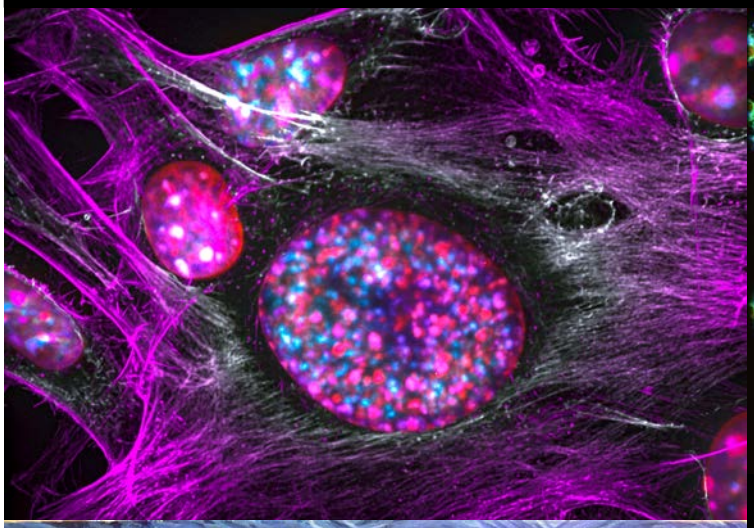
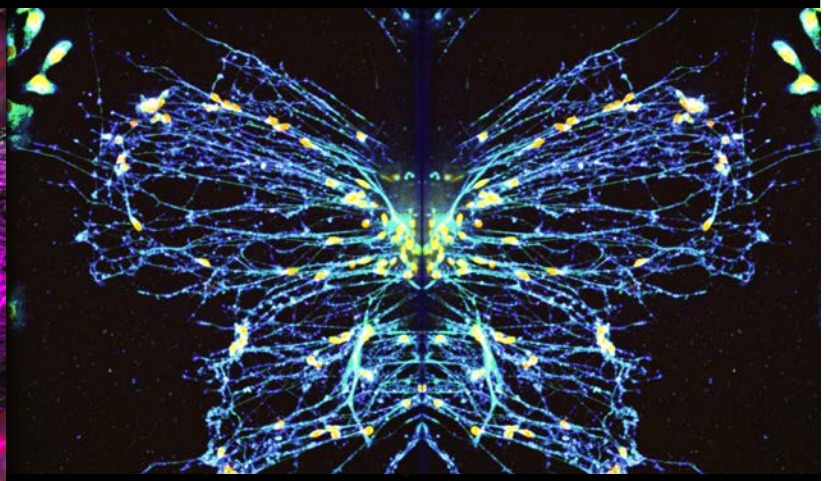
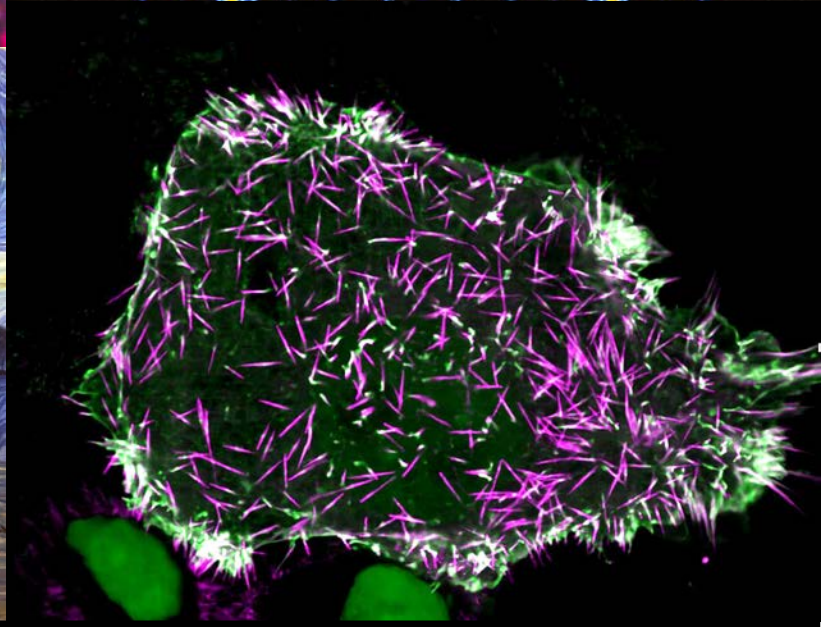
Department of Cell & Developmental Biology

Twentieth Annual Retreat
April 14, 2023





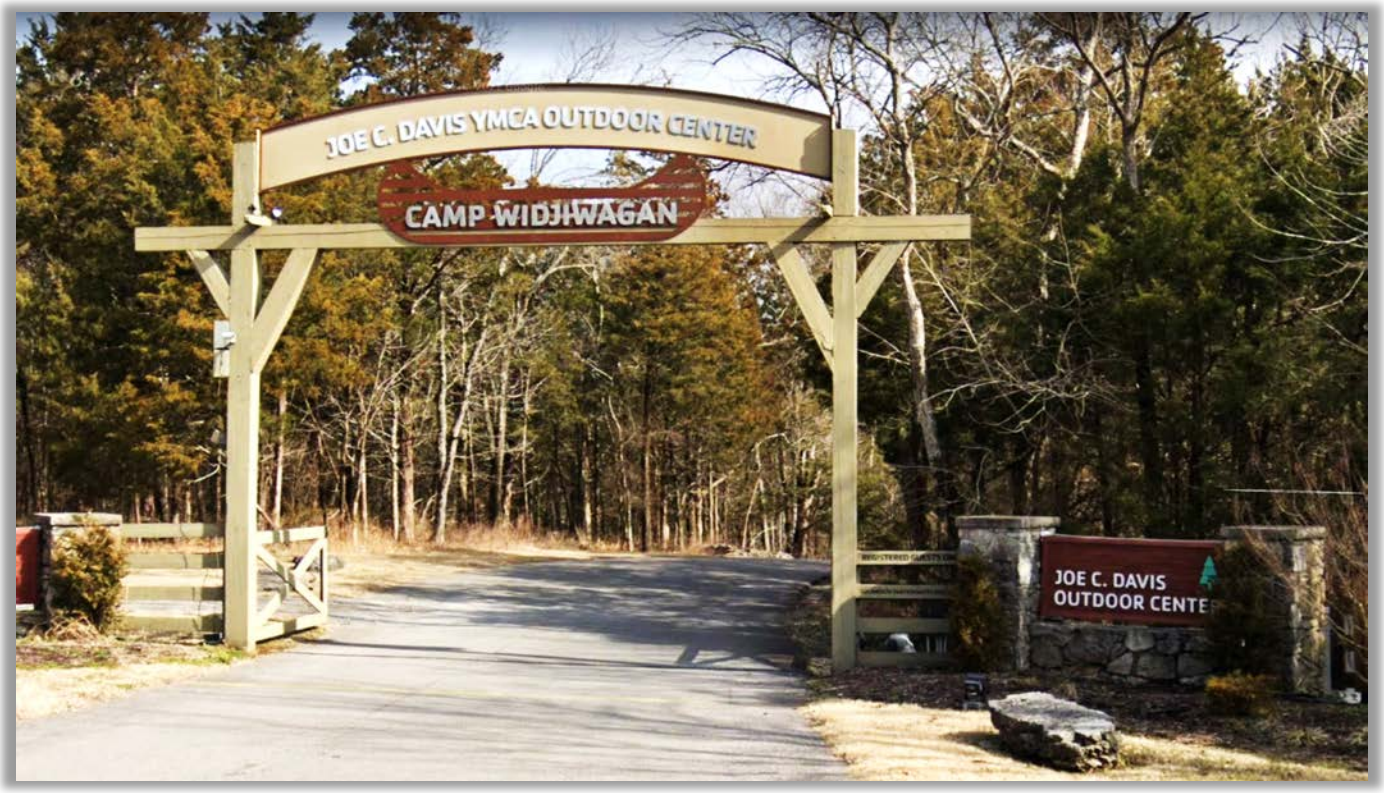


JOE C. DAVIS YMCA OUTDOOR CENTER
YMCA CAMP WIDJIWAGAN

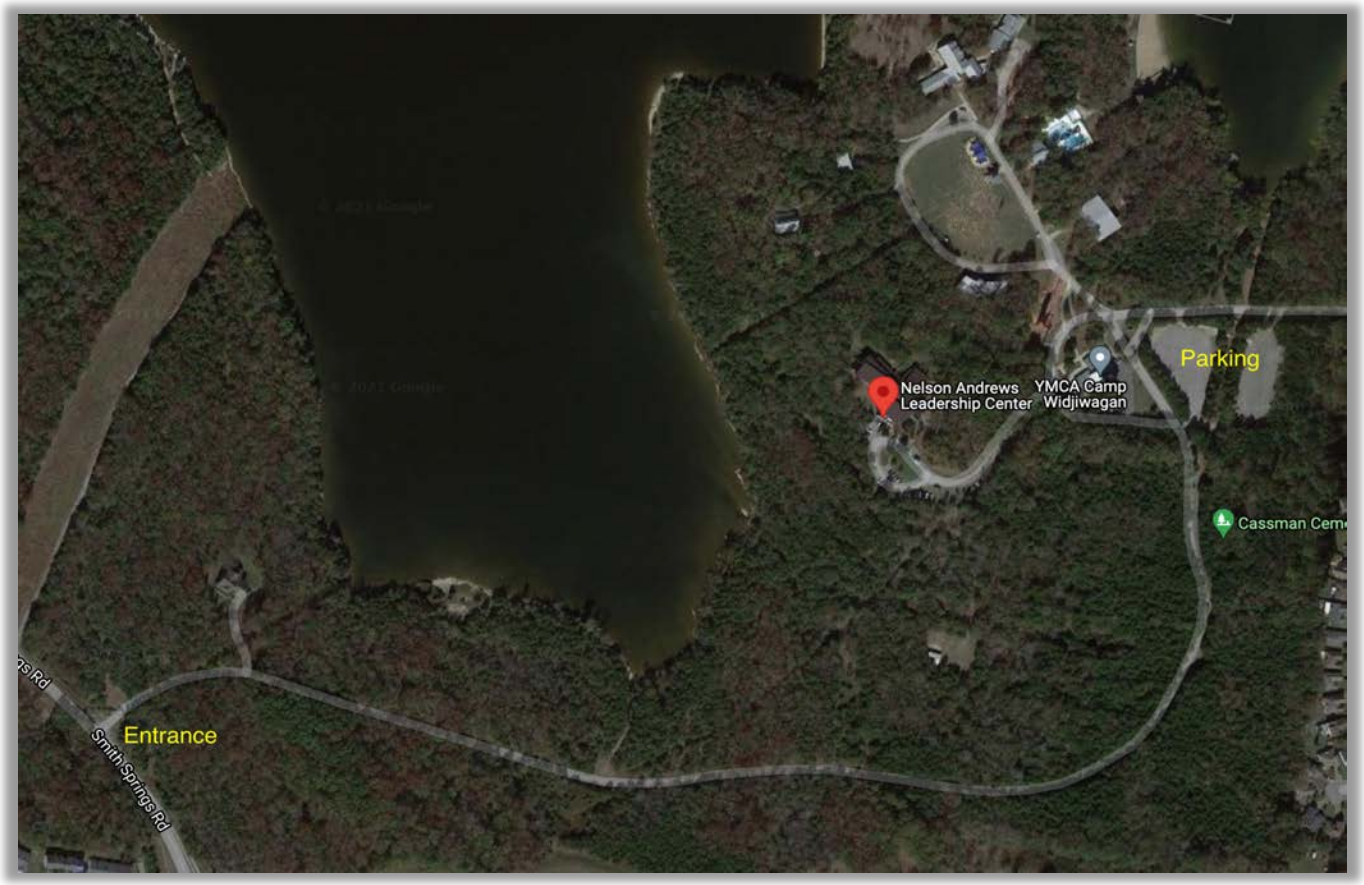
YMCA OF MIDDLE TENNESSEE
3088 Smith Springs Road, Nashville, TN 37013
P (615) 360-2267

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

Twentieth Annual CDB Retreat
April 14, 2023
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)
8:30-9:15	**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
9:20-9:50	**Graduate Student/Postdoc Award -** Presented by Andrea Page-McCaw Steve Hann Award Winner presentation Outstanding Postdoc Award winner presentation
9:55-10:55	**Poster Session I** **Breakout Session I** (Students/Postdocs only) Moderated by Indrayani Waghmare (Page-McCaw lab)- Katie Young (Reinhart-King lab), Stephanie Medina (Irish lab), Anna Cassidy (Zanic lab)
11:00-12:00	**First Session** – Moderated by Jared Nordman Eric Donahue (Burkewitz lab) Christian DeCaestecker (Macara lab) Sara Kassel (Lee lab) James Hayes (Burnette lab)
12:00-2:00	**LUNCH** (Chang Noi Thai-Lao and Urban Cookhouse) **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**
2:05- 2:50	**Second Session-**Moderated by Eunyoung Choi Dongshen Han (Nordman lab) Jenna Mosier (Reinhart-King lab) Beth Lawrence (Zanic lab)
2:55- 3:25	**CDB DEI Presentation**
3:30- 4:15	**Third Session**- Moderated by Neil Dani Tyler Kennedy (Miller lab) Ela Contreras-Panta (Goldenring lab) Tierney Baum (Gama lab)
4:20-5:20	**Poster Session II - Open bar** **Breakout Session II** (Students/Postdocs only) Moderated by Christian de Caestecker (Macara lab)- Laura Richardson (Zanic lab), Mary Chalkley (Ess and Ihrie labs), Ela Contreras-Panta (Goldenring lab)
5:20-5:30	**POSTER SESSION II TAKE DOWN**
5:30-8:30	**Reception** **Dream Events & Catering**

Oral Presentations

Steve Hann Award Winner

Outstanding Postdoc Award winner

First Session- Speaker 1

Lifespan-extending interventions protect against age related remodeling of the endoplasmic reticulum

Eric KF Donahue, Alexandra G Mulligan, Kristopher J Burkewitz

Vanderbilt University, Nashville, TN, 37027

The endoplasmic reticulum (ER) is critical for cellular homeostasis, with major roles in proteostasis, calcium signaling, lipid and membrane synthesis, and inter-organelle communication. Dysregulation of many of these functions are considered hallmarks of aging, highlighting the ER as a key player in age-related deterioration. ER functions are tightly associated with its morphological subdomains, and specific subdomain balances support specialized cellular functions. As such, disruptions in ER structure may drive its functional decline and the progression of age-related disease. However, it is unclear if ER morphology changes in aging. To understand the dynamics of ER morphology with age, we used CRISPR/Cas9 to generate ER subdomain-reporter strains of C. elegans. In vivo imaging and ultrastructural analysis revealed a striking remodeling of ER subdomains across tissues. With age, ER sheets and tubules are significantly depleted in the epidermis and intestine, and the remaining ER tubules have increased inter-organelle contact sites. Using RNAi, we found that autophagy is necessary for the age-related loss of ER sheets. Because ER sheets regulate proteostasis, which also fails with age, we then investigated whether longevity interventions protect the ER. Both dietary restriction and reduced IGF signaling, which improve proteostasis and extend lifespan, provided subdomain-specific protection of ER sheets. Overall, we show that the ER is significantly remodeled in early aging, and autophagy and metabolic signaling regulate this decline. These studies underscore the importance of ER form and function in aging and establish ER structure as a potential target to mitigate age-related decline.

First Session- Speaker 2

A Size Filter Regulates Apical Protein Sorting

Christian de Caestecker and Ian G. Macara

Department of Cell and Developmental Biology Vanderbilt University School of Medicine
Nashville TN 37205, U.S.

An essential aspect of epithelial cell biology is the polarized sorting of membrane proteins to the basolateral and apical plasma membranes. This differential sorting, which occurs at the trans-Golgi network (TGN), maintains the identities and functions of these two cortical regions, and is disrupted in multiple human diseases. Short zip codes within the sequences of basolateral membrane proteins provide sorting information, but apical sorting mechanisms are still not fully understood. Bioinformatic analysis of apical versus basolateral membrane proteins revealed that apical cytoplasmic domains are on average significantly smaller than those of basolateral proteins. However, the reason for this attribute is unknown. We asked if a size filter or diffusion barrier at the trans-Golgi network might kinetically exclude proteins with large cytoplasmic tails from apical sorting compartments. We used the polarity protein Crumbs 3 as an example of an apically targeted transmembrane protein with a short cytoplasmic C-terminus. A FKBP domain was attached to the C-terminus to enable inducible dimerization to FRB-tagged proteins of variable size. A streptavidin-binding peptide on the extracellular domain traps the protein in the endoplasmic reticulum (ER), using the RUSH system. Upon addition of biotin, this construct synchronously departs the ER, traverses the Golgi, and after about 35 minutes exits in vesicles that are delivered to the apical plasma membrane. The FKBP tag alone did not significantly impede Golgi exit, but dimerization to one or more SNAPtag-FRB moieties significantly delayed departure. In epithelia, Crumbs proteins bind through their cytoplasmic tails to the large polarity protein Pals1. We discovered that although Crb3 and Pals1 are associated at the ER and arrive simultaneously at the Golgi, Pals1 disassociates ~7 minutes prior to departure of Crb3 from the TGN. Notably, a mutant Pals1 lacking the N-terminal domain does not disassociate and impedes Crb3 exit. We conclude that a small cytoplasmic domain facilitates apical sorting and that reducing this domain size by timely disassembly of the Pals1-Crb3 complex is essential for normal kinetics of Crb3 apical delivery.

First Session- Speaker 3

The ubiquitin ligase TRIP12 ubiquitylates BRG1 to promote Wnt-β-catenin signaling

Sara Kassel1, Kai Yuan2, Nawat Bunnag2, Alex Cho1, Mary Rockouski1, Lily Goldsmith1, Andres Lebensohn3, Rajat Rohatgi4, David Robbins5, Arminja Kettenbach2, Yashi Ahmed2, Ethan Lee1

1Vanderbilt University, Nashville, TN 37232; 2Geisel School of Medicine, Dartmouth College, Hanover, NH 03755; 3National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; 4Stanford University School of Medicine, Stanford, CA 94305; 5Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057

The Wnt-β-catenin signal transduction pathway is essential for animal development and adult tissue maintenance. Wnt signaling promotes stabilization of β-catenin, which translocates to the nucleus and binds to TCF/LEF transcription factors and other cofactors to initiate a Wnt transcriptional program. For β-catenin to regulate transcription of Wnt target genes, the chromatin structure of target promoters must first be remodeled into a permissive state to allow binding of transcription factors. This process is facilitated by the SWI/SNF chromatin remodeling complex. Through a genetic screen in human cells, we identified the ubiquitin ligase TRIP12 as a positive regulator of Wnt signaling. Knockdown of TRIP12 inhibited expression of Wnt target genes, and TRIP12 acted downstream of the β-catenin destruction complex. Immunoprecipitation and mass spectrometry revealed that TRIP12 interacted with BRG1, the central catalytic subunit of the SWI/SNF chromatin remodeling complex. Upon Wnt stimulation, TRIP12 increased the ubiquitylation of BRG1 but did not alter its stability. Our data suggest a mechanism by which regulated ubiquitylation of BRG1 enhances the ability of the SWI/SNF complex to remodel the chromatin structure of Wnt target gene promoters, thereby allowing β-catenin and its cofactors to bind and regulate the transcription of Wnt target genes.

First Session- Speaker 4

A role for a "non-muscle" alpha-actinin in cardiovascular development

James Hayes, Dylan Ritter, Abigail Neininger, Ela Knapik, Dylan Burnette

Vanderbilt University, Nashville, TN, 37235

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-actinin-4 has a role during the formation of the cardiac sarcomere in vitro and during cardiovascular development in vivo. In vitro, alpha-actinin-4-depleted human cardiac myocytes subjected to a sarcomerogenesis assay are hypertrophic and assemble more sarcomeres than do controls and restoring the levels of alpha-actinin-4 overexpression restores control phenotypes. Alpha-actinin-4 depletion stabilizes the primary muscle actinin paralog, alpha-actinin-2, at the muscle sarcomere Z-line, and delays alpha-actinin-2 release from the Z-line. In vivo, zebrafish injected with morpholinos targeting alpha-actinin-4 form a heart with an abnormally dilated atrium and a thickened ventricular myocardium. Preliminary traction force microscopy data reveals that atrial cardiac myocytes depleted of alpha-actinin-4 produce more contractile force than do controls. Taken together, these results suggest that alpha-actinin-4 is a low-expressed paralog that nonetheless serves to oppose or otherwise regulate sarcomerogenesis during cardiovascular development.

Second Session- Speaker 1

BRWD3 targets KDM5/Lid for degradation to maintain H3K4 methylation levels

Dongsheng Han, Samantha Schaffner, Jonathan P Davies, Mary Lauren Benton, Lars Plate, Jared T Nordman

1Department of Biological Sciences, Vanderbilt University, Nashville, TN, 37212, USA 2Department of Chemistry, Vanderbilt University, Nashville, TN, 37212, USA 3Department of Computer Science, Baylor University, Waco, TX 76798, USA

Histone modifications are critical for regulating chromatin structure and gene expression. Dysregulation of histone modification levels may contribute to disease development and cancer. Depletion of the chromatin-binding protein BRWD3, a known substrate-specificity factor of the Cul4-DDB1 E3 ubiquitin ligase complex, results in an increase in H3K4me1 levels. The underline mechanism linking BRWD3 and H3K4 methylation, however, has yet to be defined. Here, we show that depleting BRWD3 causes a decrease in H3K4me3 levels. Using immunoprecipitation coupled to quantitative mass spectrometry, we identified an interaction between BRWD3 and the H3K4-specific demethylase 5 (KDM5/Lid), an enzyme that removes tri- and di- methyl marks from K3K4. Moreover, analysis of ChIP-seq data revealed that BRWD3 and KDM5 are significantly co-localized throughout the genome and that sites of H3K4me3 are highly enriched at BRWD3 binding sites. We show that BRWD3 promotes K48-linked polyubiquitination and degradation of KDM5/Lid. Critically, KDM5/Lid degradation is dependent on both BRWD3 and Cul4. Critically, depleting KDM5 fully restores altered H3K4me3 levels and partially restores H3K4me1 levels upon BRWD3 depletion. Together, our results demonstrate that BRWD3 regulates KDM5 to ensure the balance of H3K4me levels.

Second Session- Speaker 2

Priming in Confinement Drives Mechanical Memory of Breast Cancer Cells

Jenna A. Mosier, Catherine Ludolph, Addison White, Matthew R. Zanotelli, Cynthia A. Reinhart-King

Vanderbilt University, Nashville, TN, 37212

Breast cancer cell migration through the primary tumor microenvironment is modulated by mechanical and biochemical signals that can induce intracellular changes. Confinement, a key mechanical cue imparted on cells due to dense collagen organization in the surrounding matrix, significantly changes cell speed and behavior during migration. Specifically, confinement has been shown to induce polarization of organelles and cytoskeletal structures that promote increased migration. However, the role of confinement in future migratory ability and cell behavior, has not been fully explored. To determine how confinement modulates intracellular changes, migration, and mechanical memory, we utilized a microfabricated, collagen microtrack platform with tunable track geometries that can replicate confinement encountered by breast cancer cells in the tumor microenvironment. We created uniform tracks of high (7 um) and low (15 um) confinement, as well as temporarily confined tracks that contain a region of high confinement that then transitions into low confinement to investigate the effect of priming in confinement on mechanical memory. Agreeing with previous data, we found that cells significantly increase velocity in high confinement compared to those in low confinement. Interestingly, when primed for at least 200 um in the temporarily confined tracks, cells maintained their exit velocities even after transitioning into low confinement. This data indicates that the mechanical memory of migrating breast cancer cells is dependent on priming in confinement. Because bioenergetics and migration are intrinsically linked, we next investigated mitochondria to determine their role in confined migration and mechanical memory. When mitochondrial activity was pharmacologically inhibited with antimycin-A, an inhibitor the electron transport chain, cell velocity in confinement was significantly decreased, suggesting that mitochondrial activity is necessary for confined migration. Further, mitochondria localize significantly closer to the leading-edge of cells in high confinement compared to those in low confinement. When primed for greater than 200 um in high confinement, increased leading-edge localization of mitochondria was maintained even when cells transition to a region of low confinement, indicating a potential role of mitochondrial positioning in mechanical memory. By identifying molecular determinants of mitochondrial localization, we can better understand the relationship between confinement and mechanical memory and highlight potential therapeutic targets to inhibit breast cancer metastasis.

