performed co-culture of patient-derived metaplastic gastroids (GOs) with fibroblasts isolated from metaplastic or cancer-derived regions from the same patient. RNA sequencing showed that the metaplasia- and cancer-derived fibroblasts were enriched for FbB and FbA, respectively. Compared to GOs cultured alone, metaplastic GOs cultured with metaplasia-derived fibroblast showed disrupted multilayer growth with higher expression of the dysplasia marker, TROP2, indicating metaplasia-derived fibroblasts can promote progression of metaplasia into dysplasia. While cancer-derived fibroblasts increased expression of TROP2 in metaplastic GOs, they also significantly enhanced proliferative activity of metaplastic GOs.

Conclusion: We have identified four different fibroblast subsets with distinct geographical distributions and functional heterogeneity in gastric carcinogenesis.

P16

Exosomes in Filopodia Formation

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Exosomes are small extracellular vesicles (SEVs) that carry a variety of cargoes and have been shown to promote tumor cell motility and metastasis. Cell motility is influenced by dynamic formation and stability of filopodia: actin-rich protrusions that extend from the leading edge and perform directional sensing. Filopodia regulators such as fascin are upregulated in multiple epithelial cancers and can promote invasive phenotypes. However, how filopodia are induced and controlled by extracellular factors is poorly understood. Here, we describe a role for SEVs in regulating filopodia formation and tumor cell motility. Inhibition of exosome secretion in cancer cell lines led to decreased filopodia numbers. Specificity to SEVs was demonstrated by rescue experiments in which purified SEVs but not large EVs rescued the filopodia phenotypes of exosome-inhibited cells. Live imaging revealed that exosome secretion regulates filopodia formation and stability. Proteomics data and molecular validation experiments identified the TGF-beta coreceptor endoglin (Eng) as a key SEV cargo regulating filopodia formation, cancer cell motility, and metastasis. Additionally, THSD7A (thrombospondin type-1 domain-containing protein 7A) expression was also reduced in shEng SEVs and contributed to filopodia formation in tumor cells. Finally, trafficking of THSD7A into SEVs seems to be dependent upon endoglin. Here, we identified exosomal endoglin as a regulator of filopodia formation and metastasis. These data are relevant to cancer as endoglin expression is altered in many cancers. In addition, endoglin is the disease gene for hereditary hemorrhagic telangiectasia, and may influence angiogenesis. Overall, our data implicate SEV-carried endoglin as a key cargo regulating filopodia.

P18

Quantifying neural cell identity with mass cytometry in ex vivo cultures of primary human glioblastoma

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Background: Glioblastoma (GBM) is the most common primary brain tumor in adults and remains poorly understood at the cellular level despite being highly aggressive and nearly always fatal. Recent work in the Irish and Ihrie labs identified two GBM cell subtypes with distinct biological phenotypes whose abundance stratifies patient survival. One type, Glioblastoma Negative Prognostic (GNP) cells, was distinguished by elevated cell signaling (phosphorylation of STAT5, AKT, and S6) and abnormal co-expression of proteins normally restricted to neural stem cells or differentiated astrocytes (SOX2 and S100B, respectively). While clinically significant, the GNP cell subset is not well represented in GBM cell lines, and it is not known whether GNP cells depend on the observed signaling. Here, a mass cytometry panel focused on neural differentiation and neural stem cells was applied to track the impact of kinase inhibitors in ex vivo cultures of primary human GBM.

Methods: Mass cytometry was used to measure 47 features per cell, including neural cell identity proteins and phospho-proteins. Dimensionality reduction with t-SNE, T-REX (Barone et al., eLife2021), and biaxial gating approaches were used to quantify the abundance of neural cell types and track changes in signaling and cell identity. Cells collected with informed consent from a primary human GBM tumor were treated for 24 hours with kinase inhibitors imatinib, tofacitinib, and rapamycin to target hallmark signaling nodes active in GNP cells. over Ex vivo culture experiments also varied conditions and tracked neural stem cells and cells with features of differentiated oligodendrocytes (SOX10), neurons (TUJ1), and astrocytes (S100B) after 7 days.

Results: Following imatinib treatment, SOX2+/S100B+ GNP cells died via apoptosis (cleaved Caspase 3+). Cells persisting in cultures after treatment were phenotypically consistent with neural stem cells, seen in higher per-cell SOX2 protein and a lack of all differentiation markers. Changes in culture conditions had opposing impacts from kinase inhibition and increased expression of differentiation markers. For example, removal of supplement B27 and non-essential amino acids (NEAA) caused GBM cells to adopt multiple differentiated phenotypes, including SOX10+ cells, S100B+cells, and TUJ1+ cells.

Conclusions: These results establish a core set of protein features for single cell tracking of neural cell identity and demonstrate two ways to shift this identity in primary human tumor cells. First, inhibitor treatment killed key GBM cell subsets but resulted in a culture enriched for SOX2++ cells, supporting the idea that inhibiting multiple signaling pathways will be needed in GBM. Second, changing the culture media composition appeared to differentiate GBM cells. These results establish a framework for shifting GBM cell identity that may enable more effective targeting of therapy-resistant cells in human tumors.