Second Session- Speaker 3

CLASPs stabilize the pre-catastrophe intermediate state between microtubule growth and shrinkage

Lawrence EJ, Chatterjee S, Zanic M

Vanderbilt University, Department of Cell and Developmental Biology.

CLASPs regulate microtubules in many fundamental cellular processes. CLASPs stabilize dynamic microtubules by suppressing microtubule catastrophe and promoting rescue, the switch-like transitions between growth and shrinkage. But how CLASPs specifically modulate microtubule transitions is not understood. Here, we investigate the effects of CLASPs on the pre-catastrophe intermediate state of microtubule dynamics, employing distinct microtubule substrates to mimic the intermediate state. We find that CLASP1 promotes the depolymerization of stable microtubules in the presence of GTP, but not in the absence of nucleotide. This activity was also observed for all CLASP family members and a minimal TOG2-domain construct. Conversely, we find that CLASP1 stabilizes unstable microtubules upon tubulin dilution in the presence of GTP. Strikingly, our results reveal that CLASP1 drives microtubule substrates with vastly different inherent stabilities into the same slowly-depolymerizing state in a nucleotide-dependent manner. We interpret this state as the pre-catastrophe intermediate state. Therefore, we conclude that CLASPs suppress microtubule catastrophe by stabilizing the pre-catastrophe intermediate state between growth and shrinkage.

Third Session- Speaker 1

The transcription factor MEC-3 regulates the adhesion GPCR FMIL-1 to direct neuron-specific synapses in the PVD nociceptive circuit

Tyler Kennedy, Damilola Oje, Kylie Howerter Barbara O'Brien, Rebecca D. McWhirter, Jamie Stern, David M. Miller, III.

Vanderbilt University, Nashville, TN, 37235

Neural circuit architecture is highly reproducible within species, suggesting that genetic programs are key determinants of neuronal wiring diagrams. To investigate this question in C. elegans, we used the PVD sensory neuron as a model. PVD drives an escape response to nociceptive stimuli via selective connections with the PVC interneuron in the densely packed ventral nerve cord. We developed fluorescent, live-cell markers for visualizing PVD>PVC synapses and performed an RNAi screen of PVD-enriched transcription factors for PVD synaptic defects. This approach revealed that the LIM homeodomain transcription factor MEC-3 is necessary for PVD>PVC synapses. To identify MEC-3 targets, we used FACS to isolate PVD neurons for RNA sequencing. An RNAi and mutant screen of MEC-3-regulated genes determined that the adhesion protein FMIL-1 phenocopies the PVD miswiring defect in mec-3 mutants. fmil-1 (Flamingo-like) encodes an adhesion class GPCR, a family which has been implicated in neuronal target selection in mammalian circuits. Because the PVD nociceptive circuit is established during larval development, we can use our synaptic markers to determine the temporal requirements of fmil-1 for PVD>PVC synapses. In particular, we can ask: "Is FMIL-1 required for synapse formation or synaptic stability?" questions that are rarely accessible to direct analysis in mammalian neurons.

Third Session- Speaker 2

Interleukin 13 (IL-13) accelerates SPEM maturation and proliferation in mouse normal and metaplastic gastroids

Ela Contreras Panta1-2, Su-Hyung Lee2,4, Eunyoung Choi1-4, and James R. Goldenring1-4

1 Department of Cell and Developmental Biology, School of Medicine, Vanderbilt University, Nashville, TN, USA 2 Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN, USA. 3 Section of Surgical Sciences, Vanderbilt University Medical Center, Nashville, TN, USA. 4 Nashville VA Medical Center

Gastric cancer is one of the leading causes of cancer mortality worldwide, however, key regulators and mechanisms involved in the gastric carcinogenesis remain unclear. The onset of metaplasia phenotypes in the stomach is correlated with the development of gastric cancer. Therefore, understanding the cellular events that lead to stomach metaplasia proliferation and progression remains a priority. Spasmolytic Polypeptide-Expressing Metaplasia (SPEM) is the earliest type of metaplasia seen in the stomach. Using transgenic Mist1-Kras mice, we have established murine SPEM gastroid lines (Meta1) from metaplastic glands isolated at 1 month after induction of active KRas (G12D). We have found that these gastroids maintain their SPEM features across multiple passages in in vitro culture. Moreover, single-cell RNA-sequencing analysis revealed that Meta1 have a distinctive transcriptional profile in comparison with mouse dysplastic gastroids, established previously. These data suggest that Meta1 gastroids are a novel in vitro tool for the study of SPEM maturation and proliferation. Recent investigations from our laboratory have shown an increased number of intrinsic immune cells, type 2 innate lymphoid cells (ILC2s), in mouse SPEM tissues. ILC2s are known to release cytokines including IL-13, and we have demonstrated that IL-13 could promote SPEM development in mouse. After isolation of activated ILC2s from IL-13-tdTomato reporter mice treated with the parietal cell toxic drug L635, we co-cultured ILC2s together with Meta1. Co-culture with ILC2s significantly enhanced gastroid growth, and the gastroids co-cultured with ILC2s had a higher proportion of TFF2- and AQP5-co-expressing SPEM cells compared with gastroids cultured alone. In accordance with increased Meta1 gastroid growth, proliferative activity of MUC6- and CD44v9-co-positive cells was also upregulated in gastroids co-cultured with ILC2s. We then studied the effects of IL-13 alone in Meta1 gastroids treated with either recombinant murine IL-13 or vehicle solution (0.1% BSA) as control. Gastroids treated with IL-13 showed a significantly increased growth by diameter measurements in comparison with control. Cell proliferation was also increased as measured by the percentage of Ki67 expressing cells. Moreover, immunoblotting showed up-regulation of phosphorylation of STAT6 in gastroids treated with IL-13 in a dose-dependent manner; and RT-qPCR revealed a significant increase in SPEM-related genes including Tff2, Muc6 and He4 (Wfdc2). These results suggest that ILC2s and, more specifically, IL-13 have a direct effect on Meta1 gastroids proliferation and maturation. Further analyses are needed to understand the mechanisms of action for promoting proliferation and maturation.

Third Session- Speaker 3

Patient Mutations in Mitochondrial Fission Gene (DRP1) Perturb Synaptic Maturation of Cortical Neurons

Tierney Baum1, Caroline Bodnya1, and Vivian Gama 1,2,3

1. Department of Cell and Developmental Biology, Vanderbilt University
2. Vanderbilt Center for Stem Cell Biology, Vanderbilt University
3. Vanderbilt Brain Institute, Vanderbilt University

With the advent of exome sequencing, a growing number of children are being identified with *de novo* loss of function mutations in the large GTPase essential for mitochondrial fission - Dynamin Related Protein 1 (DRP1); these mutations result in severe neurodevelopmental phenotypes such as developmental delay, optic atrophy, and epileptic encephalopathies. Though it is established that mitochondrial fission is an essential precursor to the rapidly changing metabolic needs of the developing cortex, it is not understood how identified mutations in different domains of DRP1 uniquely disrupt cortical development and synaptic maturation. We are currently leveraging the power of both high-resolution imaging and induced pluripotent stem cells (iPSCs) harboring DRP1 mutations in either the GTPase or stalk domains to model early stages of cortical development. Time-lapse imaging of axonal transport of mutant DRP1 cortical neurons reveals disrupted mitochondrial motility of severely hyperfused mitochondrial structures. Furthermore, super-resolution imaging of mutant DRP1 synapses shows a significant decrease in post-synaptic densities, suggesting a potential reduction in pre-synapse activity. Cells with mutations at the DRP1 stalk domain exhibit increased severity of these phenotypes. Imaging of organelle dynamics in the context of disease mutations strongly suggests that altered mitochondrial morphology of DRP1 mutant neurons affects the neuronal identity of cortical populations and results in the pathogenic dysregulation of synaptic activity.

Poster Presentations

Poster Session I

Name	Poster Number
Adeniran, Francisca	(see Poster Session II- P4)
Amos, Tyler	P1
Anthony, Christin	P2
Arceneaux, Deronisha	P3
Baer, Brandon	P4
Barman, Bahnisikha	P5
Benthal, Joseph	P6
Berestesky, Emily	P7
Bowman, Deanna	P8
Bracey, Kai	P9
Brown, Monica	P10
Bryant, Jamal	P11
Burgos-Rivera, Julissa	P12
Burman, Andreanna	P13
Cario, Alisa	P14
Cassidy, Anna*	(see Poster Session II- P1)
Cencer, Caroline	P15
Cephas, Amelia	P16
Chalkley, Mary**	P17
Chen, Lei	P18
Cho, Youngwon	(see Poster Session II- P42)
Choudhary, Dharmendra	P19
Colley, Madeline	P20
Cullati, Sierra	P21
Cywiak, Carolina	P22
Dong, Xinyu	P23
Dufresne, Martin	P24
Ebert, Adam	P25
Eleuteri, Nicholas	P26
Esselman, Allison	P27
Fabiano, Emily	P28
Feng, Gaomin	P29
Finnel, Ryan	P30
Fitz, Gillian	P31
Fye, Margret	P32
Gailey, Casey	P33
Garcia, Arlene	P34
Goswami, Soumita	P35
Grub, Lantana	P36
Guenther, Alexis	P37
Hepowit, Nathaniel	P38
Hua, Junmin	P39
Islam, Mirazul	P40
Jimenez, Lizandra	P41
Richardson, Laura**	P42
LaFever , Kimberly	P43

* Breakout Session I
**Breakout Session II

Poster Session II

Name	Poster Number
Cassidy, Anna*	P1
Jung, Youn Jae	P2
Kaur, Harsimran	P3
LaFever , Kimberly	(See Poster Session I – P43)
Adeniran, Francisca	P4
Lee, Nury	P5
Liu, Jiayue	P6
McAtee, Caitlin	P7
Medina, Stephanie*	P8
Merbouche, Lilia	P9
Momoh, Michael	P10
Mulligan, Alexandra	P11
Nagai, Taylor	P12
Najam, Syeda Sadia	P13
Nevills, Simone	P14
Peebles, Katherine	P15
Peek, Jennifer	P16
Perkins, Olivia	P17
Pfannenstein, Alex	P18
Richardson, Laura*	(See Poster Session I – P42)
Rodgers, Nikki	P19
Rossi, Anthony	P20
Ruark, Elizabeth	P21
Ruelas, Amanda	P22
Sanchez, Zachary	P23
Sawant, Avishkar	P24
Schaffner, Samantha	P25
Schwarzkopf, Anna	P26
Scott, Claire	P27
Scott, Halee	P28
Siladi, Andrew	P29
Sohn, Yoojin	P30
Stricker, Aubrie	P31
Sun, Rong	P32
Tirgar, Reyhaneh	P33
Van Ardenne, Jacqueline	P34
Vega, Paige	P35
Waghmare, Indrayani	P36
Wang, Wenjun	P37
White, James	P38
Willet, Alaina	P39
Won, Yoonkyung	P40
Yao, Vincent	P41
Cho, Youngwon	P42

* Breakout Session I
**Breakout Session II

Session I – P1

A single-cell RNA-Seq strategy that uses combinatorial barcoding for whole transcriptome coverage of C. elegans neurons.

Tyler Amos, Seth Taylor, Alec Barrett, Alexis Weinreb, Marc Hammerlund, David Miller III

Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 37212; Cell Biology and Physiology, Brigham Young University, Provo, UT, 84602; Department of Neurobiology, Yale University School of Medicine, New Haven, CT, 06520

The CeNGEN project seeks to produce gene expression maps for every neuron in the C. elegans nervous system in hermaphrodites and males and across post-embryonic development. Droplet-based sequencing methods are readily scalable and ideal for generating accurate profiles of single cells but typically do not provide whole transcriptome coverage (e.g., alternative splicing, noncoding RNAs). In contrast, bulk sequencing methods that rely on FACS-isolation of specific neuron types can achieve whole transcriptome coverage but are not amenable to high throughput data collection and are less accurate due to contaminating transcripts from non-target cells. We have now utilized an alternative approach that retains the precision of single cell sequencing while expanding transcriptome coverage. Mid-L1 larvae were dissociated with established methods and ~10,000 cells processed with a Parse combinatorial barcode labeling protocol. The Parse method uses both poly(dT) and random primers to target whole transcripts and noncoding RNAs. In addition to muscle, intestine, seam cell, glia, germ cells, etc., our data set includes ~40 clusters of identifiable neuron types. In comparison to a droplet-based profile of L1 cells, the Parse data set is more sensitive (3x UMI/cell) and affords robust 3'-5' transcript coverage. We are exploring a CAS9-based strategy for depleting ribosomal RNA sequences which comprise >60% of reads in the Parse data set.

Session I – P2

Investigating the role of cadherin in canonical Wnt signaling

Christin C Anthony1, Amanda Lawson1, Vivian Weiss2, David Robbins3, Yashi Ahmed4, Ethan Lee1

1Vanderbilt University, Nashville, TN 37232; 2Vanderbilt University Medical Center, Nashville, TN; 2Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057; 4Geisel School of Medicine, Dartmouth College, Hanover, NH 03755

The transcriptional coactivator, β-catenin, is the central mediator of canonical Wnt signaling. Its early identification as a key component of cadherin junctions has led to speculation that there is an intimate cross-talk between Wnt/ β-catenin signaling and cadherin function. Several studies have shown that 1) acute release of β-catenin associated with cadherin can activate Wnt/ β-catenin signaling and 2) the rate of β-catenin turnover in the cytoplasm is more rapid than those associated with cadherin. To better understand the dynamics of cellular β-catenin in Wnt signaling and cadherin function, we initiated a careful analysis of Wnt signaling in a HEK293T cell line in which N-cadherin has been knocked out (293T NC cells). We show that 293T NC cells exhibit low levels of cytoplasmic β-catenin compared to the HEK293T parental line and demonstrate a robust increase in total β-catenin levels in response to Wnt3a treatment. In contrast, the parental HEK293T line exhibited high levels of total β-catenin and showed minimal changes in β-catenin levels in response to Wnt3a. We found the transcriptional response, as assessed by the TOPFlash assay, of 293T NC and parent cells were similar. Consistent with this finding, examining the kinetics of phospho- β-catenin showed an identical response between the two lines. Our current goals are focused on performing careful measurements of the turnover rates of cytoplasmic β-catenin and those associated with cadherins using luciferase-based strategies. In addition, we have developed PROTAC compounds that target β-catenin for degradation. To assess whether targeting β-catenin is a viable approach for developing therapeutics against Wnt-driven cancers, we will determine whether there is a reasonable window in which we can effectively target cytoplasmic β-catenin for degradation with minimal effects on cadherin-associated β-catenin.

Session I – P3

Succinate driven Atoh1-independent tuft cells are derived from absorptive cell lineage

Deronisha Arceneaux, Amrita Banerjee, Joey Simmons, Nabil Saleh, Naila Tasneem, Mirazul Islam, Janney Wang, Cody Heiser, Bob Chen, Ken Lau

Vanderbilt University, Nashville, TN

Atoh1 is known to be essential for the development of intestinal secretory cells such as goblet cells and enteroendocrine cells however, its role in tuft cell development remains unclear. Tuft cells are specialized epithelial cells that are found in the mucosal lining of various organs, including the respiratory tract, gallbladder, and intestine. These cells are characterized by apical tufts of microvilli and are involved in sensing and responding to different stimuli, with research indicating their roles in inflammation regulation, secretion of antimicrobial peptides, and modulation of the gut microbiota. Moreover, tuft cells have been implicated in the development and progression of intestinal diseases, including inflammatory bowel disease and colorectal cancer. Recent research from our lab has revealed the existence of a lineage of tuft cells, called induced tuft cells, which are triggered by the microbiome and are independent of Atoh1. Using immunofluorescence microscopy staining for DCLK1 on succinate-treated Atoh1-knockout mice, we have identified tuft cells, which were corroborated with single-cell RNA sequencing (scRNA-seq) data that identified two separate populations of tuft cells with distinct differentially represented gene programs. Furthermore, scRNA-seq analysis of cells obtained from CRISPR-cas9 barcoded mice revealed that tuft cells had identical mutations to enterocytes, suggesting a shared lineage with enterocytes. In future research, we plan to investigate the link between induced tuft cells and the microenvironment by studying the anti-inflammatory properties of PGD2 in ileitis.

Session I – P4

Overexpression of Alveolar Epithelial Tissue Factor Promotes Maintenance of Lung Barrier Integrity in ALI

Brandon Baer, Nathan D. Putz, Lee Han Noo Ri, Lorraine B. Ware, Julie A. Bastarache

Vanderbilt University Medical Center, Nashville, Tennessee, 37232

Acute Respiratory Distress Syndrome (ARDS) is a common cause of acute respiratory failure. Despite extensive research in animal models, in which the syndrome is called Acute Lung Injury (ALI), no targeted therapy has been found to reduce its high mortality rate. Two major pathologic features of ARDS are loss of lung barrier integrity and activation of the Tissue Factor (TF) pathway of coagulation in the airspace. However, as an integral membrane protein TF also serves several non-coagulant functions including promotion of cell adhesion. All systemic anti-coagulants tested have failed to show clinical benefits in ARDS, with some trials of TF pathway inhibition showing increased mortality in ARDS patients. One explanation for these clinical results is that TF in the airspace is protective in ARDS. Supporting this concept, our previously published mouse work found that loss of alveolar epithelial cell TF caused increased loss of lung barrier integrity in models of ALI. As such, we hypothesize that epithelial TF is necessary for maintaining lung barrier integrity and that its overexpression will be protective in ALI. To determine whether supraphysiologic overexpression of TF in the lung can enhance barrier integrity we created a novel transgenic mouse in which TF was inducibly overexpressed in the lung epithelium (TFEpi+). A human influenza hemagglutinin-tagged TF construct, driven by the CMV-TetO promoter and crossed with SPC-rtTA59 mice was used to produce inducible, lung epithelial-targeted TF overexpressing mice. High alveolar epithelial TF expression compared to wild-type littermates (WT) was confirmed through immunohistochemistry and western blot analysis after one week of doxycycline in drinking water. To induce ALI, mice were intranasally infected with 2000 colony forming units of Klebsiella pneumoniae or PBS. At 24-hours post infection, mice were euthanized, lung tissue was collected, and a bronchoalveolar lavage (BAL) was performed. Animal body weights were recorded pre-, and 24-hours post infection. BAL was analyzed to measure protein, clot time, and leukocyte influx. Lung tissue was utilized to calculate wet-to-dry weight ratios and bacterial burden. TFEpi+ mice infected with Klebsiella pneumonia showed lower BAL protein (400.38 vs 225.94 μg/ml; p=0.0205) and lung wet-to-dry ratios compared to WT. However, weight loss, bacterial burden, BAL clot time, and BAL inflammatory cell counts did not differ between infected TFEpi+ and WT mice. These findings suggest that alveolar epithelial TF overexpression is protective for maintaining lung barrier integrity and a non-coagulate based mechanism, potentially linked to epithelial cell adhesion.

Session I – P5

Cellular cholesterol levels regulate RNA contents of extracellular vesicles

Bahnisikha Barman, Elizabeth M Semler, Kasey Vickers, Alissa M. Weaver

Vanderbilt University, Nashville, TN, 37232

RNA carried by extracellular vesicles (EVs) has emerged as a novel mechanism for cell-to-cell communication and drives many physiological and pathological processes, including cancer. Previously, we identified the conserved endoplasmic reticulum membrane contact site (ER MCS) linker proteins VAP-A and the ceramide transfer protein CERT as significant regulators of the RNA and RNA-binding protein content of a subpopulation of small EVs. Since VAP-A also binds the cholesterol transporter Oxysterol binding protein-related protein 1L (ORP1L) and cholesterol may contribute to EV biogenesis, here we explored the role of cholesterol in regulating RNA-containing EVs. For cholesterol depletion conditions, DKs-8 cells were cultured for 96h in 10% lipoprotein-depleted serum (LDS) supplemented with 250 μM mevalonate and 10 μM mevastatin for 96h. Small EVs were purified by cushion density gradient from control and cholesterol-depleted colon cancer cells. We used confocal and electron microscopy and various biochemical techniques to analyze EV biogenesis and cargo content. We observed a substantial alteration of EVs secreted from colon cancer cells cultured in lipoprotein-depleted growth media and inhibited for cholesterol synthesis. QRT-PCR for candidate microRNAs showed a significant alteration in the RNA content of EVs purified from cholesterol-depleted cells. Knockdown of the VAP-A-binding cholesterol transfer protein ORP1L led to a substantial alteration in the RNA contents of small and large EVs. We propose that cholesterol binding and/or transfer via ORP1L at MCS affects the biogenesis of RNA-containing small and large EVs. Conclusions: These data suggest cholesterol transfer and/or sensing at ER MCS regulates RNA trafficking into small and large EVs.

Session I – P6

Single cell genomic strategies for prioritizing candidate genes in Sox10Dom Aganglionosis Modifier Intervals

Joseph T. Benthal, Justin A. Avila, E. Michelle Southard-Smith

Department of Medicine, Division of Genetic Medicine, Vanderbilt University Medical Center, Nashville, TN USA

Enteric nervous system (ENS) development requires coordinated gene expression that regulates enteric neural crest-derived cell (ENCDC) migration, enteric gliogenesis, and enteric neurogenesis. Perturbations in genes involved in these initial processes can result in gastrointestinal motility disorders, including Hirschsprung's disease (HSCR). The Sox10Dom mouse model of HSCR recapitulates the variable penetrance of the aganglionosis phenotype. To identify the genetic loci that modify severity and penetrance of the Sox10Dom aganglionosis phenotype, our group conducted a genome-wide linkage study on Sox10Dom F2 progeny. Five modifier loci intervals were identified, three of which are novel and do not coincide with known aganglionosis susceptibility loci or intervals containing genes known to participate in neural crest development. We hypothesize that genes within these Sox10Dom modifier loci will exhibit differential gene expression within ENS progenitors from wild type and Sox10Dom fetal intestine and that intervals around these differentially expressed genes will exhibit differential chromatin accessibility. To address this, we are utilizing single cell RNA-seq and single nucleus ATAC-seq generated in our lab in previously mapped modifier intervals to identify and prioritize the most relevant candidate genes in ENS development. Our approach will locate candidate genes and regulatory elements that are likely to impact the severity of the Sox10Dom aganglionosis phenotype and may point to new mechanisms in which SOX10 interacts with genetic elements to produce phenotypes relevant for human disease.

Session I – P7

Cancer-Associated Fibroblasts Increase Invasion in Aggressive Breast Cancer Cells Through Mitochondrial Transfer

Kayla F. Goliwas, Jian Zhang, Sarah Libring, **Emily Berestesky***, Shayan Gholizadeh, Samantha C. Schwager, Andra R. Frost, Thomas R. Gaborski, and Cynthia A. Reinhart-King

***Presenter**

Vanderbilt University, Nashville, TN, 37235

The tumor microenvironment, which includes both the extracellular matrix and non-cancerous cells, is known to modulate tumor growth and invasion out of the primary site. Cancer-associated fibroblasts (CAFs) are a heterogeneous and abundant stromal cell type in the tumor microenvironment with both pro- and anti-tumorigenic roles. In weakly-migratory breast cancer cells, CAFs have been shown to increase invasion by directly leading tumor cells in collective migration. However, there is a lack of understanding surrounding the cooperative nature of CAF-cancer cell interactions and their role in migration for aggressive breast cancer cells. To evaluate the impact of CAFs on collective 3D migration, spheroids were generated containing highly metastatic MDA-MB-231 breast cancer cells in monoculture or co-culture with patient-derived breast cancer-associated fibroblasts and embedded in collagen gels with or without CAFs in the surrounding collagen. We found that while collective migration was observed in all conditions, increased outgrowth was seen in co-culture spheroids when compared to spheroids containing breast cancer cells alone, even when CAFs were in the surrounding collagen. CAF leaders did not significantly promote collective migration, but we did interestingly witness the presence of CAF material within the breast cancer cells. Further exploring the method of cargo transfer, we observed that CAFs communicate with highly invasive breast cancer cells through the formation of contact-dependent tunneling nanotubes (TNTs) that allow for the exchange of cargo and alter the metabolism of breast cancer cells. TNT formation and transferred mitochondria between CAFs and cancer cells was found to increase cancer cell migration. This remained true if breast cancer cells were pre-educated with CAFs or by artificially transferring isolated CAF mitochondria to cancer cells prior to monoculture tumor spheroid formation. Metabolic changes were evaluated using ATPlite and Seahorse XF Real-Time ATP Rate Assays. Mitochondrial transfer was found to increase oxidative phosphorylation without reducing glycolysis, which increased the total ATP output. By contrast, exogenously supplementing the breast cancer cells with pyruvate increased oxidative phosphorylation to the detriment of glycolysis, leading to the same total ATP production with or without pyruvate. Overall, we identified that CAFs communicate with cancer cells through the formation of TNTs for intercellular exchange of materials to promote collective migration. The transfer of mitochondria from CAFs to cancer cells shifted energy metabolism pathways, increased ATP levels, and promoted the migration of highly metastatic cancer cells.