P20

Cell-free Hemoglobin-Oxidized LDL Axis Contributes to Microvascular Endothelial Barrier Dysfunction and Poor Outcomes During Sepsis

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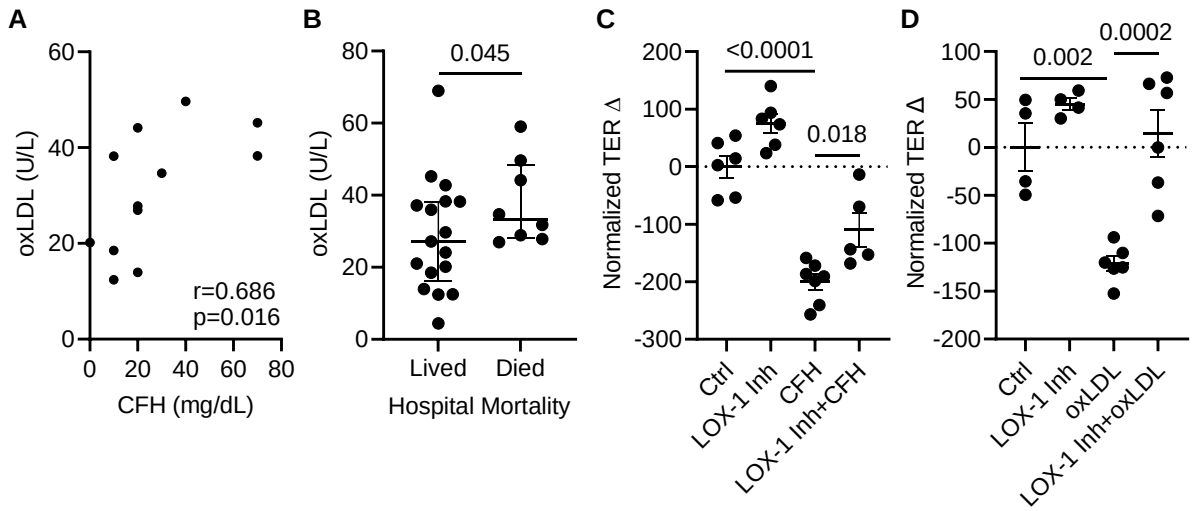
Sepsis, a dysregulated host response to infection with high morbidity and mortality, is characterized by a systemic inflammatory response and widespread vascular hyperpermeability leading to edema, organ dysfunction, and death with no specific treatments. Disruption of the microvascular endothelial barrier is a critical pathological feature of sepsis-induced organ dysfunction driven by circulating inflammatory mediators, oxidants, and proteolytic enzymes. Cell-free hemoglobin (CFH) is one such factor, released from damaged red blood cells during sepsis due to their increased deformability and fragility. Though CFH is not detectable in healthy subjects, plasma CFH is elevated in patients with sepsis and independently associated with mortality. CFH contributes to endothelial dysfunction, but the signaling mechanisms are incompletely understood. CFH can oxidize circulating low-density lipoproteins (oxLDL), which could potentiate endothelial barrier disruption. We hypothesized that elevated levels of CFH during sepsis are associated with increased oxLDL and contribute to microvascular endothelial barrier dysfunction through the oxLDL receptor, lectin-like oxidized LDL receptor 1 (LOX-1). **Methods:** Circulating levels of CFH and oxLDL were measured in 25 sepsis patients. In human lung microvascular endothelial cells (HLMVECs), transendothelial electrical resistance (TER), a measure of barrier dysfunction, was assessed by Electric Cell-substrate Impedance Sensing (ECIS). **Results (Figure):** In sepsis patients, plasma oxLDL levels correlated with CFH (r=0.686; p=0.016, n=12) and were higher in patients who died in hospital (33.24 U/L [IQR 28.08, 48.28] vs. 27.19 U/L [IQR 16.24, 38.24]; p=0.045, n=25). HLMVEC barrier dysfunction induced by CFH (p<0.0001, n=6-7) or oxLDL (p=0.002, n=4-6) was attenuated by blocking the LOX-1 receptor with small molecule inhibitor BI-0115 (Boehringer Ingelheim, 20 µM; p=0.018 vs CFH, n=5; p=0.0002 vs oxLDL, n=6). **Conclusion:** Increased plasma CFH and oxLDL are associated with HLMVEC barrier dysfunction and poor clinical outcomes during sepsis; one mechanism by which CFH may cause vascular hyperpermeability during sepsis is through oxidation of LDL which can drive signaling through the LOX-1 receptor.

Funding: NIH HL094296, HL103836, HL135849, HL158906, HL150783



P22

Investigating Dietary Restriction Induced ER Remodeling and Calcium Homeostasis

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Dietary restriction (DR) is an effective and evolutionarily conserved intervention for promoting longevity and protecting against age-related diseases. Defined as reduction of nutrient intake without malnutrition, DR confers benefits via widespread reprogramming of cell and organismal metabolism and physiology. While the key signal transduction and gene expression pathways mediating the effects of DR are increasingly well-mapped, remodeling events at the cell biological and organelle level, and their effects, remain understudied. Complex and dynamic organelle networks, such as the mitochondria and endoplasmic reticulum (ER), undergo drastic morphological changes in response to nutrients. ER structure is defined largely by its balance of smooth tubules, which perform roles in lipid biosynthesis and inter-organelle calcium signaling, with rough cisternal subdomains, which are hubs for protein homeostasis and secretion. While we know these structures correlate closely with healthy cellular function, our understanding of ER dynamics during DR and organismal aging remains limited. Using a *C. elegans* model, we find that aging causes striking alterations in ER morphology and specific ER-resident proteins. Furthermore, DR protects ER morphology and protein expression against these age-related changes. Together these results suggest that regulation of ER dynamics is a novel mechanism by which DR protects against age-dependent dysfunction, as well as a role for ER calcium in DR mediated longevity. Here I will present my hypothesis, that DR promotes longevity by protecting against ER-shaping protein loss, thus improving ER calcium signaling. I propose that protecting ER structures from age-dependent shifts preserves its function, including storage of calcium, as well as maintaining calcium dependent cytosolic signal transduction pathways. I will explore these possibilities through a combination of live fluorescence imaging and ultrastructural analysis of organelle structures via focused ion beam scanning electron microscopy (FIB-SEM) to determine gross ER quantity, subdomain shifts, and contact sites with the mitochondria and other membrane bound organelles. I also plan to introduce techniques to analyze ER calcium dynamics using fluorescent calcium reporters and microfluidics to investigate changes in ER calcium content, as well as its distribution to the cytosol and other organelles. Collectively, these experiments will inform the way DR protects ER morphology and calcium homeostasis against age-related dysfunction.

P24

Analysis of surface markers as biomarkers for small cell lung cancer

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Introduction: Small Cell Lung Cancer (SCLC) is an aggressive tumor type, usually metastatic at diagnostic leading to poor overall survival. Interestingly, SCLC tumors are composed by distinct subpopulations of cells that cooperate as an ecosystem to drive tumor survival. Since the subtype of SCLC may have prognostic significance, the aim of this study was to identify surface marker proteins as biomarkers of SCLC.
Methods: A linear discriminant analysis (LDA) model, implemented in Python via Sci-kit learn, was used to choose the best 4 markers for distinguishing subtypes. This analysis was based on RNA-seq data from a previous study. In order to identify EV-based biomarkers that would identify SCLC EVs and not normal EVs, we excluded from this analysis proteins without a verified transmembrane domain and proteins associated with EVs expected to be present in white and red blood cells, and endothelial cells (according to Exocarta and Vesiclepedia databases). We also prioritized proteins that could be pan markers for SCLC and that might have prognostic significance. To validate our findings, we performed Western blotting and Flow cytometry in SCLC cell lines from different subtypes.
Results: Our RNA analysis indicated that the best 4 surface markers to distinguish SCLC subtypes were *CEACAM5, FAM174A, LRFN1, EPHA2*. Immunoblot analysis validated CEACAM5 and EPHA2 but not FAM174A or LRFN1. We also found that NCAM1, a commonly used SCLC marker, only marks some of the subtypes. For further analysis we chose proteins with antibodies validated for flow cytometry as our chosen biomarker platform. Flow cytometry analysis of CD24 is suitable as a pan-SCLC marker. However, the expression of NON-NE cell lines was decreased compared to RNA-seq data.
Summary/conclusion: Protein analysis of CEACAM5 and EPHA2 corresponded to RNA-seq data. NCAM was not detected as a pan marker for all SCLC subtypes. However, we could see CD24 expression in all SCLC subtypes, indicating it may be a useful pan marker for SCLC. Future studies will be performed to validate the expression of other surface markers in cells, purified EVs, and plasma of SCLC patients.
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RETURN CODE: 7ML889FK

P26

The Mystery of the *Peroxidasin* Mutant: Why Does this Catalytically Dead *Drosophila* mutant survive?

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The basement membrane is a sheet-like extracellular matrix that underlies epithelia and surrounds muscles. In the gut of *Drosophila*, the stiff basement membrane surrounds the muscles used in peristalsis to keep them flat and smooth. Collagen IV is one of the main components of the basement membrane, where it adds structure and stiffness. The *Peroxidasin* (*Pxn*) gene encodes an enzyme that crosslinks collagen IV at the NC1 domain. This crosslinking supports basement membrane stability and contributes to its stiffness. Hypomorphic P-element mutations of *Pxn* exist in *Drosophila* that survive to the larval stages with decreased viability to adulthood, but no null mutation has been reported. We expected a null mutation to be lethal at the end of embryogenesis, when collagen IV mutants die. Using CRISPR, we created a mutant (Pxn^{11}) that deletes a portion of the catalytic domain, eliminating its activity. Further, this deletion also caused a frameshift mutation that inserted a stop codon soon after the deletion. Unexpectedly, about twenty percent of expected homozygotes survive and live an apparently normal lifespan. These homozygotes exhibit muscle defects in the gut consistent with loss of stiffness in the basement membrane. A few hypotheses that could account for their viability will be discussed.

P28

Proximal Tubule Targeted Gene Therapy for Cystinuria

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Monogenic kidney diseases such as cystinuria are well characterized genetically, but lack safe and effective clinical treatments. Patients with cystinuria form numerous cystine-based stones in their urinary tract due to failure to reabsorb cystine in renal proximal tubule epithelial cells. The most common subclass of cystinuria, type A, is a result of a homozygous deficiency of *SLC3A1*, which encodes an amino acid transporter (rBAT) that reabsorbs cystine in proximal tubules. We have previously shown significant reductions in urinary cystine levels in murine models of type A cystinuria through plasmid delivery of transposable elements containing *SLC3A1*. However, gene transfer was estimated to be <5% of proximal tubule epithelial cells within the injected kidney and therefore did not affect cystine stone formation. Gene therapy delivery to the kidney has been a hurdle for the renal genetics field, but recent innovations in viral vectors have allowed for improved renal transduction. With a novel adeno-associated virus (AAV) from our collaborator (Asokan, Duke University), we have shown efficient viral delivery to the proximal tubule in murine models. However, the proximal tubule-targeting AAV utilizes a reduced viral genomic packaging size due to self-complementary DNA. In order to design a gene therapy strategy for type A cystinuria using this AAV, the transgene packaging limitations of the AAV necessitate the splitting *SLC3A1* into two AAVs. Full length *SLC3A1* expression can be induced by recombination of the split *SLC3A1* transgene using homologous recombination and mRNA splicing within the host cell. We have validated this hybrid reconstitution strategy to express *SLC3A1* in in vitro models and will further characterize it in organoid and murine models of type A cystinuria. Renal phenotypic correction of disease has yet to be accomplished, but our current efforts to optimize the delivery, integration, and stable expression of desired transgenes through AAV and transposon engineering will allow for an enhanced understanding of barriers to renal gene therapy.