Session I – P8

Using MVID-Causing Mutations to Investigate MYO5B Motor Function

D.M. Bowman, M.J. Tyska, J.R. Goldenring

1Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN; Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN; Section of Surgical Science, Vanderbilt University Medical Center, Nashville, TN

The devastating autosomal recessive disorder microvilli inclusion disease (MVID) causes life-threatening diarrhea in neonates. Mutations in the non-conventional myosin motor, Myosin Vb (MYO5B), lead to MVID. 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 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. 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 predicted mutations or patient MVID-causing MYO5B motor mutations. The MYO5B motor domain constructs were co-expressed with mCherry-espin in a 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. The predicted rigor mutation (N208A) resulted in an accumulation of MYO5B motor signal at the base of microvilli. The predicted actin non-bonder (E443A) had a robust cytoplasmic pool. All the predicted mutations, including the uncoupled mutation (I439A) had a tip-to-cytoplasm ratio below one. 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. This partially functional motor indicates that the entire protein is affected, while the mutation does not impact the MYO5B motor function. Understanding the subtle differences in the phenotype of separate mutations could lead to a better understanding of MVID pathology and insights into the treatment of MVID.

Session I – P9

Glucose - stimulated KIF5B - driven microtubule sliding organizes microtubule networks in pancreatic beta cells

Kai M. Bracey1, Pi'illani Noguchi1, Courtney Edwards2, Alisa Cario1, Guoqiang Gu1,3,4, Irina Kaverina1,3

1Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37235, USA 2Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA. 3Program of Developmental Biology, Vanderbilt University, Nashville, TN 37232, USA. 4Center for Stem Cell Biology, Vanderbilt University, Nashville, TN 37232, USA.

Insulin is a critical hormone in regulating glucose metabolism in the body, and it is produced and secreted by beta cells in the pancreas. In beta cells, molecular motors use cytoskeletal polymers microtubules as tracks for intracellular transport of secretory insulin granules and regulating their positioning relative to the secretion sites. Beta-cell microtubule network has a complex architecture, which allows to provide insulin granules at the cell periphery for rapid secretion response, yet to avoid over-secretion and subsequent hypoglycemia. We have previously characterized a peripheral sub-membrane microtubule array, which is critical for withdrawal of excessive insulin granules from the secretion sites. Microtubules in beta cells originate at the Golgi in the cell interior, and how the peripheral array is formed is unknown. Using real-time imaging, photo-kinetics approaches, and single-molecule tracking in clonal mouse pancreatic beta cells MIN6, we now demonstrate that kinesin KIF5B, a motor protein with a capacity to transport microtubules as cargos, slides existing microtubules to the cell periphery and aligns them to each other along the plasma membrane. Moreover, like many physiological beta-cell features, microtubule sliding is facilitated by a high glucose stimulus. We have previously shown that in high glucose sub-membrane MT array is destabilized to allow for robust secretion. Our new data indicate that MT sliding is another integral part of glucose-triggered microtubule remodeling, likely replacing destabilized peripheral microtubules to prevent their loss over time and beta-cell malfunction.

Session I – P10

Keratin 7 is a Specific Marker for Human Intestinal Tuft Cells

Monica E. Brown, James R. Goldenring, Izumi Kaji

Vanderbilt University, Nashville, TN, 37235 ; Vanderbilt University Medical Center, Nashville, TN, 37232

Background and Aim: The epithelia of small intestine and colon contain numerous different specialized cell types to extract nutrients, maintain homeostasis, and prevent infection. One such cell type, the tuft cell, is found throughout the villi and crypts in two different sub-populations currently known as tuft-1 and tuft-2 cells. These two populations are typically grouped together in human studies due to limited human tuft cell specific markers, and no subpopulation markers are currently identified in human tissues. Identifying markers that are specific to human tuft cells and the two different populations is needed to further study the role of tuft cells in both healthy and diseased intestine. Methods: Using a single cell RNA-sequencing dataset published by Burclaff et al. that contains data of duodenum, jejunum, ileum, and colon from three human male donors, clusters were identified using Leiden clustering with a resolution of 1.5 in scanpy (v1.9.1). Well-established cell-type specific markers were used to identify population clusters and the tuft cell cluster was identified using a mixture of human and mouse marker genes. Marker candidates were then systematically parsed through before determining potential markers. Identified markers were then further analyzed through immunofluorescent staining on paraffin sections of human jejunum and colon using established protocols. Results: Leiden clustering with expression analysis identified two tuft cell clusters; one associated with small intestine samples and one associated with colon samples, through the use of established markers including POU 2 class homeobox 3 (POU2F3), Spi-B Transcription Factor (SPIB), and Keratin 20 (KRT20). Systematic analysis of markers found Keratin 7 (KRT7) to be highly expressed in a subpopulation of tuft cells both in small intestine and colon. Immunostaining for choline acetyl transferase (CHAT), phosphorylated Girdin (also known as CCDC88A), and POU2F3 confirmed co-expression with KRT7 in both human jejunum and colon sections within the cytoplasm. Conclusion: Keratin 7 is a specific marker for human small intestine and colon tuft cells that can be used in replacement of less specific markers.

Session I – P11

Examining the role of WUSP in APC-deficient Wnt pathway activation

Jamal Bryant1, Hassina Benchabane2, Mary Rockouski1, David Robbins3, Yashi Ahmed2, Ethan Lee1

Vanderbilt University, Nashville, TN 37212

1Vanderbilt University, Nashville, TN 37232; 2Geisel School of Medicine, Dartmouth College, Hanover, NH 03755; 3Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057

De-ubiquitinase (DUB) enzymes critically regulate the function and homeostasis of proteins in the canonical Wnt/β-catenin signaling pathway. We performed two independent screens for Wnt pathway regulators - a Drosophila RNA interference screen and an insertional mutagenesis screen in HAP1 cells - and identified the tripartite WUSP complex as a positive regulator of Wnt activation. Recently, our lab characterized a conserved role for this DUB in regulating LRP6 protein homeostasis. Here, we examine a receptor-independent role for USP46 in regulating the Wnt pathway. WUSP complex overexpression enhances Wnt agonist-induced TCF/LEF luciferase reporter activity in HEK293-STF cells, and the knockdown of WUSP blocks pathway activation. WUSP also blocks APC knockdown-induced Wnt-reporter activation. Additionally, we demonstrate that WUSP is required for Wnt reporter activity and target gene expression in APC-mutant SW480 cells but not HCT116 WT KO cells, indicating a role for WUSP downstream APC. Furthermore, in the Drosophila midgut, WUSP RNA interference blocks Wnt reporter expression upon APC loss. In organoids derived from APCmin mice, WUSP knockdown attenuates Wnt target gene expression as well as reduces organoid viability. Together, this data suggests a conserved role for WUSP downstream APC, and future studies aim to characterize the mechanism of WUSP regulation of APC-deficient Wnt pathway activation.

Session I – P12

Investigating the role of IRTKS in Enterohemorrhagic E. coli pathogenesis

Julissa Burgos-Rivera and Matthew J. Tyska

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

Enterohemorrhagic Escherichia coli (EHEC) is a foodborne pathogen that breaches the intestinal epithelium and causes outbreaks of bloody diarrhea and hemolytic uremic syndrome. Despite the threat, EHEC poses to public health, several gaps remain in our understanding of its unique infectious mechanism. During infection, EHEC destroys microvilli on the apical surface of enterocyte cells and attaches firmly to the host. Subsequently, EHEC reorganizes the host cytoskeleton to form dynamic actin-rich structures known as "pedestals," which are suggested to aid in cell-to-cell spread, enhancing its colonization of the intestine. Notably, EHEC remains extracellular during infection and reorganizes host cytoplasmic proteins by secreting bacterial effector proteins into the host using a Type 3 Secretion System. One of those bacterial effectors required for pedestal formation is Translocated intimin receptor (Tir). Once translocated into the cytoplasm of the host cell, Tir inserts into the plasma membrane and binds to intimin, an EHEC surface protein, to initiate intimate bacterial attachment. However, the mechanism by which Tir is inserted into the membrane is still unknown. Interestingly, Tir also binds to the host protein, Insulin Receptor Tyrosine Kinase Substrate (IRTKS) to initiate the recruitment of additional proteins involved in forming actin-rich pedestals. IRTKS is also implicated in actin assembly during microvilli biogenesis and outward membrane curvature via its Inverse BAR (I-BAR) domain. My project seeks to further understand its role in EHEC's infectious life cycle. Our preliminary data shows HeLa cells overexpressing IRTKS contained more adherent bacteria, suggesting that IRTKS may assist in Tir-mediated attachment in addition to pedestal assembly. Interestingly, in infected IRTKS knock-out (KO) Caco2BBe cells there is still Tir signal beneath bacterial attachment sites, but this signal is reduced compared to the control cells, indicating that IRTKS may enhance the delivery of Tir to the membrane. We have also captured live cell imaging of pedestals and will investigate the recruitment of IRTKS to bacterial attachment sites to form a spatiotemporal framework. Overall, this research will define how IRTKS promotes bacterial attachment and build a more comprehensive model of EHEC pathogenesis highlighting the importance of understanding the function host proteins provide to a pathogen's infectious life cycle.

Session I – P13

A Novel Mouse Model Derived from an Uncharacterized MVID Patient MYO5B Mutation

Andreanna Burman, Michael Momoh, Leesa Sampson, Jennifer Skelton, Joseph T. Roland, Cynthia Ramos, Evan Krystofiak, Sari Acra, James R. Goldenring, and Izumi Kaji

Cell and Developmental Biology, Vanderbilt University; Epithelial Biology Center, Vanderbilt University Medical Center; Section of Surgical Sciences, Vanderbilt University Medical Center; Center for Stem Cell Biology, Vanderbilt University; Vanderbilt Monroe Carell Jr. Children's Hospital; Nashville VA Medical Center, Nashville, TN 37205

Microvillus inclusion disease (MVID) is a congenital disorder characterized by severe diarrhea that presents within the first days of life. Inactivating mutations of a motor protein, myosin Vb (MYO5B), have been identified as a cause of MVID. Although a majority of MVID patients carry compound heterozygous mutations, all previously established in vivo MVID models carry homozygous mutations or deletion of MYO5B. We identified a novel set of MYO5B mutations in an MVID patient: a G519R point mutation and another that leads to early truncation. To better understand the pathogenesis of MVID from compound heterozygous mutations, we generated a mouse strain carrying this patient-based mutation. To avoid early lethality by a homozygous Myo5bG519R mutation, we employed the one-step two-cell embryo microinjection (OSTCM) method, that ensures at partial WT Myo5b contribution by inducing CRISPR-mediated mutations in only one cell of two-cell stage mouse embryos. A chimeric founder was backcrossed with WT, and generated progeny heterozygous for the Myo5bG519R allele. To complete the modeling of the patient's mutation, we crossbred a sequence-verified N1 Myo5bG519R/+ mouse with the tamoxifen-inducible intestinal epithelial-directed Villin-CreERT2;Myo5bflox/flox mouse to produce VilCreERT2;Myo5bG519R/flox (Myo5b(G519R)) mice. In the G519R patient's duodenal biopsy, enterocytes lacked DPP4+ mature brush border and Actinin-4+ terminal web structure. Multiplexed immunofluorescence analyses on the G519R patient biopsy revealed a lack of GLUT2-expressing mature enterocytes and accumulation of MYO5B together with RAB11A in the cytoplasm. Adult Myo5B(G519R) mice demonstrated a severe, watery diarrheal phenotype 4 days after the tamoxifen injection. The Myo5B(G519R) mouse intestine illustrated various characteristic features of MVID, such as microvillus inclusions, villus blunting, and subapical accumulation of PAS+ vesicles in the enterocytes. Like the G519R patient, immunostaining in the small intestine of Myo5b(G519R) mice demonstrated a mis-localization of apical nutrient transporters and cytoplasmic accumulation of Myo5b with Rab11a. Electron microscopy revealed the shortened, disorganized microvilli on enterocytes of both the villus and crypt. The crypts of Myo5b(G519R) mouse small intestine were elongated compared to control, mirrored by an expansion of PCNA+, EdU+, and OLFM4+ epithelial cell regions. This study provides a novel MVID patient-mimicking mouse model with compound heterozygous mutations in MYO5B and demonstrates the utility of combining the OSTCM and Cre/loxp systems for modeling deadly monogenic congenital diseases, like MVID.

Session I – P14

Paralog-specific role of CLASP2 in the Golgi cargo export

Alisa Cario, Briahnah Streeter, Anneke Sanders, Irina Kaverina

Vanderbilt University, Nashville, TN, 37235

CLIP-associated proteins (CLASPs) are microtubule associated proteins (MAPs) that belong to microtubule plus-end tracking proteins (+TIPs) and have known roles in cell division, cell motility, and microtubule stabilization. Interestingly, CLASPs are also known to localize to the Golgi and nucleate Golgi-derived microtubules. However, the role of CLASPs in microtubule-based cargo export from the Golgi remains unclear. Furthermore, there are two CLASP paralogs, CLASP1 and CLASP2, and their distinct functions are largely unknown. Here, we study the independent effects of CLASP1 and CLASP2 paralogs on cargo export through use of Erv29-dependent secretory cargo (ES-Cargo) assay using paralog specific knockout cell lines. We find that knockout of CLASP2 impedes cargo export while knockout of CLASP1 does not. Yet, we do not find large scale differences in the microtubule or Golgi morphology. We are currently investigating the mechanism behind this CLASP2 specific effect. Overall, our work reveals important distinctions between the functions of CLASP1 and CLASP2 as well as a role for CLASP2 in regulation of cargo export.

Session I – P15

Adhesion-based capture stabilizes nascent microvilli at epithelial cell junctions

Caroline S. Cencer 1, Jennifer B. Silverman 1, Leslie M. Meenderink 1, Evan S. Krystofiak 1, Bryan A. Millis 2, and Matthew J. Tyska 1

1 Department of Cell and Developmental Biology Vanderbilt University School of Medicine Nashville, TN 37232; 2 Department of Biomedical Engineering Vanderbilt University School of Engineering Nashville, TN 37235

Differentiated transporting epithelial cells present an extensive apical array of microvilli - a "brush border" - where neighboring microvilli are linked together by intermicrovillar adhesion complexes (IMACs) composed of protocadherins CDHR2 and CDHR5. Although loss-of-function studies provide strong evidence that IMAC function is needed to build a mature brush border, how the IMAC contributes to the stabilization and accumulation of nascent microvilli remains unclear. We found that, early in differentiation, the apical surface exhibits a marginal accumulation of microvilli, characterized by higher packing density relative to medial regions of the surface. While medial microvilli are highly dynamic and sample multiple orientations over time, marginal protrusions exhibit constrained motion and maintain a vertical orientation. Unexpectedly, we found that marginal microvilli span the junctional space and contact protrusions on neighboring cells, mediated by complexes of CDHR2/CDHR5. FRAP analysis indicated that these transjunctional IMACs are highly stable relative to adhesion complexes between medial microvilli, which explains the restricted motion of protrusions in the marginal zone. Finally, long-term live imaging revealed that the accumulation of microvilli at cell margins consistently leads to accumulation in medial regions of the cell. Collectively, our findings suggest that nascent microvilli are stabilized by a capture mechanism that is localized to cell margins and enabled by the transjunctional formation of IMACs. These results inform our understanding of how apical specializations are assembled in diverse epithelial systems.

Session I – P16

Identification of an Endocrine-type Intraductal Papillary Mucinous Neoplasm of the pancreas

Amelia Cephas, BS1; Vincent Q Trinh, MSc, MD, FRCPC2,3; Marcus C.B. Tan, MBBS, FACS3,4,5; Kathleen DelGiorno, PhD1,3,4,5

1Cell and Developmental Biology, Vanderbilt University; 2Department of Pathology, University of Montreal, Montreal, Quebec, Canada; 3Department of Surgery, Vanderbilt University Medical Center; Vanderbilt Digestive Disease Research Center; Vanderbilt-Ingram Cancer Center, Nashville, TN 37232

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths in the United States. Several types of neoplastic lesions can give rise to PDAC, including Intraductal papillary mucinous neoplasms (IPMNs). These macroscopic lesions are of ductal origin and are typically benign, though approximately 20-30% can progress to PDAC. IPMN are characterized by long finger-like protrusions of the ductal epithelium that produce mucin and can be categorized as either gastric, intestinal, or pancreata-biliary type. There are currently no markers to distinguish between IPMN that will remain benign and those that will progress to cancer. The aims of this study were to characterize the epithelial content of IPMN to identify markers of progression. 71 human IPMNs were screen by immunostaining for markers of epithelial cell types (e.g. chromogranin A) that we previously identified in pancreatic metaplasia. 20 resected human gastric-type IPMN tissue samples underwent histological staining (immunofluorescence, and Immunohistochemistry) for expression of the following endocrine markers: serotonin(5-HT), gastrin (GAST), insulin, pancreatic polypeptide (PPY), glucagon, synaptophysin (SYP), somatostatin(SST) and ghrelin. A subset of these samples were used to conduct Nanostring multiplex digital spatial profiling. Gastric IPMNs were found to be enriched (>2% positive cells) for expression of endocrine marker chromogranin A. In a subset of 20 gastric-type IPMNs, 70-80% were enriched for a second endocrine marker, SYP. In the SYP+ high lesions, we identified co-expression of 5HT in 90 % of samples and GAST in 80%. GAST+ cells co-expressed with 5-HT in 45% of all samples. Insulin expression was not found in any IPMN but was identified in fragmented islets within the stroma. Glucagon and ghrelin expression was rare. SST and PPY were identified as solitary cells in ~50% and 20-30% of SYP+ lesions, respectively. Nanostring analysis of SYP-high and SYP-low IPMN identified expression of additional hormones not identified in our initiation histological screen.We have identified a subtype of pancreatic IPMN enriched for the expression of endocrine hormones, including 5-HT and GAST. The abundance of hormone expression in gastric-type IPMN suggests a functional role in gastric IPMN formation (as compared to other subtypes) or in malignant progression. Further studies are required to evaluate the use of these markers and to thoroughly identify the functional role of these hormones in disease.

Session I – P17

Early Neurodevelopment and Cytoarchitecture is Altered in Tuberous Sclerosis

Mary-Bronwen L. Chalkley1, Lindsey Guerin2, Samantha G. Mallahan1, Asa A. Brockman1, Laura C. Geben3, Brittany P. Short8, Mustafa Sahin4, Emily Hodges2,5, Rebecca A. Ihrie1,6 & Kevin C. Ess1,6,7

Departments of (1) Cell & Developmental Biology, (2) Biochemistry, (3) Pharmacology, Vanderbilt University School of Medicine; (4) Department of Neurology, Harvard Medical School; (5) Vanderbilt Genetics Institute and Departments of (6) Neurological Surgery, (7) Neurology, and (8) Pediatrics, Vanderbilt University Medical Center

Tuberous Sclerosis Complex (TSC) is a debilitating developmental disorder characterized by a variety of clinical manifestations. While benign tumors in the heart, lungs, kidney, 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 heterozygous loss of function mutation in the TSC1 or TSC2 genes, which encode the hamartin/tuberin proteins respectively. 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 comprising the majority of cells in patients. To examine early neurodevelopmental phenotypes, we utilized TSC patient-derived induced pluripotent stem cells (iPSCs) with a heterozygous microdeletion mutation in TSC2. Within the field, it is debated whether second hits are required. To model this state, CRISPR was used to create a similar deletion mutation in the other TSC2 allele, producing a homozygous mutant line. The heterozygous mutant was also corrected to wild type, creating a set of isogenic lines. This isogenic series was compared to another allelic series with TSC2 deleted. 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. Collectively, these data suggest that mutation or loss of TSC2 has early effects on gene expression in proper neural development. Indeed, our preliminary studies have found that DNA methylation is changed with some key genes in neurodevelopment being hypermethylated in the wild type cells and hypomethylated in TSC2 mutant cells. Understanding precisely when development is disrupted in TSC1/2- mutant brain will be essential to tailoring treatment and determining whether prenatal treatment should be pursued.

Session I – P18

The Sin3a/Hdac/Neurod1 complex epigenetically regulates the differentiation of cerebellar granule cell precursors

Lei Chen, Wen Li, Chin Chiang

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37212

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 the 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 the 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. Our de novo motif analysis has identified Neurod1 as a potential candidate transcription factor that recruits Sin3a/Hdac1 complex to the 3' Atoh1 enhancer, thereby inhibiting the transcriptional activity of Atoh1.

Session I – P19

PLOD3 deficient pro-collagen processing results in autophagy-driven connective tissue disorders

Dharmendra Choudhary, Ela W Knapik

Department of Medicine, Department of Cell and Developmental Biology, and Vanderbilt Genetics Institute, Vanderbilt University Medical Center

Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3 (PLOD3, also known as lysyl hydroxylase 3 [LH3]) is a post-translational modification enzyme that plays a critical role in the stability of the triple helix configuration of collagen. Previous studies have identified pathogenic variants in PLOD3 that result in clinical phenotypes affecting the musculoskeletal, vascular, and sense organs. However, the biological mechanism linking PLOD3 function to these disease traits is unknown. To identify the cellular and molecular mechanisms of PLOD3 and its role in human disease, we utilized electronic health records (EHR), human variant fibroblast cells, and zebrafish as model systems. Using a Phenome-wide association study (PheWAS) methodology, we identified the clinical phenome associated with reduced predicted expression of PLOD3, greatly expanding on the limited phenotypes described in patients with PLOD3 deficiency. To understand the functional relevance of PLOD3 with its associated pathophysiology, we used human fibroblast cells and a mutant plod3 zebrafish model. Zebrafish mgt (plod3 -/-) line display clinically relevant phenotypes such as defects in craniofacial development, body contractures, and malformations in sensory and muscle tissue that are moderately rescued with the overexpression of human PLOD3 mRNA, suggesting PLOD3 has a conserved biological function. In human fibroblasts carrying PLOD3 variant mutations and plod3 -/- zebrafish, we observe defects in collagen processing and accumulation, leading to endoplasmic reticulum (ER) stress. Prolonged ER stress disrupts secretory pathways causing trafficking issues, eventually triggering autophagy signals, making the cells more vulnerable to apoptosis. Together, our data suggest that impaired function of PLOD3 results in improper collagen secretion, which facilitates connective tissue disorders. Understanding these mechanisms can aid in discovering new treatment options and create a framework for novel drug candidate screening for patients with PLOD3 mutations.

Session I – P20

High performance surface UHPLC coupled with PASEF enabled lipidomics allows for thousands of lipid identifications from small sample amounts

Madeline E Colley, Katerina V Djambazova, Martin Dufresne, Jamie L Allen, Angela RS Kruse, Lukasz Migas, Jeffrey M Spraggins

Mass Spectrometry Research Center, Department of Biochemistry Vanderbilt University Nashville, TN, 37240

Imaging mass spectrometry (IMS) utilizes micron thick tissue sections for surface molecular analysis but only recent advances have made large-scale fragmentation available. Traditional IMS still relies on accurate mass to make tentative identifications. One way to focus annotations in IMS is to perform lipidomics to generate a database of possible molecules. However, lipidomics must be performed on closely adjacent sections with minimal sample for the annotations to be relevant. For small tissues, we evaluated a method which couples the quantitative advantages of high performance surface (HPS) high-flow lipidomics with the speed and sensitivity of parallel accumulation-serial fragmentation (PASEF) on single 10 um thick sections of 7.5 mm2 in surface area. There were distinct differences in lipid identifications across the 3 patients which can be explained by the broad and varied functional tissue units present in each tissue as seen in the autofluorescence microscopy. The biopsy samples also showed signs of degradation and oxidation when compared to the QC which could indicate the need for improved tissue archival techniques.