P32

Cell-Free Hemoglobin Increases Leukocyte Adhesion and Mitochondrial Oxidative Damage in the Pulmonary Microvascular Endothelium

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Sepsis is a critical problem in intensive care units globally, and accounts for 20% of all annual deaths. During septic shock, the highly inflammatory state leads to the release of cell-free hemoglobin (CFH) from lysed red blood cells, which can oxidize from the normal ferrous 2+ state to methemoglobin (3+, ferric). We have shown previously that CFH is a key driver of acute respiratory distress syndrome due to the breakdown of the alveolar capillary barrier. However, the underlying mechanism of how CFH disrupts the pulmonary endothelium remains unknown. We hypothesized that oxidized CFH disrupts the endothelium due to mitochondrial oxidative damage and leads to increased leukocyte adhesion. We utilized primary human lung microvascular endothelial cells (HLMVECs) to probe the mechanism underlying CFH-induced barrier dysfunction. Endothelial barrier function was analyzed using electric cell-substrate impedance sensing (ECIS) to quantify resistance changes in real-time following treatment with CFH^{2+} and CFH^{3+} (1.0 and 0.5 mg/mL). Permeability was assessed using the SynVivo coculture system using HLMVECs and SAECs and was quantified using fluorescent 60 kDa dextrans mixed with $CFH^{2+,3+}$ in media flowed through the channels while images were taken every 15 minutes for 4 h. Adhesion of leukocytes was quantified using immunofluorescence by counting the number of fluorescent polymorphonuclear cells (PMNs) adhered to an endothelial monolayer following pretreatment with CFH^{3+} of the endothelial cells. Mitochondrial superoxide production was quantified by MitoSOX staining followed by flow cytometry analysis of CFH-treated HLMVECs. We saw a dose-dependent decrease in transendothelial resistance following oxidized CFH^{3+} treatment but not with CFH^{2+} treatment (-1270 vs 262 max TER drop, $p<0.0001$). Real-time permeability assays using the SynVivo system showed an increase in barrier permeability from CFH^{3+} treatment compared to CFH^{2+}. Surface expression of E-selectin ($p=0.037$) and ICAM-1 ($p=0.0497$) was increased following oxidized CFH^{3+} treatment versus vehicle. In addition, pretreatment of HLMVECs with CFH^{3+} increased adhesion of polymorphonuclear cells to the endothelium (1.69×10^8 vs 7.81×10^9 GFP intensity, $p=0.0014$), while pretreatment of the PMNs did not increase adhesion. HLMVECs exposed to CFH^{3+} for 6 h have significantly increased mitochondrial superoxide compared to vehicle treated cells (608 vs 283 adjusted MFI, $p=0.0014$). These data demonstrate that CFH induces endothelial dysfunction in an oxidation-state dependent manner. In summary, these studies provide new evidence to the potential mechanism in which CFH harms the endothelium during sepsis beyond the conventionally attributed oxidative cycling of the heme moiety.

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P34

Defective mitochondrial & peroxisomal fission disrupts neurogenesis

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Mitochondria and peroxisomes are both dynamic signaling organelles that constantly undergo fission. While mitochondrial fission and fusion are known to coordinate cellular metabolism, proliferation, and apoptosis, the physiological relevance of peroxisome dynamics and the implications for cell fate are not fully understood. DRP1 (dynamin-related protein 1) is an essential GTPase that executes both mitochondrial and peroxisomal fission. Patients with *de novo* heterozygous missense mutations in the gene that encodes DRP1, *DNM1L*, present with encephalopathy due to mitochondrial and peroxisomal elongation 1 (EMPF1). EMPF1 is a devastating neurodevelopmental disease with no effective treatment. To interrogate the molecular mechanisms by which DRP1 mutations cause developmental defects, we are using patient-derived fibroblasts and induced pluripotent stem cell (iPSC)-derived models from patients with mutations in different domains of DRP1 who present with clinically disparate conditions. Using super resolution imaging, we find that patient cells, in addition to displaying elongated mitochondrial and peroxisomal morphology, present with aberrant cristae structure. Given the direct link between cristae morphology and oxidative phosphorylation efficiency, we explored the impact of these mutations on cellular energy production. Patient cells display a lower coupling efficiency of the electron transport chain, increased proton leak, and Complex I/II deficiency. In addition to these metabolic abnormalities, mitochondrial hyperfusion results in hyperpolarized mitochondrial membrane potential. Intriguingly, human fibroblasts are capable of cellular reprogramming into iPSCs and display peroxisome-mediated mitochondrial adaptations that could help sustain these cell fate transitions. We introduced the DRP1 mutations into iPSCs by CRISPR-Cas9. These iPSCs showed mitochondrial and peroxisomal hyperfusion and maintained their self-renewal and pluripotency capacity. Given that EMPF1 patients present with a spectrum of neurodevelopmental abnormalities, we differentiated the mutant iPSCs into neural progenitor cells. We find that neural progenitor cells with disrupted fission express lower levels of critical identity transcription factors, such as PAX6. We are currently investigating the detailed mechanisms by which mitochondrial/peroxisomal fission maintain neural identity. Understanding these mechanisms will give insight into the role of mitochondrial and peroxisome dynamics in neurodevelopment.

P36

The Design and Fabrication of a Microfluidic Device for Studying Microtubule Mechanics *In Vitro*

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Here, we detail the design and fabrication of a microfluidic device to investigate microtubule mechanics *in vitro* using TIRF microscopy. The design basis was precipitated by our desire to utilize the intrinsic benefits of PDMS-based microfluidic devices while also prototyping a combination of features to enable robust, customizable, and high-throughput experimentation. The proposed device incorporates redundant bubble trapping capabilities and interfaces with an automated flow control system to reduce manual intervention and enable high-throughput analyses. Moreover, both COMSOL Multiphysics and MATLAB software were utilized to better develop and understand the fluid transport and microtubule mechanics investigated using this system. Overall, the engineering of this microfluidic flow system can help researchers further understand microtubule mechanics and can provide improvements to experimental design in the broader microtubule field. The synthesis of microfabrication, automated flow control, and computational modeling and analysis techniques enables a flexible system fit for probing the cellular cytoskeleton *in vitro*.

P38

The interaction between Fic1 and Cyk3 promotes septum formation in *Schizosaccharomyces pombe*

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The fission yeast *Schizosaccharomyces pombe*, like many eukaryotes, utilizes an acto-myosin based cytokinetic ring to promote membrane ingression during cytokinesis. However, unlike animal cells, *S. pombe* must coordinate septum formation with cytokinetic ring constriction to ensure the daughter cells remain encapsulated by cell wall material to prevent cell lysis. The loss of cytokinetic ring constriction prevents proper septum formation and results in failed cytokinesis. A temperature-sensitive mutation in the essential type-II myosin, *myo2-E1*, prevents cytokinetic ring constriction and offers an opportunity to study the coordination between cytokinetic ring constriction and septum formation. Fic1 is a cytokinetic ring component, and loss of Fic1 or disruption to Fic1's phosphoregulation delays cytokinetic ring disassembly and evokes cell polarity defects. We determined that the Fic1 phospho-ablating allele*, fic1-2A*, suppresses *myo2-E1*. *fic1-2A myo2-E1* exhibits a delay in cell wall deposition similar to *myo2-E1,* but unlike *myo2-E1*, *fic1-2A myo2-E1* is capable of completing cytokinesis. Loss of Cyk3, another cytokinetic ring component, abolished *fic1-2A*'s suppression of *myo2-E1.* We generated *cyk3* point mutants to identify the regions of Cyk3 that were required to support *fic1-2A*'s suppression of *myo2-E1*. We determined that disrupting Cyk3's SH3 domain (*cyk3-W43S*) or transglutaminase-like domain (*cyk3-H577A*) also abolished *fic1-2A*'s suppression of *myo2-E1*. By performing *in vitro* binding assays with Fic1 and Cyk3-SH3, we revealed that Fic1 and Cyk3-SH3 directly interact. The Fic1-Cyk3 interaction is facilitated by Cyk3's SH3 domain binding two of Fic1's PxxP motifs. These findings suggest that the Fic1-Cyk3 interaction promotes septum formation and may offer insight into how cytokinetic ring constriction and septum formation are coordinated.

P40

Tuft cells inhibit pancreatic injury through IL-25 synthesis and secretion

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Background & AIMS: In response to injury, pancreatic acinar cells transdifferentiate into ductal cells (ADM) as a protective mechanism that promotes tissue repair. We have found that ADM does not strictly consist of a homogeneous population of ductal cells, but contains differentiated cell types typically rare or absent from the pancreas, like tuft cells (TCs). TCs are solitary chemosensory cells normally found in hollow organs like the respiratory and digestive tracts. We have found that both oncogenic KRAS mutation and pancreatitis induce TC formation in the pancreas. TC secretion of cytokine IL-25 has been reported to play a role in helminth clearance in the intestine, but the role of TCs and TC-derived IL-25 in pancreatitis has not been defined. **METHODS:** Genetically engineered mouse models (GEMMs) lacking either POU2F3, the master regulator transcription factor for tuft cell formation, or Interleukin (IL-) 25 specifically in the pancreas were generated. Adult mice and controls were given pancreatitis using the cholecystokinin ortholog caerulein and the pancreas was collected to conduct histological studies. **RESULTS:** Chronic injury in mice lacking tuft cells resulted in more severe injury and greater tissue loss, as compared to controls. Pancreatitis derived tuft cells express IL-25 and IL-25 ablation also resulted in enhanced injury which included edema. **CONCLUSIONS:** Tuft cells inhibit injury under conditions of chronic pancreatitis through IL-25 synthesis and secretion. Further studies are required to determine which cells respond to IL-25 and how this suppresses inflammation.

P42

Small intestinal Tuft Cell Specification and Behavior in Homeostasis and Irritable Bowel Disease

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Crohn's disease is a debilitating disease; Patients suffer from harsh abdominal pain and severe diarrhea, resulting in weight loss due to malnutrition. While the etiology of Crohn's disease is unclear, it is likely due to a range of causes from genetic predisposition to environmental triggers and uncontrolled immune response. Treatments for Crohn's disease have greatly improved over the last few decades, moving away from broad immunosuppression strategies and focus on targeted immunomodulation.

Despite the expansion of therapeutic options, many patients fail treatment. Thus, a deeper understanding of the role of gastrointestinal epithelia in the inflammatory response is necessary. Using mouse models of Crohn's Disease, we have linked the critical role of Dclk1+ tuft cells in modulating inflammation. We show that in TNFΔARE/+ mice, which spontaneously develop Crohn's-like ileitis due to excess TNF expression, succinate supplement therapeutically reverses the inflammatory phenotype.

This amelioration of inflammation is tuft cell-dependent, and unique to tuft cells that develop independently of the secretory master regulator ATOH1. Single-cell RNA-sequencing data reveals multiple tuft cell populations in the wildtype ileum similar to previous reports, possibly consisting of a mixture of ATOH1-dependent and ATOH1-independent tuft cells. However, only a single population of tuft cells was identified in the AtohKO ileum. We investigate the hypothesis that distinct tuft cell populations, each with different functions, arise from ATOH1 -dependent and -independent mechanisms.

P44

Characterization of a New Extracellular Vesicle, Blebbisomes

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We have shown that cells leave behind large extracellular vesicles (EVs) that are 5-10 microns in diameter as they migrate. We report that these EVs are characterized by membrane blebbing. Blebbing is a well-known process that occurs at instances like cell division and cell migration. Essentially, the cell breaks down a portion of the actin cortex to relieve pressure within the cell which pushes out the plasma membrane (similar to blowing up a balloon). As these large EVs contiuously blebb, we call them "blebbisomes'. We have been able to characterize blebbisomes as a unique EV due to the presence of functional mitochondria, actomyosin cytoskeleton, Golgi apparatus, Lysomes and CD63-containing vesicles. Of note, the CD-63 containing vesicles could be exosomes; classic and much smaller extracellular vesicles. This data has led us to hypothesize that there is a population of extracellular vesicles (i.e., blebbisomes) that can take up and potentially process other populations of EVs (e.g., exosomes).