Session I – P21

Fission yeast CK1 promotes DNA double-strand break repair through both homologous recombination and non-homologous end joining

Sierra N. Cullati, Yufan Shan, Eric Zhang, Jun-Song Chen, Jose Navarrete-Perea, Zachary C. Elmore, Liping Ren, Steven P. Gygi, and Kathleen L. Gould

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 37232; Department of Cell Biology, Harvard Medical School, Boston, MA, 02115

The CK1 family are conserved serine/threonine kinases with numerous substrates and cellular functions. The fission yeast CK1 orthologues Hhp1 and Hhp2 were first characterized as regulators of DNA repair, but the mechanism(s) by which CK1 activity promotes DNA repair had not been investigated. Here, we found that deleting Hhp1 and Hhp2 or inhibiting their catalytic activity activated the DNA damage checkpoint due to persistent double-strand breaks (DSBs). The primary pathways to repair DSBs, homologous recombination (HR) and non-homologous end joining (NHEJ), were both less efficient in cells lacking Hhp1 and Hhp2 activity. In order to understand how Hhp1 and Hhp2 promote DSB repair, we identified new substrates using quantitative phosphoproteomics. We confirmed that Arp8, a component of the INO80 chromatin remodeling complex, is a bona fide substrate of Hhp1 and Hhp2 that is important for DSB repair. Our data suggest that Hhp1 and Hhp2 facilitate DSB repair by phosphorylating multiple substrates, including Arp8.

Session I – P22

Receptor-dependent Wnt activation in APC-mutant cells

Carolina Cywiak1, Anna Schwarzkopf1, Starina D'souza1, Jingjing Li1, Jamal Bryant1, Mary Rockouski1, David Robbins2, Yashi Ahmed3, Ethan Lee1

1Vanderbilt University, Nashville, TN 37232; 2Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057; 3Geisel School of Medicine, Dartmouth College, Hanover, NH 03755

Previous studies from our lab (Saito et al. 2018) demonstrated that APC loss results in the clathrin-dependent activation of the Wnt receptor independent of Wnt ligands in cultured human cells and Drosophila. Consistent with this model, siRNA knockdown of the Wnt receptor, LRP6, dominant-negative overexpression of Frizzled, and dominant-negative overexpression of Dvl, blocked Wnt pathway activation upon APC depletion. However, studies by Chen and He (2019) failed to observe the downregulation of the Wnt pathway in APC mutant cells when LRP6 is knocked out by CRISPR-Cas9 editing. We hypothesize that the discrepancy between our studies and that from the He lab (Harvard Medical School) may be due to compensation or adaptation due to genetic knockout (El-Brolosy and Stainier, 2017; Rossi et al., 2015) that maintain elevated Wnt signaling over time in individual knockout clones. To determine if this is the case, we carefully analyzed the level of β-catenin signaling immediately after LRP6 CRISPR-Cas9 treatment. Our preliminary studies indicate loss of LRP6 initially results in significantly decreased β-catenin levels, followed by a gradual increase in β-catenin to levels similar to the original APC mutant cells. These data suggest that failure to observe decreased Wnt signaling in APC mutant cells following LRP6 CRISPR-Cas9 editing is due to genetic adaptation/compensation and provides further evidence for the role of APC in Wnt receptor activation. Ongoing studies include the use of PROTAC to determine if induced degradation of LRP6 protein bypasses compensation/adaptation, RNA seq analysis to identify candidate genes mediating the compensatory mechanism induced by LRP6 CRISPR-Cas9 treatment, and biochemical mapping of the interaction between APC and the clathrin adaptor, AP2.

Session I – P23

α-Parvin, an integrin-related scaffold protein, regulates actin dynamics to facilitate kidney ureteric bud branching morphogenesis

Xinyu Dong1, Fabian Bock1, Nada Bulus1, Olga Viquez1, Reinhard Fassler2, Ambra Pozzi1, Roy Zent

1 Cell and Developmental Biology, Vanderbilt University Nephrology, Vanderbilt University Medical Center, 2 Max Planck Institute of Biochemistry, Germany

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. However, the role of α-Parvin in ureteric bud branching morphogenesis is unknown. We generated α-Parvin UB-specific knockout mice at the initiation of kidney development by crossing the α-Parvinfl/fl with a HOXB7Cre mice. We observed that the α-Parvinfl/fl:HoxB7Cre mice exhibited severely dysmorphic kidneys and died within 2-3 months. Mutant kidneys in different embryonic stages showed significant decrease in size and branching tips, along with widened tubules. Using live-organ imaging of ex vivo mouse ureteric buds with a transgenic mice line that express membrane-tethered GFP (mTmG mice), we showed that We observed that cells within the branching tip underwent repeated rounds of intercalation (interdigitation) in the Parvinfl/fl tubules, while cells in the Parvinfl/fl:HoxB7Cre went through limited reorganization. To compare cell intercalation between Parvinfl/fl and Parvinfl/fl:HoxB7Cre tubules, we tracked the movement of a group of cells that shared a same vertex. We measured the spatial dispersion over time by calculating the standard distance of the tracked cells, and we found that spatial dispersion increased more rapidly in the Parvinfl/fl suggesting active rearrangement of cells within the tubules, while cell movement in the Parvinfl/fl:HoxB7Cre tubules were restricted. A further look into the tubular structure showed excessive basal F-actin formation in the collecting ducts. Similarly, α-Parvin-null CD cells showed excessive F-actin formation, suggesting abnormal actin dynamics. Although the canonical integrin signaling such as FAK/paxillin/ERK pathway is intact, 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 activity. In addition, we observed a persistent loss of function in the actin depolymerizing factor cofilin, the Rho/Cdc42 downstream effector, in both the collecting ducts of α-Parvin-knockout kidneys and in α-Parvin-null CD cells. Inhibition of the Rho and Cdc42 GTPases were sufficient to upregulate the cofilin function, reverse the increased cell adhesion and spreading and revert the abnormal migration. In conclusion, we found that α-Parvin is required for ureteric bud development via regulating actin dynamics, a vital component of cell adhesion and migration.

Session I – P24

Aminated cinnamic acid analogues as dual polarity matrices for high spatial resolution MALDI imaging mass spectrometry of lipids

Martin Dufresne12, Nathan H Patterson12, David M Anderson1, Lukasz G Migas3, Raf Van De Plas3, Richard M Caprioli12457, and Jeffrey M Spraggins1246

1Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN 2Department of Biochemistry, Vanderbilt University, Nashville, TN 3Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands 4Department of Chemistry, Vanderbilt University, Nashville, TN 5Department of Pharmacology, Vanderbilt University, Nashville, TN 6Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 7Department of Medicine, Vanderbilt University, Nashville, TN

Matrix assisted laser desorption/ionization (MALDI) is the leading high spatial resolution (≤ 10 μm) imaging mass spectrometry (IMS) technology owing to its broad molecular coverage and ability to specifically target selected molecular classes based on different sample preparation approaches. A little over a decade ago, 1,5-Diaminonaphtalene (DAN) was proposed as a low volatility dual polarity matrix for high spatial resolution lipid IMS and has remained the commonly used matrix for such analysis. Recent reports have described alternatives such as norharman (expensive) and anthranilic acid derivatives (volatile). Here we propose the use of aminated cinnamic acid analogues (ACAA) that produce both small crystal size and provide high vacuum stability as affordable, high spatial resolution MALDI IMS matrices. ACAA compounds have shown great potential to be the primary MALDI matrix for tissue IMS of lipids at high spatial resolution. The proposed analogues have all been found to be vacuum stable for more than 48h at pressure as low as 5x10-7 mbar along with producing sub micrometer scale crystals when sprayed with either THF or ACN on tissue. UV-vis analysis of the ACAA compounds showed that these derivatives have higher extinction coefficient (1-2x104 $M^{-1}\cdot cm^{-1}$) at 355 nm compared to typical MALDI matrices (3-8x103 $M^{-1}\cdot cm^{-1}$) allowing for lower laser power and thus reducing the on-tissue laser spot size. While DAN is known to produce dimethyl phosphatidylethanolamine (DMPE) like fragment in negative ion mode the new ACAA compounds significantly reduce the formation of these fragments due to the lower laser power required for proper desorption/ionization of lipids from thin tissue sections. A final advantage for these ACAA compounds is there relatively low toxicity compared to the now proven carcinogen and mutagenic DAN. The new ACAA compounds have enabled 5 um spatial resolution IMS of both human kidneys and human eyes as part of the Human BioMolecular Atlas Program in both positive and negative ion mode allowing the visualization of hundreds of lipids at cellular resolution.

Session I – P25

Myriocin's Effect upon Sphingolipid biosynthesis and their role in cellular aging

Adam C. Ebert, Nathaniel L. Hepowit, Jason A. MacGurn

Vanderbilt University, Nashville TN, 37240

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 though the upregulation of mitochondrial selective autophagy known as mitophagy. Taken together this reveals how cells adapt and respond to the inhibition of sphingolipid biosynthesis in a way that promotes cellular longevity.

Session I – P26

Recruitment of MYC to target genes by chromatin-resident cofactors

Nicholas A. Eleuteri, Jing Wang, Brittany K. Matlock, David K. Flaherty, Qi Liu, William P. Tansey

Vanderbilt University, Nashville, TN, 37203

The MYC family of oncoprotein transcription factors (c-, N-, and L-MYC) are overexpressed in a majority of cancers and contribute to upwards of 100,000 cancer-related deaths in the USA each year. MYC dysregulation promotes tumorigenesis through the aberrant expression of its target genes, which drives processes such as cell proliferation, metabolism, and ribosome biogenesis. Central to MYC function is its ability to recognize regulatory elements within its expansive set of target genes, which it does by associating with its obligate partner MAX and binding DNA in a sequence-specific manner. Although this model for MYC function has been the accepted standard for decades, recent studies have revealed that target gene recognition by MYC can be an avidity-driven process involving interaction of MYC/MAX dimers with both DNA and chromatin-resident cofactors such as WDR5. This process of "facilitated recruitment" by WDR5 is important for binding of the c-MYC family member to a small cohort of genes (<100) required for robust protein synthesis. Importantly, mutations in c-MYC that disable interaction with WDR5 block its ability to initiate and maintain tumors, revealing that WDR5 can provide a therapeutic opportunity to block otherwise "undruggable" MYC function in cancer cells. Since the facilitated recruitment model was first proposed, studies have shown that a vast majority of chromatin targeting by MYC is likely to be a facilitated process, and the histone demethylase LSD1 has emerged as a compelling MYC recruiter that co-localizes with MYC at genes involved in cellular metabolic processes. If WDR5 and LSD1 are to open new inroads into targeting MYC in cancer, we must understand the gene networks that are controlled by facilitated recruitment through each of these factors. To achieve this objective, I will employ cutting-edge genomic and transcriptomic approaches to reveal how each of these recruiters acts to dictate target gene selection by MYC proteins. Using the "degron tag" (dTAG) system, I will acutely deplete WDR5 and LSD1 from cancer cell lines overexpressing c- and N-MYC to delineate MYC target gene networks under the control of each factor. This work will lay the foundation for new ways to therapeutically target MYC via its recruitment cofactors.

Session I – P27

Microscopy-directed Imaging Mass Spectrometry for Rapid High Spatial Resolution Molecular Imaging of Glomeruli

Allison B. Esselman1,2, Nathan Heath Patterson1,3, Lukasz G. Migas1,4, Martin Dufresne1,3, Katerina V. Djambazova1,5, Madeline E. Colley1,3, Raf Van de Plas1,3,4, Jeffrey M. Spraggins1,2,3,5*

1Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, USA 37232; 2Department of Chemistry, Vanderbilt University, Nashville, TN, USA 37232; 3Department of Biochemistry, Vanderbilt University, Nashville, TN, USA, 37232; 4Delft Center for Systems and Control, Delft University of Technology, Delft, The Netherlands; 5Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA 37232

Kidneys are complex organs that execute numerous functions including blood filtration which occurs in glomeruli. A glomerulus is a spherical structure approximately 200 μm in diameter that functions through substructures made up of fenestrated capillaries, mesangium, basement membrane, and podocytes. Diseases, such as diabetic nephropathy, can alter glomerular substructures and diminish their ability to filter blood properly. To investigate molecular changes of glomeruli on a cellular level, we utilized high spatial resolution (5 μm pixel size) matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) to map the lipid content from hundreds of individual glomeruli across human tissues. Furthermore, we demonstrate a microscopy-driven approach for targeting specific tissue structures for analysis, which substantially increases throughput for high spatial resolution MALDI IMS. To uncover molecular heterogeneity within hundreds of glomeruli in a single tissue section, rapid high spatial resolution MALDI IMS was needed, enabled by an automated localization and spatially targeted acquisition of only specific tissue structures (e.g., glomeruli). A deep learning-based machine learning model was used to recognize and segment glomeruli based on autofluorescence microscopy images, automatically finding and outlining all glomeruli from a human kidney section. The resulting glomeruli polygons were used as measurement regions for 5 μm MALDI IMS data acquisition. The acquisition of 268 segmented glomeruli took ~7h 40m, which included 747,214 pixels. Using traditional methods, it would have taken ~3.5 days and 6,645,708 pixels to image the whole tissue section. k-means clustering of the individual pixels, with k=7, enabled assessment of molecular differentiation within individual glomeruli, and each glomerulus exhibited varying mixtures and intra-glomerulus-patterning of these clusters. These clusters also captured clear differences between healthy and diseased glomeruli. For example, the mass spectral profile for a prominent cluster of diseased glomeruli reported higher intensity of sphingomyelin (SM) 34:1. Furthermore, diseased glomeruli showed a decrease of phosphatidylserine (PS) 36:1 intensity compared to healthy glomeruli. The lipidomic changes observed within diseased glomeruli may coincide with cellular changes. Experiments are underway to integrate immunofluorescence microscopy with MALDI IMS and k-means clustering segmentation to enable lipidomic profiles to be linked to specific cell types within the glomerulus.

Session I – P28

Vinculin mediates migration and bioenergetics in breast cancer

Emily D. Fabiano, Dr. Katherine M. Young, Nathaniel Seluga, Dr. Brenton Hoffman, Dr. Cynthia Reinhart-King

Vanderbilt University, Nashville, TN, 37235; Hopkins Authentic Research Program in Science, Hopkins School, New Haven, CT, 0615; Duke University, Durham, NC, 27708

Cancer cell migration, an early step of metastasis, is fueled by energy released during dephosphorylation of ATP to ADP. However, cellular energy needs in migration are poorly understood. Migration is directed by signals and mechanical anchorage from integrin mediated adhesion to the extracellular matrix. Integrins cluster to form focal adhesions (FAs), which transmit adhesive and traction forces between the cytoskeleton and the extracellular matrix. Thus, FAs are the primary mechanism through which cells interact with the extracellular matrix, vital for cell migration. Our lab has shown that silencing an FA protein, vinculin, in MDA-MB-231 metastatic breast cancer cells impairs unidirectional migration. Instead, the vinculin deficient cells oscillated when placed in our collagen microtracks that mimic the tracks cell use when metastasizing in the body. This highlights the critical role vinculin is thought to play in affecting cancer cell dissemination with potential ramifications in metastasis, as vinculin is crucial for regulating traction forces and adhesion strength to the extracellular matrix. To more precisely determine how vinculin regulates cell bioenergetics and migration, we manipulated vinculin expression and measured cellular metabolic and migratory responses. We hypothesize that with reduced vinculin, the ability of cells to transfer force from the cytoskeleton to the matrix becomes impaired, and cells upregulate their energy to compensate. We demonstrated that transiently silencing vinculin increases energy utilization, suggesting that vinculin may mediate metabolism. We also found that ATP:ADP, measured using the PercevalHR probe, was significantly upregulated on softer substrates in MDA-MB-231 cells. On stiffer substrates, cells form mature FAs, allowing the cells to adhere. Thus, weakened FAs on softer surfaces correlating with increased energy suggests that cells may be compensating for reduced adhesion ability by increasing ATP:ADP. We generated a vinculin knockout MDA-MB-231 cell line using CRISPR/Cas9 to further probe the role of vinculin in bioenergetics and migration. Bioenergetic results suggest that vinculin deficient cells experience increased glucose uptake and mitochondrial membrane potential. Based on migration measurements in 3D collagen, our data suggests that the vinculin deficient population may migrate more slowly through the extracellular matrix. By uncovering the mechanisms that mediate metabolic changes that play a role in cancer cell migration, we can pave the way for a therapeutic avenue by targeting metabolic mechanisms.

Session I – P29

The InsP3R coordinates mitochondrial stress adaptation to promote longevity

Gaomin Feng, Erica Olfson, Elizabeth Ruark, Kristopher Burkewitz

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

Mitochondrial function plays a central role in aging and age-onset diseases. While mild inhibition of mitochondrial function can extend lifespan from yeast to mammals, more severe disruption is toxic. In order to therapeutically leverage the beneficial effects of mitochondrial reprogramming, we must therefore understand the mechanisms controlling this delicate balance between mitochondrial protection and dysfunction. Using a C. elegans model, we discover 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. Although we find that the InsP3R indeed stimulates bioenergetic tone in invertebrates during both normal and stress contexts, surprisingly, mitochondrial calcium import is not required for longevity. This finding led us to hypothesize that the InsP3R promotes adaptation to mitochondrial stress via additional, matrix-independent pathways, and we have now identified multiple routes by which InsP3R function coordinates mitochondrial health and longevity. First, we have identified that the InsP3R plays novel roles in regulating retrograde transcriptional responses and expression of genes associated with mitochondrial metabolic functions. Secondly, the InsP3R regulates age-dependent mitochondrial dynamics in C. elegans, and we demonstrate that this latter role for the InsP3R is essential for its effects on mitochondrial-mediated longevity. Loss of InsP3R function leads to an increasingly hyperfused mitochondrial network in aging animals. Restoring the balance in mitochondrial fission/fusion dynamics by promoting fission is sufficient to rescue longevity in InsP3R mutants. Collectively, these results suggest that the InsP3R plays a central role in reprogramming mitochondrial functions and promoting longevity when ETC function is impaired.

Session I – P30

Distal regulatory elements that regulate Sox17 expression

Ryan Finnel, Linh Trinh, Anna Osipovich, Leesa Sampson, Mark Magnuson

Vanderbilt University, Nashville, TN, 37235

Organismal development requires the combinatorial interaction of transcription factors at both proximal and distal regulatory elements within and near genes. These interactions may involve regulatory elements that can be separated by over a million base pairs. The formation of topological associated domains (TADs) is mediated by binding of the architectural protein CTCF and a cohesion-mediated loop extrusion mechanism, which facilitates distant interactions between regulatory elements. The Magnuson Lab and others have obtained data indicating that the Sox17, a gene necessary for the formation of endoderm and vascular endothelium, exists within a TAD of approximately 350 kilobase pairs that is bounded by two strong CTCF binding sites. Deletion of a SOX17 TAD boundary prevents the differentiation of hPSCs into endoderm, suggesting that distal regulatory elements in a SOX17-specific TAD may be essential for regulating SOX17 expression. Indeed, many transcription factors important for both endoderm and endothelial cell development have been shown to bind within the putative Sox17 TAD. Based on the binding of FoxA2, Gata4, and ATAC-seq defined open chromatin, we chose two conserved regions in the Sox17 TAD for in vivo functional analysis. The first region, which we call conserved region 3 (CR3) is located 10 kb upstream of Sox17. The second region, termed CR4, is located 230 kbp upstream. Since FOXA2, a pioneer transcription factor important for endodermal differentiation, is known to cooperate with GATA4 to facilitate stable chromatin opening, we hypothesize that CR3 and CR4, sites of open chromatin where both FOXA2 and GATA4 bind, contribute to the temporal and spatial regulation of Sox17 during endoderm development as distal regulatory elements. In Aim 1 of my proposed studies, I will determine the function of CR3 and CR4 by deleting both regions in mice using CRISPR/Cas9 mutagenesis, and by testing their activity as putative enhancers using a LacZ reporter. In Aim 2, I will insert a Sox17 endodermal preferential promoter driven reporter gene into a distal location within the Sox17 TAD to test whether distal elements will activate a fluorescent protein reporter gene. If so, we will also test to see if the same reporter construct, when placed at a location outside the Sox17 TAD, is activated. These two approaches will provide us with both a targeted and global way to test the function of distal regulatory elements within the Sox17 TAD and their role in the regulation of Sox17.

Session I – P31

Protrusion growth driven by myosin-generated force

Gillian N Fitz, Meredith L Weck, Caroline Bodnya, Olivia L Perkins, Matthew J Tyska

Vanderbilt University, Nashville, TN 37237

Actin-based protrusions extend from the surface of all eukaryotic cells, where they support diverse activities essential for life. Models of protrusion growth hypothesize that actin filament assembly exerts force for pushing the plasma membrane outward. However, membrane-associated myosin motors are also abundant in protrusions, although their potential for contributing, growth-promoting force remains unexplored. Using an inducible system that docks myosin motor domains to membrane-binding modules with temporal control, we found that application of myosin-generated force to the membrane is sufficient for driving robust protrusion elongation in human, mouse, and pig cell culture models. Protrusion growth scaled with motor accumulation, required barbed-end-directed force, and was independent of cargo delivery or recruitment of canonical elongation factors. Application of growth-promoting force was also supported by structurally distinct myosin motors and membrane-binding modules. Thus, myosin-generated force can drive protrusion growth, and this mechanism is likely active in diverse biological contexts.

Session I – P32

Secreting adhesions are stabilized via the cytoskeleton in high glucose

Margret A. Fye, Guoqiang Gu, Irina Kaverina

Vanderbilt University, Nashville, TN, 37212

Pancreatic β cells are responsible for secreting insulin to maintain normal blood glucose levels. Our lab has previously shown that the microtubule (MT) cytoskeleton plays a negative regulatory role in this process, preventing insulin granule (IG) secretion by both retention of IGs and trafficking of IGs away from the membrane. It is also established that β cells primarily secrete insulin towards the vasculature using specific "hot spot" proteins, often cytoskeleton-related and mechanosensitive proteins, to accomplish this. However, the specific mechanisms underlying hot spot formation and regulation remain unexplored. We propose that hot spots would be better defined as "secreting adhesions": mechanosensitive subcellular domains which use cytoskeletal regulation to accomplish directed and clustered secretion. This project seeks to determine 1) the precise structure of the cytoskeleton at β cell hot spots and 2) the role of the MT-associated Rho GEF, GEF-H1, in regulating hot spot assembly and function. MT minus and plus ends are close in proximity to, but do not co-localize with hot spot proteins ELKS and LL5β. Studies using TIRF microscopy of mouse islets and the Zn2+-binding dye FluoZin-3 indicate that MT minus ends also do not co-localize with active sites of hot spot secretion. Studies using a similar method but with islets expressing Halo-Lifeact also indicate that there is enhanced actin polymerization at hot spots of secretion. Additionally, our TIRF-FRAP assays in islets expressing GFP-ELKS show reduced ELKS fluorescence recovery in high glucose, suggesting ELKS is stabilized at hot spots in high glucose. To assess the role of GEF-H1 in mediating this stability, we have recently validated a GEF-H1 FRET biosensor, which shows an increase in GEF-H1 activity upon MT depolymerization with nocodazole. Interestingly, cells expressing a dominant negative GEF-H1 show a decrease in ELKS patches. These results point to a role for GEF-H1, and the cytoskeleton, in stabilizing hot spot architecture in high glucose to promote directed and clustered secretion to the vasculature.

Session I – P33

Piezo initiates transient production of collagen IV NCAM-1 promotes synaptic remodeling in developing GABAergic neurons

Casey Gailey, Leah Flautt, Andrea Cuentas-Condori, John Tipps, Siqi Chen, Eleanor Rodgers, Seth R. Taylor, and David M. Miller, III

Department of Cell and Developmental Biology, Program in Developmental Biology, and Program in Neuroscience, Vanderbilt University, Nashville, TN 37235; Saint Cecilia Academy, Nashville, TN 37205

Neural circuits are actively restructured during development as synapses are dismantled in some locations and assembled in others. To investigate the underlying cell biological mechanism, we are exploiting the DD-type GABAergic motor neurons 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. The conserved homeodomain protein, IRX-1/Iroquois orchestrates DD remodeling. An IRX-1 target, the sodium epithelial channel (ENaC), UNC-8, is upregulated in remodeling DD neurons to trigger a Ca2+-dependent mechanism of presynaptic disassembly. Additional downstream effectors are likely required, however, because UNC-8 dismantles a subset of presynaptic components (RAB-3, v-SNARE, liprin-α, endophilin) whereas IRX-1 also acts in a parallel pathway to remove additional presynaptic proteins (UNC-13, ELKS, Clarinet). To identify additional remodeling genes, we used single cell RNA-Seq (scRNA-Seq) to profile D-class GABAergic neurons at periodic intervals spanning the remodeling period. Analysis of this data set revealed 93 genes that are transiently expressed in remodeling DD neurons. An RNAi screen detected a necessary role for the neural cell adhesion protein, NCAM-1, in DD synaptic remodeling. A genetic mutant of ncam-1 impairs both the removal of ventral GFP::RAB-3 and its reassembly at dorsal DD neurites. Interestingly, the ncam-1 mutant also delays remodeling of CLA-1/Clarinet, an active zone component that is not regulated by UNC-8. Thus, our results suggest that NCAM-1 functions in parallel to UNC-8 to promote DD presynaptic remodeling. Because NCAM functions as a key regulator of synaptic plasticity in mammalian neurons, we are intrigued with the possibility that NCAM-1 drives synaptic remodeling in C. elegans in a conserved mechanism that also governs circuit refinement in the developing brain.
NIH Funding: 5T32HD007502 (CG), R01NS10695 (DMM).

Session I – P34

Elucidating the mechanism of mitochondrial DNA copy number regulation

Arlene Garcia, Maulik Patel

Vanderbilt University, Nashville, TN, 37203

Mitochondria are unique among organelles in that they contain their own genome. The mitochondrial genome is regulated independent of the cell cycle, allowing it to reach levels of hundreds to thousands of copies per cell. This cell-independent regulation is especially important in cells with high energy demands, such as muscle cells and neurons, where cells require thousands of copies of mtDNA to generate sufficient cellular energy. Therefore, mtDNA copy number must be tightly regulated yet the mechanisms controlling mtDNA copy number remain unclear. It has been previously demonstrated that mtDNA copy number declines throughout life. In fact, there is a precipitous decline in mtDNA copy number that occurs with age, which correlates with various age-related disorders including cardiovascular disease, neurodegenerative disorders, and cancer. However, the cause of this decline in mtDNA copy number and whether preventing this loss can improve age-related phenotypes and extend longevity remain unknown. Many studies in humans have demonstrated this age-related decline in mtDNA copy number, but these studies are limited in that they are correlative and provide no mechanistic insight. Further, these studies are limited in that are unable to distinguish whether this age-related decline in mtDNA copy number occurs through an active mechanism or a passive mechanism since physiological age cannot be uncoupled from chronological age in humans. However, the nematode Caenorhabditis elegans is a metazoan model organism that overcomes this challenge. C. elegans have an alternate stage of development called dauer that allows us to uncouple chronological aging and physiological aging. Dauer is induced by stressful conditions of starvation or overcrowding and, during dauer, many aging phenomena, including age-dependent senescence, are suspended. I have found that mtDNA copy number declines with age in C. elegans, similar to findings in humans. However, I have found that this decline in mtDNA is suspended during dauer, suggesting that the mechanism of mtDNA copy number decline is not a passive, time related process but rather a physiological process that is suspended in dauer. C. elegans thus serves as a powerful tool to investigate the regulation of mtDNA copy number with age because we can recapitulate the age-related decline observed in humans and, with the use of dauer animals, can pause this decline to investigate the mechanism underlying these opposing phenotypes.

Session I – P35

Anthropoid-specific interaction of the chromatin associated protein WDR5 with the DNA helicase HELB

Soumita Goswami, Alissa D. Guarnaccia, Gregory C. Howard, Brian C. Grieb, Shelly Lorey, William P. Tansey

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA; Genentech, San Francisco, CA 94080, USA; Division of Hematology/Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA.

WDR5 is a highly-conserved protein that performs a variety of functions in the nucleus. Its best-known role is scaffolding MLL/SET complexes that catalyze histone methylation, but WDR5 acts outside this setting to promote ribosomal protein gene transcription, recruit MYC to chromatin and bookmark genes for reactivation after mitosis. WDR5 is also overexpressed in cancer and is an auspicious target for pharmacological inhibition in malignancy. Current WDR5 inhibitors target the WIN (WDR5-interaction) site on WDR5, a deep pocket that binds to an arginine containing WIN motif [A-R- (A/S/T)] in partner proteins. The mechanism through which WIN site inhibitors function is largely unknown. Using quantitative proteomics, we identified ~25 proteins whose interaction with WDR5 is altered by WIN site inhibition. My project centers on one of these proteins, a DNA helicase known as HELB. I have identified a WIN motif at the amino terminus of HELB that is required for its interaction with WDR5. I have shown this WIN motif is post-translationally processed to generate an unusually high affinity WIN site, likely orders of magnitude tighter than most WDR5 WIN site binders. Interestingly, this WIN motif is only present in anthropoid HELB proteins, suggesting the interaction arose late in evolution for a specific molecular function in these animals. My current work is geared to understanding the functional significance of the WDR5-HELB interaction and its contribution to WIN site inhibitor function in cancer cells.

Session I – P36

The mitochondrial genome of C. elegans is functionally 6mA methylated

Lantana K. Grub, James P. Held, Samantha S. Schaffner, Marleigh R. Canter, Tyler J. Hansen, Maulik R. Patel

Vanderbilt University, Department of Biological Sciences, Nashville, TN, 37232; Vanderbilt University, Department of Cell and Developmental Biology, Nashville, TN, 37232

Epigenetic modifications provide powerful molecular means for the transmission of conditional information from parent to progeny. As a maternally inherited genome that encodes essential components of the electron transport chain, the mitochondrial genome (mtDNA) is ideally positioned to serve as a conduit for the trans-generational transmission of metabolic information. We, therefore, set out to establish Caenorhabditis elegans as a model to study mtDNA epigenetics. Here we provide evidence that mtDNA of Caenorhabditis elegans is methylated. We performed bioinformatic analysis of publicly available SMRT sequencing data and methylated DNA IP (MeDIP) sequencing data, both of which revealed that C. elegans mtDNA is adenine methylated at high levels. We further confirmed that mtDNA contains 6mA by leveraging highly specific anti-6mA antibodies. To directly assess mtDNA 6mA, we designed MeDIP assay followed by droplet digital PCR using mtDNA specific primers. This confirmed, with high specificity, that mtDNA is methylated. Combined, these assays provide evidence that supports the presence 6mA in C. elegans mtDNA. Additionally, mtDNA methylation can be dynamically regulated and increases in response to the mitochondrial stressor antimycin. This discovery provides an excellent model for future studies to investigate the regulation and inheritance of mitochondrial epigenetics.

Session I – P37

Loss of Sox9 in SPEM cells suppresses metaplasia progression during gastric carcinogenesis

Alexis Guenther, Eunyoung Choi

Vanderbilt University Medical Center, Nashville, TN, 37232

Gastric cancer develops through a series of glandular pre-cancerous lesions in the stomach mucosa. The first stage is pyloric metaplasia which is developed by the transdifferentiation of gastric chief cells into spasmolytic polypeptide-expressing metaplastic (SPEM) cells. These SPEM cells are then seen throughout the final two pre-cancerous stages, intestinal metaplasia and dysplasia. However, it is currently unclear what roles they play in the progression of metaplasia during carcinogenesis. We have previously observed that SPEM cells display strong expression of the transcription factor Sox9 in both human and murine precancerous gastric tissues. Sox9 is expressed in adult intestinal stem cells and is believed to control stem cell dynamics. We therefore hypothesize that Sox9 may act to control proliferation and differentiation in SPEM cells and regulate metaplasia progression. To investigate this question, we utilized a mouse model (GCK) expressing activated Kras under the chief cell-specific driver (GIF) with a doxycycline-inducible rtTA-Cre system, which drives gastric carcinogenesis. Using the GCK mouse, we generated a new mouse allele which deletes the Sox9 gene only in gastric chief cells (GCK-Sox9KO) and performed immunostaining for cell lineage markers to examine cellular and morphological differences in stomach mucosas. At three and six weeks after Kras-induction, the GCK mice display pyloric metaplasia in nearly 100% of their glands which can be identified by a population of CD44v9+/Sox9+/GSII+ SPEM cells in the base of the gland, loss of parietal cells in the neck, and hyperplasia of surface cells. However, GCK-Sox9KO mice display a significant reduction in SPEM cell-populated gland bases and the appearance of a previously unobserved gland type that has parietal cells populating the base of the gland instead of SPEM cells. By ten weeks, over 80% of glands in the GCK-Sox9KO mouse stomachs appear to have recovered to normal types of gastric glands containing both chief cells and parietal cells, while nearly 100% of the glands in the GCK models progressed to dysplasia with no chief cells or parietal cells. These results show a significant disruption in the progression of metaplasia by the loss of Sox9 in SPEM cells and a possible recovery phenotype after failure of pyloric metaplasia to progress to more advanced stages. Therefore, this study suggests that Sox9 in SPEM cells may control the cell lineage progression in gastric carcinogenesis.

Session I – P38

Ede1, a ubiquitin-binding endocytic receptor, reveals a non-canonical role in mitochondrial swelling

Nathaniel L. Hepowit, Jason A. MacGurn

Dept. of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37232

Mitochondrial swelling plays a central role in the pathogenesis of many human diseases such as diabetes, neurodegeneration and cardiomyopathy. Therefore, it is important to elucidate the mechanisms of the swelling process to aid the development of mitochondria-targeted drug delivery to maintain their morphological and functional integrity. In the yeast model system, we observed that the analogous mitochondrial swelling is induced in starved chronologically aging cells. Interestingly, in the absence of Ede1, the yeast homolog of mammalian Eps15, the mitochondria appear fragmented at mid-log phase and fail to undergo swelling in aging cells. Similarly, the expression of an Ede1 variant (ΔUBA domain) incapable of binding K63-linked polyubiquitin suppresses mitochondrial swelling despite of unaltered mitochondrial morphology at mid-log phase. Notably, when Ede1 is fused with a deubiquitinating enzyme UL36, the mitochondria of aging cells form a hyperfused meshwork without apparent indication of swelling. By using the fluorescence protein complementation assay, we found that a subpopulation of Ede1 physically interacts or in close proximity with mitochondrial proteins Tom70 and Dnm1 in a mechanism that requires a K63-linked polyubiquitin. Overall, our findings demonstrate that ubiquitin and the ubiquitin-binding protein Ede1 have regulatory roles in mitochondrial dynamics.

Session I – P39

A New Model of Cell-Cell Fusion Mediated by Plasma Membrane Damage and Repair

Junmin Hua, Andrew D. Pumford, Lilia Nassar, James White, M. Shane Hutson, Andrea Page-McCaw

Vanderbilt University, Nashville, TN, 37203

Restoration of tissue integrity after injury is essential to the survival of all organisms. We use Drosophila pupal notum damaged by laser ablation as a model system to study the wound healing mechanisms. Starting about 10 minutes after laser ablation, mononuclear cells of the pupal epidermis fuse and form multinucleated syncytia to assist with re-epithelialization. The objective of this study is to investigate the mechanisms of wound-induced syncytia formation. Based on our data, we hypothesize that syncytia fuse in response to plasma membrane damage triggered by the wound. All trauma wounds generate a mixture of damage, but pulsed laser ablation creates a highly reproducible pattern of cellular damage and response. An expanding cavitation bubble is generated within microseconds after wounding, exerting shear mechanical force on cells under its footprint, damaging their plasma membranes and allowing an influx of extracellular calcium within milliseconds after ablation. Interestingly, syncytia form after this type of ablation, called single-shot ablation; but syncytia are absent after scanning ablation, when cells are destroyed by a low power laser scanning multiple times over a selected area of cells so that no damaged cells survive. Using Ca2+ entry as a proxy for plasma membrane damage in single-shot ablation, we found that approximately 96% of fused cell borders co-localize with sites of Ca2+ entry as early as 30 ms after wounding, and all fused cell borders are within the region of plasma membrane damage, as indicated by elevated level of Ca2+ within 60 ms after wounding. We thus conclude that plasma membrane damage primes wound-induced cell fusion. Current models of plasma membrane repair are based on the finding that vesicles fuse with microtears and form membrane patches. We demonstrate that overexpressing the dominant negative form of dynamin, even at a low level, impairs wound-induced cell-cell fusion. Hence, we propose a new model of cell-cell fusion, which, rather than depending on fusogens, is mediated by the plasma membrane remodeling and the contact among membrane patches during the process.

Session I – P40

Temporal recording of mammalian development and cancer using Cas9

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

Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN, USA;
Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA

CRISPR-Cas9-based single-cell technologies have been applied to address a wide range of biological questions, including molecular recording of embryonic development, genetic screening, and tracking tumor evolution at the single-cell resolution. Molecular events can be recorded into DNA as genetic edits to the genome using Cas9. Cellular events can be dynamic and stochastic in nature, and thus, developing a molecular clock system is very useful to order and quantify these events. Here, we develop a custom platform called NSC-seq that can be used for cell lineage and cell division tracking in a temporal fashion but also with single-cell resolution. With simultaneous integration of single-cell transcriptomics with lineage barcoding and temporal recording information, NSC-seq facilitates the generation of multidimensional datasets for elucidating functional heterogeneity and clonal events in vivo. We apply this platform (i) to decipher lineage branching of mouse embryonic development, (ii) to record clonal dynamics of the adult intestinal epithelium, and (iii) to track clonal composition of the mouse intestinal tumors. 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. Overall, NSC-seq enables in vivo temporal recording of mammalian development and cancer at single-cell resolution.

Session I – P41

Evaluation of culture conditions on the presence of Ago2 and RNA in extracellular vesicle preparations

Lizandra Jimenez 1, Youn Jae Jung 1, 2, Bahnisikha Barman 1, Lauren Cocozza 1, Cherie Saffold 4, Roxanne J. Pelletier 1, 3, Alissa Weaver 1, 4

1 Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee; 2 Department of Chemical and Biomolecular Engineering, Vanderbilt University School of Engineering, Nashville, Tennessee; 3 Laboratory of Systems Pharmacology, Harvard Medical Center, Boston, Massachusetts; 4 Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee

Extracellular vesicle (EV)-carried miRNAs can influence gene expression and functional phenotypes in recipient cells. Argonaute 2 (Ago2) is a key miRNA-binding protein that could influence RNA silencing in recipient cells and has been identified in EVs. However, Ago2 is present in a non-vesicular form in serum and has been reported as a major non-vesicular contaminant. In addition, other RNA-binding proteins and RNAs are often minor components of EVs that may be regulated by cell context and purification conditions. To address this problem, we evaluated the effect of growth factors, oncogene signaling, and serum on the vesicular and nonvesicular content of Ago2, other RBPs and RNA in small EV (SEVs) preparations. Media components affected both the intravesicular and extravesicular levels of Ago2, other RBPs, and miRNAs in EVs, with serum contributing strongly to extravesicular contamination. Furthermore, isolation of EVs from hollow fiber bioreactors revealed more complex preparations, with multiple EV-containing peaks and a large amount of extravesicular Ago2/RBPs. Finally, KRAS mutation impacts the detection of intra- and extra-vesicular Ago2. These data indicate that multiple cell culture conditions and cell states impact the presence of RBPs in EV preparations, some of which can be attributed to serum contamination.

Session I – P42

Elucidating the Effect of SSNA1 on Microtubule Self-Repair

Laura Richardson1, Beth Lawrence2, Marija Zanic1,2,3

1 Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN 37240, 2 Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37240, 3 Department of Biochemistry, Vanderbilt University, Nashville, TN 37240

SSNA1 (Sjögren's Syndrome Nuclear Autoantigen 1) is a microtubule-associated protein (MAP) associated with Sjögren's Syndrome, an autoimmune disease prevalent in women over 40 years old. The role of SSNA1 in Sjögren's Syndrome remains unknown and little work has been done to investigate the function of SSNA1 in the context of the cellular cytoskeleton. Our lab has shown that in vitro SSNA1 can modulate microtubule dynamics, localize to microtubule lattice damage, and protect against lattice damage from a microtubule severing enzyme, spastin. Recently, great attention has been directed toward the ability of the microtubule lattice to undergo damage and "self-repair", a process by which GTP (guanosine triphosphate)-bound tubulin dimers incorporate into the damage sites. Intriguingly, certain MAPs have been shown to enhance the self-repair process by recruiting tubulin dimers to damage sites. However, the role of SSNA1 in microtubule self-repair is not known. We hypothesize that SSNA1 localization on lattice damage sites limits the efficiency of microtubule self-repair via steric hindrance. Given that SSNA1 forms fibrils along the microtubule lattice, we theorize that SSNA1 forms bridges across lattice damage sites and hence, strengthens the microtubule lattice. To test this hypothesis, we use an in vitro reconstitution approach with purified protein components and total internal reflection fluorescence (TIRF) microscopy. Our preliminary results suggest that the localization of SSNA1 at lattice damage sites does not inhibit the incorporation of free tubulin in the process self-repair. However, our data does not indicate that SSNA1 promotes microtubule self-repair, prompting further questions on the role of SSNA1 in the damage and repair mechanism. By understanding the influence of MAPs on microtubule self-repair, our work aims to provide insight into the mechanisms underlying the regulation of microtubules and the cytoskeletal networks in their essential cellular functions.

Session I – P43

A novel RNAi screen to identify candidates involved in basement membrane maintenance in the Drosophila gut

Kimberly S. LaFever, Katherine E. Peebles, Jonathan Chung-Bruehl, Aubrie Stricker, Arnav Reddy, Laney Hirt, Satori McCormick, Greer Fraser, Andrea Page-McCaw

Vanderbilt University, Nashville TN 37240

Basement membrane is a sheet-like extracellular matrix that underlies epithelial cells and surrounds muscles. It is responsible for the stabilization of the epithelia, muscles and other tissues, so maintenance of this tissue is crucial. The gut of the Drosophila is an ideal tissue to study basement membrane homeostatis due to the well-characterized structure as well as the numerous genetic tools that are available. Previously, we have found that several genes must be continually transcribed in adult tissues to preserve the function of the gut basement membrane, suggesting that this structure is dynamic. Drawing from a curated list of hundreds of genes encoding components of the extracellular matrix, we are using an RNAi approach to identify more genes that are required for maintaining the basement membrane in the Drosophila gut.

Session II – P1

Investigating the role of the GTP-cap in the regulation of microtubule dynamics in mammalian cells

Anna Cassidy, Veronica Farmer, Göker Arpag, Marija Zanic

Vanderbilt University Department of Cell and Developmental Biology; Department of Chemical and Biomolecular Engineering; Department of Biochemistry Vanderbilt University, Nashville, TN USA

Microtubules are dynamic cytoskeletal polymers essential for cell division, cell motility, and intracellular transport. To execute a multitude of cellular functions, microtubule networks are remodeled via tight regulation of microtubule dynamics. An important feature of microtubule dynamics is dynamic instability-the ability of microtubules to rapidly transition between phases of growth and shrinkage. Dynamic instability can be explained by the GTP-cap model, suggesting that a 'cap' of GTP-bound tubulin subunits at the growing microtubule end has a stabilizing effect, protecting against microtubule catastrophe-the switch from microtubule growth to shrinkage. Loss of this stabilizing GTP-cap exposes the unstable GDP-tubulin lattice, leading to catastrophe. Although the GTP-cap is thought to protect the growing microtubule end, whether the GTP-cap size affects microtubule stability is not known. Notably, a family of microtubule end binding proteins, EBs, were recently found to recognize the nucleotide state of tubulin, and can thus be used as a proxy for the GTP-cap. Indeed, previous work from our lab demonstrated that EB comet size increases with increasing microtubule growth rates, achieved using microtubule polymerase XMAP215 in an in vitro reconstitution system using purified proteins. Surprisingly, the rate of microtubule catastrophe also increased, in spite of the presence of a larger GTP-cap. To what extent these relationships observed in vitro relate to microtubule dynamics in complex cellular environments in the presence of many different microtubule associated proteins is not known. Here, we use high spatiotemporal resolution imaging to directly compare the relationship between EB comet size and microtubule growth rate in LLC-PK1 cells to that measured in vitro. We find that the scaling between the EB comet size and the microtubule growth rate is the same both in cells and in vitro. However, we observe more variability in microtubule growth rates measured in cells than in vitro. We also measure the loss of the EB comet intensity at the onset of catastrophe in cells. Our data reveal that, in spite of the increased complexity of the cellular vs. in vitro environment, the GTP-cap size is similarly modulated in response to microtubule growth rate in both contexts. Furthermore, our results emphasize the necessity to investigate the role of the GTP-cap in modulating microtubule stability in cells.

Session II – P2

Biomanufacturing of RNA-containing extracellular vesicles via a hollow fiber bioreactor

Youn Jae Jung1,2, Jeffrey L. Franklin2, Lauren Cocozza2, Lizandra Jimenez2, Hayden Pagendarm1, John T. Wilson1* and Alissa M. Weaver2*

1.Department of Chemical and biomolecular engineering, Nashville, TN, 37212; 2. Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA

Introduction 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 system 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 commercial hollow fiber bioreactors, which allow cells to grow to high density under 3D-like conditions, to produce high yield of RNA-containing EVs without serum contamination. Methods We investigated the production yield and the characteristics of small EVs (sEVs) purified from DLD-1 cells and hTERT-MSCs via commercial hollow fiber bioreactors. sEVs were collected by iodixanol density gradient ultracentrifugation and characterized by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blot analysis. In addition, we evaluated the expression levels of small non-coding RNAs (ncRNAs) in sEVs after treatment with RNase and with or without detergent. Results: 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. DLD-1 and hTERT-MSC derived sEVs from hollow fiber bioreactor displayed the expected round shape and size (50-200 nm in diameter) along with EV marker proteins, including TSG101 and CD63. Small ncRNAs in sEVs collected from bioreactors, including U6, miR-100, miR-125b, and let-7a, were resistant to treatment with RNase only, but digested after treatment with RNase and detergent. Conclusions: Our results demonstrate that hollow fiber bioreactors can enhance the production of RNA-containing EVs from cells while also preserving the integrity. We are planning to use hollow fiber bioreactors to isolate engineered EVs for developing EV therapeutics

Session II – P3

Consensus tissue domain detection in spatial multi-omics data using MILWRM

Harsimran Kaur,1,2,* Cody N. Heiser,1,2,* Eliot T. McKinley,1,3 Lissa Ventura-Antunes,4 Coleman R. Harris,5,6 Joseph T Roland,1,7 Martha J. Shrubsole,8,9 Robert J. Coffey,1,3,8,10 Ken S. Lau,1,2,3,6,7,8** and Simon N. Vandekar5,6,11, **

1Epithelial Biology Center, Vanderbilt University Medical Center, 2213 Garland Avenue, 10475 MRB IV, Nashville, TN 37232, USA 2Program in Chemical and Physical Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA 3Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA 4Department of Neurology, Vanderbilt University Medical

Spatially resolved molecular assays provide high dimensional genetic, transcriptomic, proteomic, and epigenetic information in situ and at various resolutions. Pairing these data across modalities with histological features enables powerful studies of tissue pathology in the context of an intact microenvironment and tissue structure. Increasing dimensions across molecular analytes and samples require new data science approaches to functionally annotate spatially resolved molecular data. A specific challenge is data-driven cross-sample domain detection that allows for analysis within and between consensus tissue compartments across high volumes of multiplex datasets stemming from tissue atlasing efforts. Here, we present MILWRM - multiplex image labeling with regional morphology - a Python package for rapid, multi-scale tissue domain detection and annotation. We demonstrate MILWRM's utility in identifying histologically distinct compartments in human colonic polyps and mouse brain slices through spatially- informed clustering in two different spatial data modalities. Additionally, we used tissue domains detected in human colonic polyps to elucidate molecular distinction between polyp subtypes. We also explored the ability of MILWRM to identify anatomical regions of mouse brain and their respective distinct molecular profiles.