P46

Dynamics of Golgi positioning in interphase cells

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The Golgi complex undergoes dynamic morphological changes throughout the cell cycle. Numerous studies have shown the mechanisms involved in the changes in the Golgi complex during mitosis. However, there are limited studies which have explored the morphological transitions of Golgi during the interphase. A recently published work from our lab has shown that the Golgi transits between a highly compact configuration around the centrosomes in the G1 phase to an extended configuration around the nucleus in S and G2 phases. The data also indicated that the rearrangements rely on microtubules and microtubule-based transport. In the current study, we observe that the reassembly of Golgi stacks in the G2 stage occurs mostly around the nuclear envelope even though the radially organized microtubules produced by centrosomes persist in this stage. Moreover, our data suggest that the interplay between the minus-end and plus-end directed microtubule motors is essential in this process. Dynein inhibition leads to fragmentation and dispersal of Golgi stacks, whereas inhibition of kinesin-1 prevents detachment of Golgi around the centrosomes and blocks its redistribution around the nuclear envelope. Based on these findings, we think it is likely that in the G2 stage, dynein is necessary to connect the Golgi stacks but fails to deliver the Golgi towards the centrosomes. The plus-end directed kinesin-1 overpowers dynein to move the Golgi away from the centrosomes and stretch the Golgi ribbon around the nuclear envelope using specific perinuclear microtubule tracks.

P48

Recruitment of Foxl1+ Telocytes During Metaplasia Development in the Stomach

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Telocytes are interstitial cells present in the connective tissue of multiple organs, including the gastrointestinal tract. Telocytes have distinct ultrastructural characteristics, with small cell bodies and several long cytoplasmic processes, called telopodes. Telocytes have immunophenotypic heterogeneity depending on their anatomical location, and recent studies demonstrated that Foxl1+ subepithelial telocytes in the intestine act as the source of specific Wnt ligands that are critical for maintaining the stem cell niche. We examined telocytes in the stomach and confirmed the expression of telocyte markers using immunofluorescence for Foxl1, PDGFRα and F3. We are studying if telocytes, as putative potent sources of telopode-delivered intercellular signaling molecules, are involved in the development of stomach metaplasia. When there is loss of stomach acid-secreting parietal cells, mature chief cells at the base of the gastric gland undergo mucous-cell metaplastic conversion, designated as spasmolytic polypeptide-expressing metaplasia (SPEM), and these cells also become proliferative. Using mouse stomach tissues from various SPEM models, we examined the changes in telocyte networks in the stomach. In a normal stomach, Foxl1+ telocytes are located near the isthmal region of the gland, where proliferative progenitor cells are present (Figure 1). However, with SPEM induction, either with the parietal cell toxic drug L635 or in Mist1-Kras mice 1 month after tamoxifen induction, more telocytes were observed proximate to the base of the gland, where proliferative SPEM cells arise (Figure 1). Using Foxl1-CreERT2;R26RtdTom mice to label Foxl1+ telocytes before SPEM induction with L635, we found that the large numbers of “basally emergent telocytes” were not labeled, suggesting that they did not originate from isthmal-region telocytes, but were recruited from another source during the development of metaplasia. In human stomach, we made similar findings—telocytes are present near the isthmal region in normal glands, but in larger numbers cupping the bases of metaplastic glands. These findings suggest that telocyte recruitment produces a nascent metaplastic niche during metaplasia induction, plausibly supporting the metaplastic proliferative zone.

P50

Basement membrane repair dynamics in the *Drosophila* midgut

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Basement membranes are the oldest, most conserved forms of extracellular matrix and serve to separate tissue layers, provide mechanical support, direct signals to neighboring cells, and insulate tissues from signals. Additionally, basement membranes are subject to mechanical damage and require dynamic repair mechanisms. Faulty basement membrane repair mechanisms can aid in the progression of diseases such as asthma and diabetes, and diseases of the basement membrane itself, including Alport's syndrome and Goodpasture's syndrome. Therefore, understanding how basement membranes repair will be vital to treating these conditions. Our work utilizes the *Drosophila* midgut basement membrane to probe repair dynamics. In *Drosophila*, all major basement membrane components have been conserved but with less redundancy than mammals. Our lab has developed an assay to reproducibly damage the basement membrane and study the repair process. Previously we reported that many aspects of basement membrane repair are shared during homeostasis. Thus, it is unclear whether basement membrane damage is actively detected, or instead, passively repaired by homeostatic mechanisms.

My data suggests basement membrane damage is actively detected. Following damage, a subset of cells in the Drosophila midgut begins transcribing matrix components. We have termed these cells “Matrix Makers.” To identify the cellular identity of the Matrix Makers, I performed lineage tracing and found these cells are likely enteroblasts that differentiate into enterocytes. Further, I have found the mechanosensitive ion channel, Piezo, needs to be activated for basement membrane repair to occur. However, its exact role in basement membrane repair remains unknown.

P52

Supramolecular organization of the postsynaptic density for trans-synaptic alignment in excitatory synapse observed by cryo-electron tomography

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Nanoscale organization of proteins within synapses are critical for maintain and regulate synaptic transmission and plasticity. Revealing the supramolecular structure of synapses will lead to better understanding of synaptic functions in the brain, which feeds through to new therapeutic strategies and clinical trials. In this study, we used cryo-electron tomography to directly visualize *in situ* three-dimensional architecture and supramolecular organization of trans-synaptic nanocolumns in their native cellular context. Our findings strongly suggested a standard trans-synaptic nanocolumn contains a synaptic vesicle at release site, a cluster of postsynaptic receptors and a PSD nanodomain, together creating a physical transcellular alignment. Quantitative analyses revealed PSD nanodomain is the basic unit and form different sizes of PSD clusters. Furthermore, high-resolution tomograms obtained from the synaptosome sample allows us to detect the glutamate receptor-like particles and putative adhesion molecules. These results clarify unresolved issues regarding the ultrastructural features of synapses and support the idea that trans-synaptic alignment exists to carry out synaptic functions. The supramolecular architecture of the basic PSD nanodomain and its clusters suggests a higher-order organization of trans-synaptic nanocolumn of central nervous system synapses to maintain and regulate synaptic transmission.