Session II – P4

Heterogeneity of monocarboxylate transporter function in gastrointestinal mucosa

Francisca Adeniran, Cynthia Ramos, Izumi Kaji

Section of Surgical Sciences, Epithelial Biology Center, Vanderbilt University Medical Center; Cell & Developmental Biology, Vanderbilt University, Nashville, TN, 37213

Short-chain fatty acids and ketone bodies are important energy fuels and signaling molecules for some types of cells, such as neurons and proliferative cells. A solute carrier 16 gene (*Slc16*) family encodes proton-coupled monocarboxylate transporters (MCTs) that mediate cellular metabolism of those energy fuels. Increasing evidence demonstrates that malignant cells with overexpression of MCTs are better able to maintain microenvironmental pH and high energy metabolism for cancer growth. Although overexpression of MCTs in esophageal and gastric adenocarcinoma have been implicated, the precise distribution and function of each MCT subtype in normal epithelial or metaplastic cell lineages are mostly unknown. Utilizing selective antibodies against MCT1 and MCT2, we investigate cell type-specific expressions of MCT subtype in healthy and *Mist1-Cre*$^{ERT2Tg/+}$*; LSL-K-Ras*$^{(G12D)Tg/+}$ (Mist1-Kras) mice, which develop spasmolytic polypeptide-expressing metaplasia (SPEM) within 16 weeks after tamoxifen induction (Choi *et al*. Gastroenterology 2016). Based on immunofluorescence (IF) staining, we discovered that MCT2 was present on the basolateral membrane of gastric chief and parietal cells in control mice (tamoxifen-injected *Mist1-Cre*$^{ERT2Tg/+}$ or *K-Ras*$^{(G12D)Tg/+}$ littermates). However, in the SPEM glands of Mist1-Kras experimental mice, MCT2 was absent along with the decrease in chief and parietal cell populations. Intriguingly, MCT1 was reciprocally expressed in MCT2-negative cells, such as surface mucus cell, mucus neck cell, ChgA^{+} endocrine cell, and Ki67^{+} proliferating cell. With reference to control mice, we identified that Mist1-Kras mice had increased MCT1^{+} mucus neck cell population and that Ki67^{+} proliferative cells of metaplastic glands are more dependent on MCT1. ChgA^{+} endocrine cell population that co-expressed MCT1 was decreased in metaplastic glands compared to control tissues. These observations indicate that different cell types require different MCT subtype depending on energy metabolic pathways. The increase in MCT1-dependency in proliferative metaplastic glands is consistent with the predicted effect of MCT1 inhibitors for cancer treatment. Alterations in MCT subtype expression might be a marker for early diagnosis of metaplastic cells.

Session II – P5

Mechanisms of sepsis-induced inflammation in the aging brain

Han Noo Ri Lee, Fiona E. Harrison, and Julie A. Bastarache.

Vanderbilt University, Nashville, TN, 37235; Vanderbilt University Medical Center, Nashville, TN, 37232.

Background. Sepsis affects millions worldwide with adults over the age of 65 at particularly high risk. Up to 70% of sepsis patients develop delirium and the severity and length of delirium are strong predictors of acquisition of Alzheimer's Disease and Related Dementias (AD, ADRD). We hypothesize that older adults have augmented neuroinflammation during sepsis that correlates development of delirium and persistence of long-term cognitive impairment. Methods. Male and female wild-type (young, 3 months; old, 18 months) mice were used to test the effects of polymicrobial sepsis on neuroinflammatory markers. Sepsis was induced by intraperitoneal injection of a slurry of cecal contents. Plasma and brain were collected 24 hours after infection, and pro-inflammatory cytokines (IL-6, KC/GRO, TNF-α) were measured through multiplex protein quantification system while neuroinflammatory markers (S100B, GFAP, neurogranin, neurofilament light, and c-reactive protein) were measured through enzyme-linked immunosorbent assays (ELISAs). Results. Older mice are more susceptible to sepsis; they experience increased severity of illness and higher mortality. Sepsis increases markers of acute inflammation in young and old mice; they show increased levels of both the circulating and brain tissue expression of proinflammatory cytokines 24 hours after infection. The effect is diminished in the brain, with contrasting pattern between young and old mice; young mice show increased cytokines in cortical tissues while old mice show increased cytokines in hippocampal tissues. Additionally, older mice with sepsis express increased neuroinflammatory markers. Conclusion. Sepsis is accompanied by marked acute neuroinflammation that is aggravated in aged animals. Persistent microglial activation may underlie this pathology and prime the aged brain to develop an ADRD-like syndrome.

Session II – P6

Pancreatitis induces changes in gut microbiota and short chain fatty acid uptake

Jiayue Liu, Kathleen E. DelGiorno

Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232; Department of Surgery, Nashville, TN 37232; Vanderbilt University Medical Center. Vanderbilt Digestive Disease Research Center, Nashville, TN 37232

Background & Aims: Pancreatitis, or inflammation of the pancreas, is a major public health burden as well as a risk factor for pancreatic ductal adenocarcinoma (PDAC), the third leading cause of cancer-related deaths in the United States. Short-chain fatty acids (SCFAs) are produced by the gut microbiota through the metabolic fermenting of partially and indigestible polysaccharides. Previous studies have shown that SCFAs can facilitate the resolution of injury in the gut. The aims of this study were to investigate changes in SCFA levels and microbiota composition in pancreatitis. Methods: Pancreatitis was induced in both male and female mice (wild type or carrying a Ptf1aCre/+ allele) using a 2-week caerulein treatment protocol. Pancreatic injury was assessed by the pancreas weight: body weight ratio (PW/BW), H&E immunostaining, and immunohistochemistry for markers of injury and inflammation. Fecal samples were collected for microbiome whole-genome sequencing (WGS) and both fecal and cecal samples underwent mass spectrometry analysis for SCFAs. Imaging mass spectrometry was performed on injured and control pancreata to screen for metabolites. Results: The PW/BW ratio and immunohistochemistry staining confirmed the formation of pancreatitis and immune cell infiltration into the pancreas. Mass spectrometry of cecal and fecal content revealed a decrease in SCFAs (C2-C4) and a slight increase in isovaleric acid in mice with pancreatitis as compared to the control group. WGS results showed an increase in Bacteroides and Bifidobacterium in the feces of in mice with pancreatitis. Conclusions: The formation of pancreatitis results in a decrease in SCFA content in the cecum and colon as well as changes in SCFA-producing gut microbiota. These data are consistent with published studies showing a decrease in microbiota complexity in disease and suggest an increase in SCFA uptake. We hypothesize that SCFAs are necessary to resolve injury in the pancreas and that supplementation of SCFAs or treatment with probiotics will accelerate this process, potentially offering a new route for treatment in patients. Keywords: ADM, Pancreatitis, Short Chain Fatty Acids, Metabolism

Session II – P7

The role of exosomal endoglin in filopodia and tumor cell motility

Caitlin McAtee1, Daisuke Hoshino2, Nan Hyung Hong3, Bong Hwan Sung1, Evan Krystofiak1, Anthony Maldonado1, Anna Young1, Ariana Von Lersner4, Andries Zijlstra5, and Alissa M. Weaver1,4

1Department of Cell and Developmental Biology, Vanderbilt University, USA; 2Cancer Cell Biology Division, Kanagawa Cancer Center Research Institute, Japan; 3Fred Hutchinson Cancer Research Center, USA; 4Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, USA; 5Genentech, USA

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. We utilized B16F1 melanoma cells and HT1080 fibrosarcoma cells for fixed- and live-cell imaging to quantify filopodia numbers and dynamics in control and exosome-deplete conditions. iTRAQ proteomics was used to identify SEV protein cargoes that contribute to filopodia formation. In vivo experiments were performed using a chick embryo model for metastasis. Inhibition of exosome secretion in cancer cell lines, via Rab27a or Hrs knockdown, 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 of Hrs-KD cells revealed that exosome secretion regulates formation and stability of filopodia. 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 presence of wild type endoglin protein containing an intact RGD integrin binding motif. Overall, our data implicate SEV-carried endoglin and THSD7A as key cargoes regulating filopodia dynamics and metastasis.

Session II – P8

Primary human glioblastoma cells instruct healthy monocytes to adopt M2 macrophage phenotypes.

Stephanie Medina, Niraj Rama, Amanda Kouaho, Madeline J. Hayes, Todd Bartkowiak, Rebecca A. Ihrie, Jonathan M. Irish

Vanderbilt University, Nashville, TN, USA

Background: Glioblastoma (GBM), the most common primary brain tumor in adults, remains incurable and has not yet widely benefitted from immunotherapies. Macrophages are the most abundant immune cell in GBM tumors and may play a key role in blocking anti-tumor immune responses. Due to GBM tumors' ability to remodel immunosuppressive macrophages in the tumor microenvironment, we hypothesized that GBM tumor cells secrete factors that generate these macrophages, and they would have an M2-like suppressor cell phenotype. Here we used ex vivo culture of primary human GBM tumor cells to dissect factors from the GBM tumor microenvironment that instruct monocytes into different macrophage subtypes. Methods and Results: Blood monocytes from healthy donors were isolated and polarized over 6 days with control cytokines or with supernatant from ex vivo cultured primary GBM tumor cells. Control cytokines included IL-4, IL-6, and IFNγ, which respectively generate M2-like M_IL_4 and M_IL-6 suppressive macrophages or M1-like M_IFNγ inflammatory macrophages. Spectral fluorescence flow cytometry with a 4-laser Cytek Aurora quantified macrophage identity proteins by measuring 14 macrophage features. Macrophage subtypes were quantified both by traditional manual gating and with the T-REX computational workflow, which compares a pair of samples using t-SNE for dimensionality reduction, KNN clustering around every cell, and then marker enrichment modeling (MEM) to label enriched proteins on cells specific to macrophages from control or experimental conditions. GBM tumor supernatant polarized macrophages in an M2-like phenotype that T-REX scored as 98% similar to macrophages generated with IL-6 (M_IL-6), 93% similar to M_IL-4, and completely contrasting with M_IFNγ (only 2% similarity). Specific proteins expressed on macrophages polarized by GBM supernatant included CD16, CD32, HLA-DR, CD163, CD206, and CD123. The expression of key suppressive macrophage markers, in response to stimuli present in tumor-conditioned media is consistent with the signature of canonical suppressive macrophages and closely resembled the signature of previously described macrophages that have been associated with worse patient survival. Conclusions: Overall, the findings here indicate that factors secreted by GBM cells cultured ex vivo are sufficient to instruct monocytes from a healthy donor to adopt the phenotype of M2-like tumor-associated macrophages previously observed in patients. This ex vivo system will now be used to test the function of macrophages generated with tumor supernatant (e.g., suppression of T cell proliferation) and ultimately to identify new targets for GBM immunotherapy.

Session II – P9

The MYC-HCF-1 Interaction

Lilia Merbouche, William Tansey

Vanderbilt University. Nashville, TN,37212

The MYC family of transcription factors is over-expressed in about half of all malignancies and results in the death of thousands of Americans a year. MYC is a highly validated but challenging drug target due to its disordered nature and lack of adequate binding pockets. There is, however, a way to target MYC through its various cofactors. Host Cell Factor-1 (HCF-1) is an abundant nuclear protein that is involved in controlling the cell cycle as well as having multiple roles in transcription. HCF-1 has also been implicated in cancer. Our lab has previously shown that HCF-1 and MYC interact through the HCF-1 Binding Motif (HBM) in MYC box IV. When this interaction is perturbed through the degradation of HCF-1, there is a decrease in the expression of tumor maintenance genes. We know that the MYC-HCF-1 interaction regulates transcription without altering the recruitment of either MYC or HCF-1 to chromatin. When HCF-1 is associated with specific client proteins, it acts as a repressor of transcription, whereas when it associates with others, it acts as an activator of transcription. The adaptability of HCF-1 leads to the hypothesis that the interaction with MYC selects for a particular 'flavor' HCF-1 that allows for the activation of genes. The ability to define the mechanism used by MYC and HCF-1 to regulate gene expression is based on our ability to understand the protein environment around HCF-1 or the Proximitome of HCF-1. I will utilize proximity labeling techniques and proteomics to determine the proteins with which HCF-1 interacts. This is critical to our understanding of the mechanism of action between MYC and HCF-1 and how HCF-1 functions in general. The protein environment around HCF-1 influences how it functions, but no robust study into the interaction proteome of HCF-1 has been conducted in recent years. APEX2 proximity labeling is a robust tool that will resolve the HCF-1 interacting proteome. The APEX2 data will be compared with a flag-tagged HCF-1N IP and MudPIT proteomic technique. These experiments will showcase even the most transient HCF-1-protein interactions and allow for further understanding of HCF-1 and the MYC-HCF-1 interaction.

Session II – P10

Alteration of secretory cell lineages and immune cell migration induced by acute tuft cell ablation in mouse intestine

Michael Momoh, Cynthia Ramos, Izumi Kaji

Section of Surgical Sciences, Epithelial Biology Center, Vanderbilt University Medical Center; Cell & Developmental Biology, Vanderbilt University, Nashville, TN, 37213

Background: Intestinal tuft cells have only been recently investigated thoroughly due to their roles in type 2 immunity and chemosensory function. This rare population of intestinal epithelium is implicated in extensively interacting with other cell types and involved in nutrient absorption. Doublecortin-like kinase 1 (DCLK1) is widely used for tuft cell identification in the mouse intestine. However, the effects of tuft cell deletion on mouse intestines have not been fully understood. We investigated the effects of tuft cell deletion on the epithelial cell lineages and overall impact on mouse intestine. Methods: Tuft cell deletion was induced in Dclk1-IRES-GFP-CreERT2/+; Rosa-DTA (Dclk1-DTA) mice by a single dose of tamoxifen injection. DTA/+ mice and Dclk1-CreERT2 mice of littermates received tamoxifen injection at same time as controls. Mouse bodyweight was monitored daily, and intestinal tissues were sampled 2, 4, or 7 days after the tamoxifen injection. Immunostaining of intestinal tissue sections was performed for lysozyme (LYZ), trefoil factor 3 (TFF3), cluster of differentiation 3 (CD3), DCLK1, mast cell protease 1 (MCPT1), Ly6G, and F4/80. Image analysis was conducted using Qupath. Results: Dclk1-DTA mice showed a significant decrease in bodyweight to 11% of their original weight on day 4 after tamoxifen injection. They showed significantly shortened small intestinal length compared to control mice, however, colonic length showed no significant difference. Immunofluorescent staining of induced Dclk1-DTA mouse tissues revealed that DCLK1+ tuft cells were decreased on day 2, almost completely deleted on day 4, and recovered on day 7. Overall brush border structures defined by villin and SGLT1 staining were intact. Correlated with the tuft cell reduction, mislocalized Paneth-like cells, identified with LYZ+ granules, were observed in the middle or upper part of villi with increasing frequency on day 4 and decreasing on day 7. To confirm goblet/Paneth intermediate cells, multiplexed immunofluorescent (MxIF) staining was employed with LYZ and TFF3. Double-positive (LYZ+/TFF3+) cells were significantly increased in the small intestine of tuft cell-depleted mice on day 4. Intraepithelial lymphocytes, mucosal neutrophils, and F4/80+ macrophages/dendritic cells did not show significant changes after the tuft cell deletion. This indicates that there is no inflammatory reactions following tuft cell deletion. Conclusion: Acute ablation of intestinal tuft cell may cause nutrient malabsorption and changes in mucosal defense response in vivo due to the alteration of cell differentiation lineage and the decrease in absorptive area. Our findings support the importance of tuft cell function in nutrient sensing and mucosal defense mechanism.

Session II – P11

Directed coordination of the organelle interactome in aging

Alexandra G Mulligan, Eric KF Donahue. Kristopher Burkewitz

Vanderbilt University, Nashville, TN, 37235

Metabolic dysfunction is a central mechanism of age-related pathology. Maintenance of cellular metabolic homeostasis requires tight coordination between distinct subcellular compartments. Recent research has revealed that organelles achieve this metabolic coordination partly by forming a highly interconnected network of contact sites with the endoplasmic reticulum (ER) as the central hub. However, age-related pathologies often correlate with aberrant morphology and organization of certain organelles, such as mitochondria. These observations lead us to hypothesize that the interactions between organelles become dysregulated during aging and contribute to metabolic decline. To begin testing this model, we developed tools for live imaging of the organelle interactome's hub, the ER. We have observed significant alterations in ER morphology and mass during aging. To accurately investigate the ultrastructure of ER in aging, we used cutting-edge electron microscopy (EM) techniques to generate images with nanometer-level resolution. The most striking differences between young and aged samples were a decrease in ER, an increase in lipid droplets (LDs), and an increase in contact sites between organelles. Based on these findings, we hypothesize that aging leads to an increase in contact sites between ER, mitochondria, and LDs, and that these interactions are directed by the cell rather than being stochastic. I propose that the ER is prioritizing contacts with lipid droplets to try and remove excess lipids via autophagy. I aim to further characterize these interactions using the EM data, and by mutating tethers between organelles to demonstrate that they are indeed functional contact sites. I also plan to investigate the role of autophagy as a potential driver of the shift in ER and LD content. Collectively, these experiments will provide insight into how the organelle interactome changes during aging, and its potential as a target to mitigate age-related dysfunction.

Session II – P12

Computational and functional analysis identifies AP3D1 as a potential candidate gene associated with epilepsy

Taylor Nagai, Ela Knapik, Eric Gamazon

Vanderbilt University, Nashville, TN, 37235; Vanderbilt University Medical Center, Nashville, TN, 37232

Epilepsy is a chronic neurological disease that affects millions of people worldwide. Current treatments for epilepsy are focused on controlling seizure activity through the use of antiepileptic drugs (AEDs). However, despite the dozens of AEDs that have been developed, a large proportion of epilepsy cases remain drug resistant. Genomic analysis has proven to be useful tool for identifying novel candidate genes beyond the ion channel inhibitors and GABA potentiators that make up most AED targets. In our study we apply PrediXcan, a transcriptome-wide association study (TWAS) method, on summary statistics from a previous epilepsy genome-wide association study (GWAS) done by the International League Against Epilepsy in order to identify genes associated with epilepsy based on predicted expression. The machine learning models used for this analysis were trained using expression quantitative trait loci (eQTL) data from the GTEx consortium combined with tissue similarity data from ENCODE and Roadmap consortiums. We then used quantitative polymerase chain reaction (qPCR) data in a zebrafish seizure model as a method to identify functionally relevant genes from the TWAS results. PrediXcan identified several genes associated with epilepsy, both known epilepsy genes such as GABRA2 ($p=2.67x10-7$) and STX1B ($p=1.92x10-6$), as well as novel genes such as CDK5RAP3 ($p=7.63x10-10$), C1QL3 ($p=2.59x10-8$), and AP3D1 ($p=4.06x10-7$), all of which were significant after false discovery rate correction. The qPCR experiment then found that ap3d1 exhibited over two-fold decreased expression in seizure-induced zebrafish, indicating that AP3D1 expression is reduced in seizure response pathways. A transient ap3d1 knockout zebrafish model generated using CRISPR-Cas9 also exhibited increased sensitivity to the seizure inducing agent pentylenetetrazole (PTZ), providing further functional evidence that AP3D1 is involved in underlying seizure mechanisms. This combination of computational and functional data shows that AP3D1 is a promising candidate gene whose expression is directly involved with seizure activity.

Session II – P13

Transcription factor Atf4 is required for islet β cell function under normal and metabolic stress

Syeda Sadia Najam, Aisha Nour, Mahircan Yagan, and Guoqiang Gu

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

Type 2 diabetes (T2D) is a disease of dysregulated blood glucose absorption and metabolism. This disease usually starts with obesity-associated insulin resistance, which needs higher insulin output for glucose homeostasis. The higher insulin secretion increases unfolded proinsulin in the endoplasmic reticulum (ER) and reactive oxygen species (ROS) in the cytoplasm of the β cells, which, if not removed, causes β-cell dysfunction and death. To counter this process, β cells activate stress response to remove the unfolded proteins and ROS. One effector in this response is activating transcription factor 4 (Atf4). Atf4 is a basic leucine zipper (bZIP) stress-responsive transcription factor that belongs to the cAMP-response element-binding (CREB)/Atf family of transcription factors. Here I aim to identify the roles of Atf4 in pancreatic β-cell normal and stress conditions by loss-of function analysis. Islet β-cell specific Atf4 knockout mice (Atf4βKO) were obtained by crossing Ins-CreKI mice with Atf4 floxed mice (Atf4F/F). Glucose tolerance tests and gene expression assays showed that the Atf4βKO mice are normal. Yet islets isolated from these mice have compromised insulin secretion upon glucose and KCl-induced depolarization. Intriguingly, the Atf4βKO islets showed decreased resistance to high glucose- or fatty acid-induced dysfunction. Surprisingly, the overactivation of Atf4 also compromised β-cell function and gene expression. These studies showed that proper levels of Atf4 is required for β-cell function under normal physiology and extreme metabolic stress. Both absence or over-abundance of Atf4 will impair β-cell function, contributing to the development of T2D. The ongoing experiments include the induction of ER stress by high-fat diet (HFD) in control and Atf4βKO mice. Moreover, the single-cell RNA sequencing data is being analyzed to identify Atf4-dependent genes.

Session II – P14

Determining the Mechanistic Relationship Between Maternal Diet and Postnatal Beta Cell Function

Simone A. Nevills, Guoqiang Gu, Ph.D.

Vanderbilt University, Nashville, TN, 37235

Previous studies have shown that intrauterine nutrient levels impact the risk of diabetes in offspring postnatally, but the molecular mechanisms underpinning this phenomenon remain poorly characterized. We hypothesize that maternal malnutrition during gestation may disrupt the fetus' epigenetic program during development, leading to insufficient functional beta-cell mass postnatally. To this end, we show that: 1) endocrine progenitor cells with different DNA methylation patterns give rise to beta-cell subsets with different functional and proliferative capacity; 2) de novo DNA methyltransferases Dnmt3a and Dnmt3b are downregulated in embryonic endocrine progenitors exposed to a low-protein or high-fat diet in utero, while DNA demethyltransferases Tet1 and Tet2 are upregulated; 3) beta cells in offspring of dams fed a low-protein or high-fat diet during gestation demonstrate reduced expression of genes associated with beta cell maturation and function both during development and postnatally (e.g. Pdx1, MafA); 4) islets from adult mice exposed to maternal high-fat diet in utero have compromised insulin secretion when treated with sustained high glucose or free fatty acids in vitro. These findings are consistent with a model in which abnormal DNA methylation in islet progenitors is a key mechanism by which maternal nutrients mediate postnatal functional beta-cell mass and diabetes risk.

Session II – P15

Unraveling multiple mechanisms of maintenance in the adult basement membrane

Katherine Peebles, Junmin Hua, Andrea Page-McCaw

Vanderbilt University, Nashville, TN, 37240

The basement membrane is a sheet-like extracellular matrix that underlies epithelia and surrounds muscles. In the gut of Drosophila, the basement membrane surrounds the muscles used in peristalsis to keep them flat and smooth. The basement membrane is maintained and repaired by poorly understood mechanisms. To begin to characterize these mechanisms, we describe an assay to analyze homeostasis of the basement membrane by separating the mechanisms of assembly and disassembly of collagen IV. The amount of collagen IV incorporated is assayed by measuring the fluorescence of GFP-tagged Collagen IV, the most prevalent component of the basement membrane. Starting with a Collagen IV-GFP heterozygote, with one labeled and one unlabeled allele, we control the expression of Collagen IV-GFP using genetic tools that initiate GFP knock down in adulthood. The loss of GFP-tagged Collagen IV in the basement membrane can be quantified utilizing a novel pipeline of analysis including modules of the interactive machine learning program ilastik and python coding. This novel method for analysis shows a half-life of 15.87 days for Collagen IV in the gut basement membrane during maintenance. When reversing the assay, an immediate increase followed by a steady level of fluorescence is observed. Hypotheses explaining this unexpected trend will be discussed, including the possibility that a RITS complex may inhibit the effectiveness of this experiment. An alternative way to observe Collagen-IV assembly into the basement membrane is by overexpressing Collagen-IV-Scarlet in adulthood. The Collagen-IV-Scarlet localizes to the basement membrane between the muscles before spreading.

Session II – P16

Hybrid Dual scAAV Delivery of Transgenes to the Kidney

Jennifer Peek, Alan Rosales, Richard Welch, Thomas M. Beckermann, Lauren Woodard, Trevor J. Gonzalez, Aravind Asokan, Matthew Wilson

Vanderbilt Medical Scientist Training Program, Nashville, TN, 37212; Vanderbilt University, Nashville, TN, 37212; Vanderbilt University Medical Center, Nashville, TN, 37212; VA Medical Center, Nashville, TN, 37212; Duke University, Durham, NC, 27710

Gene therapy for kidney disease remains a challenge primarily due to lack of gene delivery to the kidney. Cystinuria, the most common inherited kidney stone disorder, results from a deficiency of an amino acid transporter (rBAT) that reabsorbs cystine in proximal tubular cells. Cystinuria patients suffer from cystine stones, obstruction, and development of chronic kidney disease; current effective treatments are lacking for this lifelong disease. 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, which encodes rBAT. However, gene transfer was estimated to be 1-5% of proximal tubular epithelial cells within the injected kidney and therefore did not affect cystine stone formation. Recent innovations in viral vectors have allowed for improved renal transduction. With a novel adeno-associated virus, AAV.cc47, we have shown efficient viral delivery to the proximal tubule in mice. 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 hybrid homologous recombination and mRNA splicing within the host cell. We have validated this hybrid reconstitution strategy to express Slc3a1 in in vitro, in human kidney organoids, and in in vivo models of type A cystinuria. Further characterization of the cystinuria phenotype is necessary to assess potency and long-term efficacy of the dual-AAV gene delivery of Slc3a1 to type A cystinuria mice. Phenotypic correction of a kidney disease has remained a challenge in animal models, but our current efforts to optimize the delivery, integration, and stable expression of desired transgenes through AAV and transposon engineering provide hope for overcoming the barriers to kidney gene therapy.

Session II – P17

Dynamin-dependent endocytosis drives microvillar assembly

Olivia L. Perkins, Matthew J. Tyska

Vanderbilt University, Nashville, TN, 37203

Intestinal epithelial function and homeostasis depend on the assembly of apical microvilli. Although many of the molecules which comprise microvilli have been identified through proteomic and biochemical studies, mechanisms that promote protrusion growth remain poorly understood. The F-bar protein, Pacsin2 has been determined to be critical for microvillar growth and is also linked to endocytosis through its binding of the large GTPase, Dynamin2, which drives vesicle scission in clathrin-mediated and caveolar endocytosis. Previous studies have shown that Pacsin2-mediated endocytic pits form at the inward curving membrane between microvilli and are essential for maintaining intestinal brush border morphology. Knockout of Pacsin2 in mice leads to reduced membrane coverage and shorter microvilli, and inhibition of endocytosis reduces membrane coverage in cell culture. However, it remains unclear whether endocytosis is required for microvillar growth or is just involved in microvillar maintenance. Capitalizing on advancements in detector technology and refinement of epithelial cell culture model systems, using live cell imaging we are now able to resolve individual microvillar growth events in live cells. Preliminary data indicate that microvillar growth occurs at sites of clathrin-mediated endocytosis, as indicated by the apical localization of clathrin light chain A (LCA) and the AP-2 ß2 subunit. Both clathrin- and caveolar-mediated endocytic components localize to microvilli, however, the caveolar endocytic protein EHD2 localizes to microvilli during elongation, suggesting a distinct role for caveolar endocytosis. Inhibition of endocytosis via Dynasore, a selective dynamin2 inhibitor, leads to reduced microvillar growth and elongation of existing microvilli, indicating that dynamin-dependent endocytosis drives microvillar growth. Importantly, sites of secretion, marked by Neuropeptide Y do not predict sites of growth. Together, these preliminary results suggest that endocytosis plays a critical role in priming sites on the plasma membrane that will eventually support microvillar growth. Further investigation is required to fully understand the role of the distinct endocytic pathways in this process.

Session II – P18

Oriented Cell Division and Epithelial Organization in the Mammary Gland

Alex Pfannenstein and Ian Macara

CDB Vanderbilt University

Most human cancers arise in epithelial tissues. Mis-orientation of epithelial cell division has been postulated to play a role in the initial stages of tumorigenesis by disrupting tissue organization however the results of division misorientation independent of other transforming perturbations is unknown. Here we disrupt normal planar-aligned division orientation in mammary cells by ablating the astral microtubule binding protein Kif18b. Kif18b loss caused random division orientation in Eph4 and MDCKII cells in 2D and disrupted MDCKII cyst morphogenesis in 3D. Remarkably, cells correct daughter cell displacement after division misorientation through re-integration into the monolayer. This result was also seen in primary mammary luminal cells grown in (3D) acinar culture. Live imaging reveals transformed cell monolayers do not require misorientation of cell division to induce multilayering and may instead be extruded or directly migrate out of the monolayer. Overall, we show misoriented divisions tend to be corrected by re-integration and transformed cells may favor other mechanisms to disrupt the normal architecture of the epithelium.

Session II – P19

CLASP2 facilitates dynamic actin filament organization along the microtubule lattice

NC Rodgers, EJ Lawrence, AV Sawant, N Efimova, G Gonzalez-Vasquez, TT Hickman, I Kaverina, and M Zanic

Vanderbilt University, Nashville, TN, 37232

Coordination between the microtubule and actin networks is essential for cell motility, neuronal growth cone guidance, and wound healing. Members of the CLASP (Cytoplasmic Linker-Associated Protein) family of proteins have been implicated in the cytoskeletal crosstalk between microtubules and actin networks, however, the molecular mechanisms underlying CLASPs role in cytoskeletal coordination are unclear. Here, we investigate CLASP2α's crosslinking function with microtubules and F-actin. Our results demonstrate that CLASP2α crosslinks F-actin to the microtubule lattice in vitro. We find that the crosslinking ability is retained by L-TOG2-S, a minimal construct containing the TOG2 domain and serine-arginine rich region of CLASP2α. Furthermore, CLASP2α promotes the accumulation of multiple actin filaments along the microtubule, supporting up to 11 F-actin landing events on a single microtubule lattice region. CLASP2α also facilitates dynamic organization of polymerizing actin filaments templated by the microtubule network, with F-actin forming bridges between individual microtubules. Finally, we find that depletion of CLASPs in vascular smooth muscle cells results in disorganized actin fibers and reduced co-alignment of actin fibers with microtubules, suggesting that CLASP and microtubules contribute to higher-order actin structures. Taken together, our results indicate that CLASP2α can directly crosslink F-actin to microtubules, and that this microtubule-CLASP-actin interaction may influence overall cytoskeletal organization in cells.

Session II – P20

The fission yeast cytokinetic ring component Fic1 promotes septum formation

Anthony M. Rossi, K. Adam Bohnert, and Kathleen L. Gould

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37240;Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

In Schizosaccharomyces pombe septum formation is coordinated with cytokinetic ring constriction but the mechanisms linking these events are unclear. In this study, we explored the role of the cytokinetic ring component Fic1, first identified by its interaction with the F-BAR protein Cdc15, in septum formation. We found that the fic1 phospho-ablating mutant, fic1-2A, is a gain-of-function allele that suppresses myo2-E1, the temperature-sensitive allele of the essential type-II myosin, myo2. This suppression is achieved by the promotion of septum formation and required Fic1's interaction with the F-BAR proteins Cdc15 and Imp2. Additionally, we found that Fic1 interacts with Cyk3 and that this interaction was likewise required for Fic1's role in septum formation. Fic1, Cdc15, Imp2, and Cyk3 are the orthologs of the Saccharomyces cerevisiae ingression progression complex, which stimulates the chitin synthase Chs2 to promote primary septum formation. However, our findings indicate that Fic1 promotes septum formation and cell abscission independently of the S. pombe Chs2 ortholog. Thus, while similar complexes exist in the two yeasts that each promote septation, they appear to have different downstream effectors.

Session II – P21

InsP3R coordinates transcriptional responses to promote mitochondrial homeostasis and longevity

Elizabeth M. Ruark, Gaomin Feng, Kristopher Burkewitz

Vanderbilt University, Nashville, TN, 37235

Maintaining proper mitochondrial homeostasis is critical for health and longevity. Indeed, a variety of lifespan-extending interventions target different aspects of mitochondrial function. One such intervention is mild inhibition of the electron transport chain (ETC). One mechanism by which cells adapt to ETC inhibition is transcriptional remodeling, but our lab has recently shown that the inositol triphosphate receptor (InsP3R), an ER calcium efflux channel, is required for lifespan extension of the complex I mutant. While investigating how the InsP3R mediates longevity, we found that the InsP3R is required for full activation of the mitochondrial unfolded response target gene, hsp-6, during longevity. Thus, indicating that the InsP3R modulates transcriptional targets during longevity. We then utilized RNA-seq to identify further transcriptional targets regulated by the InsP3R during mitochondrial stress-induced longevity. We identified that many transcripts related to ETC components and even actin cytoskeletal remodeling were differentially regulated during longevity. Altogether, these data suggest that the InsP3R coordinates adaptive response pathways during mitochondrial stress. I propose that the InsP3R coordinates transcriptional responses during mitochondrial stress to promote mitochondrial homeostasis and extend lifespan. To explore mechanisms by which the InsP3R mediates transcriptional responses during chronic mitochondrial stress, I will screen candidate transcription factors regulated by the InsP3R and identify downstream calcium targets. A recent study has shown that acute mitochondrial stress triggers a transient actin response around mitochondria, highlighting actin-mitochondrial interactions as an adaptive response to stress. The actin cytoskeleton has important roles in maintaining mitochondrial structural integrity and facilitating fission events, therefore, I will explore the role of mitochondrial homeostasis in longevity via the actin cytoskeleton. To determine the role of actin remodeling on mitochondrial homeostasis during longevity, I will test the necessity of actin remodeling to promote longevity and identify the effect on mitochondrial bioenergetics and fission/fusion events. Taken together, these experiments will reveal new insights into the cellular response to mitochondrial stress as well as establish further connections for ER-mitochondrial communication during aging.

Session II – P22

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

Amanda Ruelas, Jennifer M. Bailey-Lundberg, and Kathleen E. DelGiorno

Vanderbilt University, Nashville, TN, 37235; The University of Texas Health Science Center at Houston, Houston, Tx, 77030

Background In response to injury, pancreatic acinar cells undergo ductal metaplasia (ADM) as a protective mechanism that promotes wound repair. We have found that ADM does not strictly consist of a homogeneous population of ductal cells, but instead 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 throughout the respiratory and digestive tracts. We have found that both oncogenic KRAS 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 TC formation, or Interleukin (IL-) 25 specifically in the pancreas were generated. Adult GEMMs and controls were given pancreatitis using the cholecystokinin ortholog caerulein and the pancreas was collected to conduct histological studies. CYTOF mass spectrometry was conducted to profile the immune infiltrate and a cytokine array was used to evaluate changes in pro-inflammatory signals. Results Caerulein treatment of mice lacking TCs 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 and edema. Both TCand IL25 KO mice displayed enhanced infiltration of macrophages, activated fibroblasts, and t-regulatory cells in the injured tissue. Cytokine array analysis identified an increase in several cytokines in both models, including IL-33. Conclusions TCs inhibit injury under conditions of chronic pancreatitis through IL-25 synthesis and secretion. The immune landscape changes during injury when there is a lack of TCs or IL-25. Further studies are required to determine the mechanism(s) by which immune cells respond to IL-25 and how this inhibits inflammation and injury.

Session II – P23

Blebbisomes, extracellular vesicle that can take up other extracellular vesicles

Zachary Sanchez, Dennis Jeppesen, Noah Kelley, Evan Krystofiak, Kevin Dean, Robert Coffey, Dylan Burnette

Vanderbilt University, Nashville, Tennessee, 37235

It is currently known that extracellular vesicles (EVs) can play various roles and are often defined by both their contents and overall size. Recently using overexpression, an EV known as a migrasome has been characterized and been shown through to be left behind when cells migrate. By utilizing multiple cancer cell lines and mouse fibroblasts, we have found a different type of EV that is a remnant of cell migration but much larger in size as well as energetically active. This EV dubbed a blebbisome ranges from 6-15 microns wide and can be found exhibiting the same behavior of a blebbing cell, for these EVs also develop circular protrusions that can recede and reform. We have performed a series of biochemical assays to not only purify these EVs but also survey their contents. Our data shows that blebbisomes contain many organelle specific markers and have been found containing exosomes. We have been able to demonstrate that blebbisomes are able to take up exosomes from the environment as well as contain them natively. These unique EVs are able to last in culture for multiple days and maintain their blebbing behavior. By using EM and live imaging, blebbisomes were found to have active mitochondria. These unique EVs could be a potential way that cells can communicate, and future experiments are aimed at elucidating this further.

Session II – P24

A Cell-cycle-dependent Switch in Microtubule-dependent Molecular Motors Driving Golgi Positioning in Interphase Cells

Avishkar V. Sawant, John Lee, Claire F. Scott, Irina Kaverina

Vanderbilt University, Nashville, Tennessee, 37235

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 compact configuration around the centrosomes in the G1 phase to an extended configuration around the nucleus in S and G2 phases. The data indicated that the rearrangements rely on microtubules and microtubule-based transport. In the current study, we aim to dissect the mechanisms of this cell-cycle-dependent transition. We take advantage of the fact that microtubule depolymerization leads to a random scattering of Golgi fragments in the cytoplasm. We utilize the process of Golgi reassembly during microtubule regrowth as an assay to determine the destinations of microtubule-dependent Golgi transport. We observe that the reassembly of Golgi stacks in G1 occurs around the centrosomes, whereas in S/G2, the reassembly occurs around the nuclear envelope even though the radially organized microtubules produced by the centrosomes persist. Interestingly, our data suggest that the interplay between the minus-end and plus-end directed microtubule motors is essential for this distinction between G1 and S/G2. Inhibition of dynein, a minus-end directed motor, leads to fragmentation and dispersal of Golgi stacks in both G1 and S/G2 phases. In contrast, inhibition of kinesin-1, a plus-end directed motor, does not affect Golgi positioning in the G1 phase. However, in S/G2, kinesin-1 inhibition prevents its redistribution from the centrosomes to the nuclear equator. Subsequent washout of the kinesin inhibitor reverses Golgi configuration to the extended perinuclear configuration typical for G2. The importance of kinesin-1 for Golgi configuration in S/G2 was confirmed by the depletion of the kinesin light chain, which phenocopies the inhibition of kinesin-1. Based on these findings, we propose a model where dynein facilitates the connection of Golgi stacks into a continuous ribbon throughout the interphase. In contrast, kinesin-1 exerts a cell-cycle stage-specific action on the Golgi complex. In G1, kinesin-1 does not significantly contribute to Golgi complex positioning, allowing dynein to condense Golgi around the centrosome. Upon transition to S/G2, kinesin-1 overpowers dynein to move the Golgi away from the centrosomes and stretches the Golgi ribbon along the nuclear envelope, possibly utilizing specifically modified perinuclear microtubule tracks. Our data suggest a potential cell-cycle-dependent pathway tuning kinesin-1 capacity to transport Golgi stacks.

Session II – P25

C. elegans Meiotic and Mitotic Function is Regulated by a Mitochondrial Localized Protein with Puzzling Essential Function

Samantha H. Schaffner, Maulik R. Patel

Vanderbilt University, Nashville, TN, 37235

Mitochondria play many essential cell biology roles, such as in apoptosis and immune regulation, that go beyond their canonical function of energy production. SPD-3 is a nuclear-encoded mitochondrial-localized protein in the model organism Caenorhabditis elegans that could be playing a non-canonical role in mitochondrial function. This protein is essential for the fertility of the animal as spd-3 mutants have a sterile phenotype due to disruption of meiotic and mitotic spindle regulation which results in abnormal polar body extrusion and chromosome segregation. Despite its function in the nucleus, previous research has shown that ectopically introduced SPD-3::GFP localizes to the mitochondria. While it might be assumed that SPD-3 has some function in mitochondrial ATP production, such specialized effects on fertility are not typically seen in nuclear-encoded elements of cellular respiration function. Therefore, we hypothesize that spd-3 is a mitochondrial-localized regulator of meiosis and mitosis that must have a specific and novel mitochondrial function. Using a split-fluorescent protein labeling system, we have tagged the endogenous locus of spd-3 to learn more about its sub-organellar localization and how it can signal from the mitochondria to the nucleus. Upcoming results of a forward genetic screen will also provide more information about what signaling pathways spd-3 is involved with. Additionally, despite the essential function of spd-3 on the fertility of the organism, spd-3 is seemingly lost in some closely related Caenorhabditis species. Due to this unique protein evolution, we investigated spd-3 as a possible element of a toxin-antidote (TA) system. TA systems are comprised of a "toxin" element which is fatal without its tightly linked "antidote" element. While TA systems are well studied in bacteria, their function in animals are just beginning to be studied. We hypothesized that instead of spd-3 having its own essential function, it could instead function as an "antidote" element that is essential to combat the lethal effects of a linked "toxin" element. Through RNAi and CRISPR knockouts of these putative "toxin" and "antidote" elements, we investigated if their function was dependent on each other. However, initial results show that these genes do not seem to function as a TA system despite the puzzling evolutionary history of this essential meiotic gene.

Session II – P26

Targeting a Newly Identified Binding Site in KRAS(G12D)

Anna Schwarzkopf, Laura Friggeri, Sabina Leonard, Mary Rockouski, Kathleen DelGiorno, Ethan Lee

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

Activating mutations in the Ras family of small GTPases are major drivers of ~30% of all human cancers. *KRAS* is the most frequently mutated isoform of the Ras family and is prevalent in lung, colon, and pancreatic cancers. Over 99% of mutations in *KRAS* occur at glycine12 (G12), glycine13 (G13), and glutamine61 (Q61), each of which promote oncogenesis by locking KRAS in its active, GTP-bound state. Ras was widely believed to be undruggable until a recent breakthrough identified the Ras Switch II pocket, which provides a binding site for small molecules to stabilize KRAS in its inactive, GDP-bound state. This discovery has led to some success in developing inhibitors specific to a single glycine-to-cysteine mutation, KRAS(G12C), through the formation of a covalent adduct, but this strategy is not effective for targeting high-affinity molecules against non-cysteine G12, G13, or Q61 KRAS mutants. Thus, there remains an urgent need for alternative strategies to effectively and specifically target other common mutants of KRAS. Using the crystal structure of the G12D-specific inhibitory peptide KRpep-2d bound to KRAS(G12D) as a guide, our lab identified a new pocket of KRAS, Distal Deep Ras Pocket (DDRP). The DDRP is proximal to the G12 and Q61 sites and possesses an extensive binding surface, making it an ideal candidate for high-affinity binding of small molecule inhibitors. We used the Molecular Operating Environment (MOE) software to conduct a multi-step virtual screening approach, and after filtering for molecular weight, commercial availability, known liabilities, and drug-like properties, we identified 50 candidate molecules predicted to bind specifically to the DDRP of KRAS(G12D). Cell-based screens of these 50 candidates revealed three compounds that show inhibitory activity against KRAS signaling. Follow-up experiments with each of these three compounds showed a dose-dependent decrease in ERK phosphorylation, a downstream target of active KRAS, in KRAS(G12D) pancreatic and colorectal cancer cells. In contrast, these compounds did not possess inhibitory activity in KRAS wild-type pancreatic cancer cells. These results suggest that small-molecule targeting of the DDRP represents a novel method for inhibiting major KRAS-activating mutants, which could provide insight into the development of novel therapeutic strategies for treating a large percentage of KRAS-driven cancers.

Session II – P27

Mapping lipid landscapes present in Alzheimer's-associated neuropathologies

Claire F Scott 1,2; Cody Marshall 1,2; Allison B Esselman 1,2; Martin Dufresne 1,2; Matthew S Schrag 1,3; Jeffrey M Spraggins 1,2

1 Vanderbilt University, Nashville, TN, 37235; 2 Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN 37235; 3 Cerebral Amyloid Angiopathy Clinic, Vanderbilt University Medical Center, Nashville, TN 37235

Uncovering the molecular underpinnings of Alzheimer's disease (AD)-associated brain tissue features will allow us to better understand disease progression and determine effective treatment strategies. Molecular imaging of AD tissues via targeted immunofluorescence (IF) microscopy can reveal the cellular neighborhoods present across tissue sections, including AD-associated features. MALDI IMS can be used for untargeted molecular mapping of human brain tissue sections. We establish a multimodal imaging workflow that combines IF and MALDI IMS to comprehensively study cellular and molecular landscapes of human brain tissue. Whole brain lipid levels are altered in those diagnosed with AD and here we will examine how lipids are impacted within AD-associated features in situ. AD and control human brain tissues were sectioned at 10μm thickness onto indium tin oxide (ITO) glass slides. Sections imaged using IF were photobleached prior to the application of antibodies and subsequent imaging. Prior to MALDI IMS, serial tissue sections were washed with 150 mM ammonium formate and sublimed with 5 mg of an aminated cinnamic acid analog using an in-house developed sublimation device. MALDI IMS data were acquired at 5 and 10μm spatial resolution in positive and negative ionization mode on a Bruker timsTOF FleX (Bruker Daltonics). Data analysis was performed using SCiLS, ZEN microscopy software, and in-house software. IF microscopy images give cellular context to molecular data acquired with MALDI IMS by marking AD-associated features. Initial studies were performed to optimize methods for high spatial resolution MALDI IMS. AD frontal lobe brain tissue samples from a 68-year-old female donor were used. Experiments were conducted at 5 μm and 10 μm spatial resolution with a scan range of m/z 400-2500. Various lipid species were found to localize to specifically the white matter or grey matter (e.g., m/z 885.552, m/z 734.562), and molecular heterogeneity within these regions was also detected (e.g., m/z 610.224). In future studies, we expect to correlate pathological cellular features with altered lipid landscapes and discover information about the molecular drivers of AD. In conclusion, combining MALDI IMS with IF microscopy allows for detailed mapping of the cellular and lipid landscape of Alzheimer's disease pathologies.

Session II – P28

Alterations in Dnmt1 expression during metaplasia progression

Halee Scott, Brianna Caldwell, Eunyoung Choi

Vanderbilt University, Nashville, TN, 37235; Vanderbilt University Medical Center, Nashville, TN, 37235

Gastric cancer is one of the most common cancers and the fourth leading cause of cancer-related death worldwide. Progression of the disease can be attributed primarily to the rise of metaplastic cell lineages that develop upon parietal cell loss or injury of the gastric mucosa. During the metaplastic process, chief cells located in the base of the stomach gland undergo trans differentiation into spasmolytic polypeptide-expressing metaplastic (SPEM) cells and contribute to the progression of metaplasia to neoplastic stages such as dysplasia. Our lab has recently reported that considerable gene expression changes occur in the dysplastic cell population, however, no significant mutations are acquired to describe this phenomenon, suggesting that the transcriptional changes during metaplasia progression to dysplasia might be controlled by epigenetic regulation. In this study, we examine the patterns of DNA methyltransferase 1 (Dnmt1) expression in mouse stomachs with metaplasia or dysplasia induced by Kras activation. We used a novel mouse model (Gapple-Kras) by crossing a novel chief cell specific Cre-driver expressed with the red fluorescent protein mApple in GIF transcript-expressing cells (Gapple). The Gapple mice were crossed with the LSL-Kras mouse allele to constitutively activate Kras expression in chief cells after Tamoxifen treatment. H&E and immunofluorescent staining of the stomach tissues were performed at multiple time points to determine the stages of gastric carcinogenesis. Changes in mApple and Dnmt1 expression were then examined during the metaplasia progression to dysplasia in the Gapple-Kras mouse tissues. The Gapple-Kras mouse allele displayed metaplasia development within one month after tamoxifen treatment and the metaplasia progressed to low-grade dysplasia by four months. The mApple expression indicating Gif transcription activity was already lost within one month while AQP5 and CD44v9 co-positive SPEM cells appeared at the base of the gland, confirming the transdifferentiation of chief cells after Kras activation. Immunofluorescence staining for Dnmt1 in the Gapple-Kras stomach tissue sections showed that both the location and expression of Dnmt1 changed during metaplasia progression to dysplasia. The Dnmt1 was observed at the very base of the stomach gland, co-positive with SPEM cell markers CD44v9 and GSII in metaplastic glands. However, the Dnmt1 expression was no longer present at the very base of the gland and spreads closer to the neck of the gland, where proliferating cells and dysplastic cells are located. Therefore, these results suggest that DNA methylation by Dnmt1 may play a role in dynamic transcription regulation during metaplasia progression.