P54

Single-cell RNA sequencing reveals sexually dimorphic gene expression in the *C. elegans* nervous system

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Key differences in neural circuits are defined by sex but the underlying genetic programs that specify sexual dimorphisms in the nervous system are largely unknown. To address this question, we are exploiting the model organism *C. elegans* because sexually dimorphic differences in connectivity have been catalogued for the entire nervous systems of both sexes. In addition to neurons that are unique to each sex, the wiring diagrams also revealed striking differences in connectivity for neuron types shared by both males and hermaphrodite worms. In our approach, we are using single-cell RNA Sequencing to define sexually dimorphic gene expression across the *C. elegans* nervous system. To begin, we optimized methods for simultaneous profiling of adult males and hermaphrodites grown under identical conditions. We used FACS to isolate subsets of neurons for 10X Genomics single-cell RNA sequencing. Using the expression of known sex-specific transcripts, including DMTR family transcription factors, we identified male and hermaphrodite-specific subclusters for several sex-shared neuron classes. Differential gene expression tests revealed dozens of highly dimorphic transcripts. Using endogenously tagged CRISPR reporters, we have validated the sexually dimorphic expression of a homeodomain transcription factor and several neuropeptide encoding genes in sex-shared neurons. Thus, our approach is capable of uncovering novel sexually dimorphic genes with individual neuron type resolution. Interestingly, some sex-shared neuron types show very little dimorphic expression despite being well-represented from both sexes, indicating strong cell-type specific variation in the extent of sexual dimorphic gene expression. We are currently expanding this dataset with additional experiments to capture the entire nervous system in adults of both sexes. In the future, we will expand our approach to profile male vs hermaphrodite neurons during development to include larval males in order to uncover the developmental timing of sexually dimorphic gene expression. These data will provide a foundation for interrogating the molecular basis of sexually dimorphic gene expression, connectivity, and behavior.

P56

Variant stem cell clones are transcriptionally related to mature goblet cells in COPD

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Background: Goblet cell hyperplasia is common in the airways of patients with chronic obstructive pulmonary disease (COPD) and contributes to pathologic impairment in mucociliary clearance. Recent data suggests that a transcriptionally distinct subset of lung basal (stem) cells preferentially generate goblet cells in COPD, but the mechanism through which this occurs is unclear.
Methods: We performed single-cell RNA sequencing (scRNA-seq) on distal lung tissue from 11 COPD patients who underwent lung transplantation and 10 non-diseased organ donors whose lungs were rejected for transplantation (e.g. controls) yielding a total of 23,075 epithelial cells. Recursive clustering of the secretory cell population was used to isolate and transcriptionally characterize 302 goblet cells. Individual basal cell clones were isolated from COPD and control lungs and analyzed by RNA-sequencing. A portion of cells from each clone was transferred to air-liquid interface (ALI) culture to stimulate terminal differentiation. Goblet cells in mature epithelia were identified by immunostaining for Muc5ac.
Results: Using scRNA-seq, we identified known (*MUC5AC*, *TSPAN8*) and novel (*TFF3*, *PRSS23*) markers which distinguish human lung goblet cells from other transcriptionally-related secretory cells. Transcription factors enriched in goblet cells compared to other types of secretory cells included *CREB3L1*, *TSHZ2*, and *EPAS1*. Basal cells from COPD patients were transcriptionally distinct from control basal cells, were more transcriptionally heterogeneous, and had downregulation of multiple pathways related to mitosis and the cell cycle. Basal cells from one COPD patient were characterized by increased expression of several of the same markers (*TFF3*, *MUC5AC*, *MUC5B*, *WFDC2*, *SLPI*) and transcription factors (*CREB3LI*, *EPAS1*, *TSHZ2*) enriched in mature goblet cells, and mature epithelia from these clones had increased numbers of Muc5ac+ goblet cells.
Conclusions: We identified multiple new markers and transcription factors which are enriched in COPD goblet cells and found that some GCH-primed basal cells share markers and transcription factors with mature goblet cells. These findings suggest that epigenetic modulation of goblet cell-specific transcription factors may explain the predilection for some basal cells to generate goblet cells.

P58

Cancer-associated fibroblasts and squamous epithelial cells constitute a unique microenvironment in a mouse model of inflammation-induced colon cancer

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The tumor microenvironment plays a key role in the pathogenesis of colorectal tumors and contains various cell types including epithelial, immune, and mesenchymal cells. Characterization of the interactions between these cell types is necessary for revealing the complex nature of tumors. In this study, we used single-cell RNA-seq (scRNA-seq) to compare the tumor microenvironments between a mouse model of sporadic colorectal adenoma ($Lrig1^{CreERT2/+}$;$Apc^{2lox14/+}$) and a mouse model of inflammation-driven colorectal cancer induced by azoxymethane and dextran sodium sulfate (AOM/DSS). While both models develop tumors in the distal colon, we found that the two tumor types have distinct microenvironments. AOM/DSS tumors have an increased abundance of two populations of cancer-associated fibroblasts (CAFs) compared with APC tumors, and we revealed their divergent spatial association with tumor cells using multiplex immunofluorescence (MxIF) imaging. We also identified a unique squamous cell population in AOM/DSS tumors, whose origins were distinct from anal squamous epithelial cells. These cells were in higher proportions upon administration of a chemotherapy regimen of 5-Fluorouracil/Irinotecan. We used computational inference algorithms to predict cell-cell communication mediated by ligand-receptor interactions and downstream pathway activation, and identified potential mechanistic connections between CAFs and tumor cells, as well as CAFs and squamous epithelial cells. This study provides important preclinical insight into the microenvironment of two distinct models of colorectal tumors and reveals unique roles for CAFs and squamous epithelial cells in the AOM/DSS model of inflammation-driven cancer.

P60

The Role of Polyploidy During *Drosophila* Epithelial Wound Repair

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In the past decade, there has been increased awareness of cellular polyploidy in development, homeostasis, and cancer. In addition to being a conserved developmentally programmed behavior, polyploidy is also induced in response to injury. Work by Vicki Losick has shown adult *Drosophila* epithelia repair by becoming polyploid through endoreplication and cell-cell fusion. In Zebrafish epicardial explants, regeneration is led by a wavefront of polyploid cells. We have found that both endocycling and cell fusions occur after laser wounding in the mitotically competent pupal notum, a system that supports live imaging. Live imaging allowed us to determine that cell-cell fusions are initiated within the first 20-30 minutes after wounding, and cell borders begin to break down within the first 3 rows of cells from the wound. Thus, multinuclear syncytia form very rapidly and are positioned at the front lines of wound repair. Interestingly, mononucleate cells that do not fuse are pushed out, so that they do not contribute to the leading edge by the time the wound is actively closing. Most fusion events occur between cells at different distances from the wound, as opposed to neighbors in the same row. We hypothesize fusions of sequential rows of cells allows distal cellular resources to be pooled at the leading edge of wound repair. We have observed syncytia constructing large actin-rich contractile structures which could increase wound closure rate. Further, we are investigating whether fusion allows the proteostatic machinery of distal, more healthy, cells to aid highly damaged wound proximal cells preventing their death. By reducing the initial area of cell death the tissue may be able to repair more quickly.

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EP4 Perturbation Resulting in Mouse Patent Ductus Arteriosus may be Dependent on Developmental Timing

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The ductus arteriosus (DA) is a vascular shunt which allows oxygenated blood to bypass the developing lungs *in utero*. After birth, normal DA closure may be disrupted, leading to persistent patency of the DA (PDA). COX-derived PGE2 signaling via the prostanoid receptor EP4 appears to be critical for the normal closure process, with neonatal lethality in EP4 KO and COX double KO mice due to PDA. Previous studies have examined the EP4 KO phenotype, but questions of developmental timing and vascular function remain. **We hypothesize that EP4 signaling in late gestation is critical for establishing the contractile properties of the mature DA, including its myogenic tone, biomechanical properties, and responsiveness to key molecular signals.** For developmental timing, a gavage model using a selective EP4 antagonist (ONO-AE3-208) was employed. Mice treated from D15-D19 showed partially open DAs at 4hrs of age. Contractile properties of EP4 KO mice were examined through pressurized vessel myography. KO vessels lacked the myogenic response to pressure increase typical of the WT. KO vessels also showed a decreased dynamic range compared to WT. Mathematically this translated to a decrease in compliance of the KO vessel. In addition, term gestation KO vessels showed vasomotion characteristic of the preterm DA. Mounted DAs were exposed to signaling compounds of various pathways. Significant differences were seen in responsiveness to KCl, PGE2, and SNP. Together, these data indicate that EP4 signaling is required during the D15-D19 window for proper DA development, and that EP4 signaling is required for establishing the myogenic tone, compliance, and various signaling properties of the mature DA.

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Investigating the Mechanism of Action of the Poorly Studied Transcription Factor, *Mnx1,* During β-cell Development

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Motor Neuron Homeobox 1 (*Mnx1; a.k.a. Hb9, Hlxb9*), a homeodomain-protein-encoding gene, encodes a poorly studied pancreatic β-cell (insulin-producing cell) transcription factor (TF). *Mnx1* is expressed early in tissues producing intercellular signals that induce formation of the pancreatic anlagen. Later, *Mnx1* is produced in pancreatic cells after endocrine-lineage commitment, beginning at the $Pax6^+$ post-mitotic stage. Previous studies indicate *Mnx1* as a core TF regulating β-cell specific differentiation and function, but its lack of molecular analysis is surprising considering the profound phenotypes accompanying mouse or human mutations. When *Mnx1* is inactivated early in mouse β-cell development, ∆Mnx1 cells still have the "momentum" to follow the β-cell differentiation track, but in the early postnatal period they all transform into somatostatin-producing δ-like cells. We are testing if ∆Mnx1 cells undergo a progressive accumulation of cryptic deflections from the differentiation-guidance program, leading to an inability to maintain the b-cell GRN (gene-regulatory network) when faced with "checkpoint control" in early postnatal life. An alternate model is that the GRN and epigenetic control mechanisms undergo large-scale paroxysmal alteration only postnatally, triggering rapid β-to-δ transdifferentiation. Human Mnx1 point mutations cause severe permanent neonatal *diabetes mellitus* (PNDM). A CRISPR-editing mouse model mimicking the human *Mnx1* PNDM (F272L in helix 2 of the DNA-binding homeodomain) should allow complete study of the abnormal developmental program. The profound phenotype, including postnatal death, suggests an extreme effect on the pancreas and its endocrine-cell population.

We are investigating the *Mnx1* GRN and epigenetic-guidance patterns at various stages of β-cell development. Defining Mnx1 target genes, and activation vs. repression effects, will be paramount in understanding how it drives and maintains β-cell fate. Because experiments such as ChIP-Seq, CUT&Tag, scRNA-seq will unveil key chromatin-Mnx1 interactions, we need effective and high signal:noise antibodies. Currently, we have rabbit, goat, and alpaca polyclonal affinity-purified antibodies, with alpaca nanobodies being made. As triangulation methods, we created gene-edited epitope-tagged mouse strains carrying either 3x[FLAG] or the novel ALFA-tag at the Mnx1 C-terminus. Both strains seemingly have normal *Mnx1* function, and we are checking that these additions are entirely passive by testing for the absence of any endocrine-cell phenotype in embryonic, postnatal, and adult tissue. By inactivating *Mnx1* using Pdx1-CreER at various stages of lineage allocation, differentiation, and maturation, we will determine if the beta-cell GRN collapse occurs differently according to developmental stage – for example, if adult-stage inactivation causes the same beta-to-delta conversion described above, or a highly moderated phenotype.

“They thought I was crazy, absolutely mad.”

Barbara McClintock

The response (1944) of the National Academy of Sciences, to her (later Nobel prize-winning) theory that genes could 'jump' to new locations on a chromosome.

See ya next year!