Session II – P29

WIN Site Inhibition Disrupts a Subset of WDR5 Function

Andrew J. Siladi, Jing Wang, Andrea C. Florian, Lance R. Thomas, Joy H. Creighton, Brittany K. Matlock, David K. Flaherty, Shelly L. Lorey, Gregory C. Howard, Stephen W. Fesik, April M. Weissmiller, Qi Liu & William P. Tansey

Vanderbilt University Nashville TN

WDR5 is a highly-conserved nuclear protein which facilitates the assembly of histone-modifying complexes involved in a variety of chromatin-based, gene regulatory processes. WDR5 is best known for its role in scaffolding the assembly of MLL/SET histone methyltransferase complexes that catalyze histone H3 lysine 4 (H3K4) di- and tri-methylation (Me2/Me3), but WDR5 acts outside this setting to promote ribosomal protein gene transcription and recruit the oncogenic transcription factor MYC to chromatin. WDR5 is also a target for pharmacological inhibition in cancer, and most drug discovery efforts aim to block the WIN site of WDR5, an arginine binding cavity that engages MLL/SET enzymes. Therapeutic application of WIN site inhibitors is complicated by the disparate functions of WDR5, but is generally guided by two assumptions-that WIN site inhibitors disable all functions of WDR5, and that changes in H3K4me drive the transcriptional response of cancer cells to WIN site blockade. Here, we test these assumptions by comparing the impact of WIN site inhibition versus WDR5 degradation on H3K4me and transcriptional processes in Burkitt's lymphoma cells. We show that WDR5 regulates transcription widely, yet WIN site inhibition disables only a specific subset of WDR5 activity, and that H3K4me changes induced by WDR5 depletion do not explain accompanying transcriptional responses. These data recast WIN site inhibitors as selective loss-of-function agents, contradict H3K4me as a relevant mechanism of action for WDR5 inhibitors, and point to distinct clinical applications of WIN site inhibitors and WDR5 degraders. Further investigation of WDR5 degradation in CHP134 neuroblastoma cells validates our findings and forecasts WDR5 degraders as destructive agents in human cells, their use having the potential to unintentionally destabilize other essential proteins like MLL/SET.

Session II – P30

Recruitment of Foxl1+ Telocytes During Metaplasia Development in the Stomach

Yoojin Sohn1,2, Blake Flores Semyonov1, Christopher V.E. Wright2, Klaus H. Kaestner5, Eunyoung Choi1,2,3,4, James R. Goldenring1,2,3,4

1Epithelial Biology Center, 2Department of Cell and Developmental Biology, 3Department of Surgery, 4Nashville VA Medical Center, Vanderbilt University School of Medicine, Nashville, TN; 5Department of Genetics and Center for Molecular Studies in Digestive and Liver Diseases, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

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. However, with metaplasia 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. To study the origin of basally emergent telocytes, we utilized Foxl1-CreERT2;R26R-tdTom mice to label Foxl1+ telocytes and trace them through SPEM development. With whole-mount staining and comparing untreated vs L635-treated mouse stomach, we found that labeled telocytes show extended telopodes and migration toward the base of the gland during the development of metaplasia, suggesting the telocytes are recruited to the site of proliferation. 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.

Session II – P31

Piezo initiates transient production of collagen IV to repair damaged basement membranes

Aubrie Stricker, Shane Hutson, Andrea Page-McCaw

Vanderbilt University, Nashville, TN, 37212

Basement membranes are sheets of extracellular matrix that serve to separate tissue layers and provide mechanical support. Their mechanics are determined largely by collagen IV, the most abundant protein in basement membranes. Basement membranes are subject to mechanical damage and require repair, but repair must be highly regulated because collagen is a long-lived molecule, and too much causes fibrosis. Little is known about how repair occurs, and it is unknown how basement membrane damage is detected. Previously we found there are homeostatic mechanics of basement membrane replacement, raising the question of whether basement membrane damage is actively detected or passively repaired. Here we show that repair is an active process, requiring the mechanosensitive channel Piezo. To repair the basement membrane around the Drosophila midgut, short-lived cells in the gut epithelium transcribe matrix components specifically after damage. Lineage tracing indicates these "matrix maker" cells are a subset of enteroblasts that die shortly after repair is complete. Piezo is activated by basement membrane damage and is required to upregulate collagen IV synthesis in the matrix makers. Further, most of the collagen IV in the gut originates from cells expressing Piezo, suggesting that mechanical sensitivity is a general property of collagen-producing cells.

Session II – P32

Cryo-electron tomography reveals ferrosome organelles in Clostridioides difficile

Rong Sun 1234, Hualiang Pi 56, James R. McBride 7 , Eric P. Skaar 56, Qiangjun Zhou 1234

1.Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37232, 2 Vanderbilt Brain Institute, Vanderbilt University, School of Medicine, Nashville, TN, 37232, 3 Center for Structural Biology, Vanderbilt University, School of Medicine, Nashville, TN, 37232, 4 Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN, 37232, 5 Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University, Nashville, TN, 37232, 6 Department of Pathology, Microbiology, & Immunology, Vanderbilt University Medical Center, Nashville, TN, 37232, 7 Vanderbilt Institute of Nanoscale Science and Engineering, Vanderbilt University, Nashville, TN, 37235

Iron is indispensable for almost all forms of life but toxic at elevated levels. To survive within their hosts, bacterial pathogens have evolved iron uptake, storage, and detoxification strategies to maintain iron homeostasis. Emerging studies have described ferrosome organelles as a potential iron storage mechanism in Gram-negative environmental anaerobes, however, little is known about the structure and formation of ferrosomes. Here, we use cryo-electron tomography (cryo-ET) to investigate the ferrosomes in Gram-positive human pathogen Clostridioides difficile. We found C. difficile ferrosomes are bound by lipid membranes and might originate from the cellular membrane. Our results also reveal the existence of proteins on the ferrosome membrane. Together with other evidence shown in our experiments, these proteins are required for ferrosome formation and sufficient to form vesicular structures in Escherichia coli.

Session II – P33

The histone chaperone NASP maintains H3- H4 reservoirs in the early Drosophila embryo

Reyhaneh Tirgar, Jonathan P. Davies, Lars Plate, Jared T. Nordman

Vanderbilt University, Nashville, Tennessee, United States of America,

Histones are essential for chromatin packaging, and histone supply must be tightly regu- lated as excess histones are toxic. To drive the rapid cell cycles of the early embryo, how- ever, excess histones are maternally deposited. Therefore, soluble histones must be buffered by histone chaperones, but the chaperone necessary to stabilize soluble H3-H4 pools in the Drosophila embryo has yet to be identified. Here, we show that CG8223, the Drosophila homolog of NASP, is a H3-H4-specific chaperone in the early embryo. We dem- onstrate that, while a NASP null mutant is viable in Drosophila, NASP is maternal effect gene. Embryos laid by NASP mutant mothers have a reduced rate of hatching and show defects in early embryogenesis. Critically, soluble H3-H4 pools are degraded in embryos laid by NASP mutant mothers. Our work identifies NASP as the critical H3-H4 histone chap- erone in the Drosophila embryo.

Session II – P34

Uncovering Molecular Heterogeneity in S. aureus Abscesses with Multimodal Molecular Imaging

Jacqueline M Van Ardenne1,2, Lukasz G Migas3, Martin Dufresne2,4, Madeline E Colley2,4, Jeffrey A Freiberg5,6, Valeria M Reyes Ruiz6,7, Andy Weiss6,7, Katherine N Gibson-Corley7, Raf Van de Plas2,3,4, Eric P Skaar6,7,8, Jeffrey M Spraggins1,2,4,9

1Department of Chemistry, Vanderbilt University, Nashville, TN ; 2Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN ; 3Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands ; 4Department of Biochemistry, Vanderbilt University, Nashville, TN ; 5Division of Infectious Diseases, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN ; 6Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University Medical Center, Nashville, TN ; 7Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN ; 8Vanderbilt Institute for Chemical Biology, Vanderbilt University, Nashville, TN ; 9Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN

Staphylococcus aureus (S. aureus) is the leading cause of death associated with bacterial infections and is most often associated with formation of soft tissue abscesses. These abscesses have a complex organization forming the interface between bacteria and the host immune response. Bacteria group together at the center of the abscess in staphylococcal abscess communities (SACs). SACs are surrounded by both viable and necrotic neutrophils. As the infection progresses, the abscess is encapsulated by fibrin deposits to limit further tissue damage. Characterization of the host-pathogen interactions in S. aureus abscesses is critical to the development of preventative measures and therapeutics, but conventional microscopic assays provide limited information about the molecular composition of the abscess. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) is a sensitive, label-free, highly multiplexed technology for mapping biomolecules in situ. Advancements in spatial resolution for MALDI IMS have enabled the correlation of these molecular signatures to specific tissue structures and cell types allowing for more in-depth analysis of abscess pathology. Histological staining provides complementary information about cellular and morphological organization in and around the abscess, such as identification of viable and necrotic neutrophils and fibrosis. By combining stained microscopy with MALDI IMS, these data reveal trends in molecular distributions as they relate to changes in host cellular neighborhoods due to infection. Using this approach, we have identified a variety of phospholipid classes including both odd and even chain saturated cardiolipin and lysyl-phosphatidylglycerol species with distinct localizations in the SACs, as well as phosphatidylinositol and phosphatidylserine species which localize to regions containing neutrophils. These lipids play important roles in both host immune signaling pathways as well as in S. aureus defenses. Understanding the heterogeneity of lipid profiles between abscesses with different host cellular neighborhoods is essential to building a more detailed molecular model of S. aureus abscess progression.

Session II – P35

Epithelial, stromal, and microbial remodeling in a TNF-drive mouse model of Crohn's-like intestinal inflammation

Paige N. Vega, Alan J. Simmons, Jiawei Wang, Yanwen Xu, Yilin Yang, James Ro, Amrita Banerjee, Mariah H. Harned, Qi Liu, & Ken S. Lau

Vanderbilt University Nashville, TN 37232

Crohn's disease is characterized by chronic inflammation of the gastrointestinal tract and can be modeled by TNF overexpression in the TnfΔARE/+ mouse model. We use droplet-based single-cell RNA-sequencing to profile epithelial and stromal cells in wildtype mice and TnfΔARE/+ mice with mild to severe ileal and colonic inflammation. We found an emergence of innate immune cell types and transcriptomic changes in lymphocytes in both stages of inflammation in the ilea and colon of TnfΔARE/+ mice. Surprisingly, we identified a loss of normal fibroblast populations and an emergence of pro-inflammatory fibroblasts in TnfΔARE/+ mice at both stages of inflammation. Absorptive enterocytes, but not colonocytes, in TnfΔARE/+ with severe inflammation displayed major transcriptomic changes when compared to normal or TnfΔARE/+ mice with mild inflammation. Enterocyte transcriptomic changes reflected a switch from normal absorptive function toward an upregulation of inflammatory response genes. Shotgun DNA sequencing on intestinal lavages from the ileum and colon revealed increased abundance of the obligate intracellular bacterium Chlamydia muridarum in TnfΔARE/+ mice compared to wildtype littermates and cagemates. While ileitis is spontaneous, colonic inflammation was dependent on the housing facility, where "clean" facility mice were free of colonic inflammation. We transferred and co-housed mice from the "clean" facility with those in the "dirty" facility and found TnfΔARE/+ mice developed colitis, suggesting that TNF-induced colitis is microbiome-dependent. We provide a full characterization of cell types in inflamed ilea and colon from the TnfΔARE/+ model of Crohn's-like disease, and moreover, identify cell types and microbes that are involved in chronic inflammation. Future studies aim to reveal the epithelial cell type that harbors Chlamydia muridarum and the role of Paneth cells and their antimicrobial products in persistent infection. Revealing mechanisms by which host cell-cell and host-microbe interactions drive intestinal inflammation is critical for understanding Crohn's disease and will lead to novel therapeutic targets.

Session II – P36

Understanding glypican-based mechanisms of extracellular Wnt distribution

Indrayani Waghmare and Andrea Page-McCaw

Vanderbilt University School of Medicine, Nashville, TN 37240

Wnts are evolutionarily conserved secreted ligands that form extracellular gradients to direct several cellular behaviors at short- and long-ranges. The Drosophila germarium, a tissue where oogenesis initiates, is an excellent model to study mechanisms of Wnt distribution as this tissue expresses several Wnts, which are required for proper egg production. My postdoctoral research shows that the distribution of extracellular Wnts is important for Wnt function and is primarily dependent on cell-surface localization of glypicans, which distribute Wnts by continual binding and release in the plane of the tissue. Further, because glypicans play an important role in modulating extracellular Wnt availability, their cell-surface levels are likely regulated by other factors. In the fly germarium, the glypican Dally-like protein (Dlp) promotes long-range distribution of extracellular Wg, the Drosophila ortholog of mammalian Wnt1, from Wg-producing cap cells to Wg-responsive follicle stem cells inducing their proliferation, required for egg development. Both knockdown of dlp or tethering Wg to cap cell membrane disrupts follicle stem cell proliferation. In genetic experiments, Matrix Metalloproteinase 2 (Mmp2) inhibits Dlp's long-range Wg distribution to restrict Wg signaling in follicle stem cells. Thus, Mmp2 acts as a molecular brake on Dlp's long-range function. Mechanistically, I discovered that in S2R+ insect cells, Mmp2 cleaves Dlp on the cell surface to induce a conformational change. Further cleavage of Dlp causes it to be destabilized and internalized from the cell surface, and cleaved Dlp sequesters more Wg than intact Dlp, suggesting that cleavage by Mmp2 alters Wg-Dlp binding affinities to inhibit Wg distribution. Taken together, my research identifies that Dlp modulates the extracellular availability of several Wnts and identifies the molecular basis of protease-mediated inhibition of cell-surface Dlp, which modulates Wnt ligand distribution and function.

Session II – P37

Matrix Stiffness Regulates Tumor Cell Intravasation through Expression and ESRP1-mediated Alternative Splicing of MENA

Wenjun Wang1, Paul V. Taufalele1, Martial Millet2, Kevin Homsy2, Kyra Smart1, Emily D. Berestesky1, Curtis T. Schunk1, Matthew M. Rowe1, Francois Bordeleau2,3 *, Cynthia A. Reinhart-King1 *

1 Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA; 2 CHU de Québec-Université Laval Research Center (Oncology division), and Université Laval Cancer Research Center, Québec, Canada; 3 Département de biologie moléculaire, de biochimie médicale et de pathologie, Université Laval, Québec, Canada; *Corresponding author

Intravasation is a critical step of metastasis where tumor cells cross the endothelial barrier and migrate into the bloodstream. During this process, tumor cells encounter a complex microenvironment which provides both chemical and physical cues to cells. Pathological changes in the microenvironment, including extracellular matrix (ECM) stiffening, have been correlated with increased metastatic potential. However, the mechanism by which matrix stiffening regulates each step of tumor metastasis, especially intravasation, is still relatively unclear. Noting that both increased expression and alternative splicing of MENA regulate intravasation, we hypothesize that matrix stiffening promotes tumor cell intravasation through enhancing expression and ESRP1-mediated alternative splicing of MENA. We first investigated the impact of matrix stiffness on MENA by analyzing MENA and MENA11a expression of MDA-MB-231 cells on compliant or stiff substrates via RT-qPCR. We found that ECM stiffness promoted MENA expression and its alternative splicing, resulting in a decreased expression of MENA11a which could be prevented by FAK inhibition. Matrix stiffening promoted intravasation as downmodulation of MENA11a further increased this effect; knockdown of MENA decreased intravasation in both soft and stiff conditions. To further understand the mechanism by which matrix stiffness regulates splicing of MENA, we tested expression of ESRP1, the MENA alternative splicing regulator. We showed that heightened matrix stiffness decreased ESRP1 expression in vitro and in vivo. ECM stiffness and ESRP1 expression were inversely correlated within patient specimens. In turn, overexpression of ESRP1 upregulated MENA11a expression. Altogether, our data indicate that matrix stiffness regulates intravasation through enhanced expression and ESRP1-mediated alternative splicing of MENA. Our study provides a mechanism by which ECM stiffness regulates tumor cell intravasation, and also supports the connection between matrix stiffness and epithelial-mesenchymal transition (EMT) by showing that matrix stiffness regulates the expression of ESRP1, an RNA-binding protein that participates in the regulation of EMT.

Session II – P38

Wound-Induced Syncytia Outpace Mononucleate Neighbors during Drosophila Wound Repair

James White, Jasmine Su, Elizabeth Ruark, M. Shane Hutson, Andrea Page-McCaw

Vanderbilt University, Nashville, TN, 37212

Injury is a constant reality of life making wound repair critical for survival in all organisms. Once an injury is detected tissues must rapidly induce wound repair behaviors such as proliferation, migration, and invasion. When these behaviors become dysregulated, they lead to cancers. So, understanding how these behaviors are induced and regulated in the context of wounding could lead to better understanding of how they become dysregulated. Drosophila have been used as a foundational model system to study wounding as they have a simple monolayer epithelium that is easily accessible. Previous studies by the Losick lab identified the formation of wound induced multinucleated syncytial cells that act as a plug for the post mitotic adult epithelium after wounding. We have observed using the mitotically capable pupal notum that these multinucleate cells form first by a rapid breakdown of individual cell borders 10-40 minutes after wounding. We confirmed cell-cell fusion using individual cells labeled with GFP where we can observe the diffusion of GFP rapidly from source cells into unlabeled cells, followed by the corresponding border breakdowns. We also observe a later fusion event which we have dubbed "cell shrinking" where the apical border of a cell will shrink away near a syncytium. Clonal GFP analysis also revealed source cells shrinking and contributing their GFP, including nuclei, to a neighboring syncytium. Syncytia outpace mononucleate cells to effectively close the wound. Cell borders break down primarily on borders tangential to the leading edge, suggesting syncytia may allow distal resources that would otherwise be trapped to move towards the leading edge. To test this hypothesis we generated Actin-GFP clones within the pupal notum. Upon wounding we saw a rapid relocalization of Actin from distal clones through syncytia and into migratory structures at the leading edge. It is likely the pooling of actin from many cells at the syncytial leading edge allows syncytia to outpace their unfused neighbors and completely take over the leading edge during wound repair.

Session II – P39

Long-tailed myosin-1 membrane binding is inhibited by a class of ankyrin repeat proteins

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

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

Myosin-1s are monomeric actin-based motors that promote cellular processes at membranes. Myo1 is the single myosin-1 isoform in fission yeast that functions redundantly with Wsp1-Vrp1 to activate the Arp2/3 complex for endocytosis. Here, we identified Ank1 as an uncharacterized binding partner and found that Myo1 dramatically re-localizes from endocytic patches to along the entire plasma membrane in ank1Δ cells. Co-immunoprecipitation experiments and structural predictions suggested that the Ank1 ankyrin repeats associate with the Myo1 lever arm and the Ank1 acidic tail binds the Myo1 TH1 domain. These results along with our finding that Ank1 is cytoplasmic suggest that Ank1 sequesters Myo1 in the cytoplasm by precluding Myo1 TH1 domain-dependent membrane binding. In accordance, Ank1 over-expression caused a redistribution of Myo1 into the cytoplasm. In budding yeast and humans, we propose that another uncharacterized ORF and OSTF1, respectively, are functional Ank1 orthologs and that cytoplasmic sequestration by small ankyrin repeat proteins is a conserved mechanism of regulating myosin-1s in endocytosis.

Session II – P40

Metabolic rewiring activates fatty acid metabolism to fuel dysplastic cells in gastric carcinogenesis

Yoonkyung Won, Bogun Jang, Su-Hyung Lee, Michelle L. Reyzer, Kimberly S. Presentation, Hyesung Kim, Brianna Caldwell, Changqing Zhang, Hye Seung Lee, Cheol Lee, Vincent Q. Trinh, Marcus C.B. Tan, Kwangho Kim, Richard M. Caprioli, Eunyoung Choi

Department of Surgery, Vanderbilt University Medical Center, Nashville, TN, 37232, USA; Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN, 37232, USA; Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37232 USA.

Gastric cancer develops within a carcinogenic process of a sequential cascade from pre-cancerous metaplasia to dysplasia and adenocarcinoma. The dysplastic stage is considered the key transition state between pre-cancerous and cancerous stages. Cancer cells rewire numerous metabolic pathways to meet their high energy demand for augmented growth and proliferation. However, it is not yet understood how metabolic pathways are involved in the progression of metaplasia to dysplasia. We have examined roles and patterns of metabolic reprogramming during gastric carcinogenesis using a novel mouse model which induces Kras activation in gastric chief cells. We utilized a Gif-rtTA;TetO-Cre;KrasG12D (GCK) mouse model that continuously induces active Kras expression only in chief cells following doxycycline treatment. The GCK mice were sacrificed at 2-14 weeks after the Kras induction and immunostaining was performed to examine mucosal changes in the stomachs. Imaging mass spectrometry was performed for spatial profiling of bioactive metabolites highly accumulated in the GCK mouse stomachs. Human and mouse gastric organoids were used for metabolic enzyme inhibitor treatment. The GCK mice were treated with A939572 to block the fatty acid desaturation pathway in dysplastic cells. We examined the GCK mouse stomachs developed pyloric metaplasia and progressed to high-grade dysplasia within 4 months after the active Kras expression in chief cells. Glycolytic metabolism was first activated in the development of metaplasia and sequentially shifted toward fatty acid metabolism in the progression of metaplasia to dysplasia. In particular, the stomach with high-grade dysplasia showed highly accumulated patterns of monounsaturated fatty acid (MUFA). Stearoyl-CoA desaturase (SCD), a rate-limiting enzyme for the biosynthesis of MUFAs, was specifically expressed in the transitioning area where the cell lineage converts from metaplastic to dysplastic cells. Inhibition of fatty acid desaturation pathway using SCD inhibitor killed 100% of mouse dysplastic organoids within 3 days after treatment, while the dysplastic organoids did not display any significant changes after inhibition of glycolysis. Human gastric organoids exhibited a differential drug sensitivity to the SCD inhibitor depending on the level of SCD expression. Moreover, mouse dysplastic organoids mostly restored their viability by supplementation with the MUFA in the presence of the SCD inhibitor. Finally, the SCD inhibitor selectively killed dysplastic cells in the GCK mouse stomachs. Collectively, our study indicates that active Kras expression only in gastric chief cells drives the full spectrum of gastric carcinogenesis and the metabolic reprogramming from glycolysis to fatty acid metabolism is critical for metaplasia progression during gastric carcinogenesis.

Session II – P41

Exploring the WDR5-mTORC2 Interaction

Vincent Yao, Soumita Goswami, Gregory Caleb Howard, Alissa Guarnaccia, William Tansey

Vanderbilt University, Nashville, TN

WDR5 is a scaffold for multiprotein complexes. It directly interacts with over a dozen proteins and participates and various unrelated functions. Our laboratory has previously discovered that components of the mTORC2 complex also interact with WDR5 and are displaced when a WDR5 inhibitor is used. Sin1 and Rictor both contain several putative WDR5-interacting motifs, indicating that they may directly bind to WDR5. Preliminary evidence indicates that arginine 1459 in Rictor interacts with WDR5. It is currently unknown how this interaction modulates WDR5 or mTORC2 function.

Session II – P42

ROLES OF ALTERATION IN APC AND SMAD4 GENE EXPRESSION DURING GASTRIC CARCINOGENESIS INDUCED BY KRAS ACTIVATION

Youngwon Cho1,2, Brianna Caldwell1,2, Eunyoung Choi1,2,3

1Department of Surgery, 2Epithelial Biology Center, 3Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA

Gastric cancer arises from progression of pre-cancerous metaplastic and dysplastic lesions and numerous pathway alterations through genetic alterations are commonly observed in patients with gastric cancer. Previous studies have shown that oncogenic Kras expression in gastric chief cells can lead to the development of metaplasia and its progression to intestinal metaplasia and dysplasia. However, it is unclear whether alterations in other oncogenic genes such as Apc and Smad4 has any unique effects or functions on the metaplasia progression.

To evaluate combinatorial roles of oncogenic genes, we utilized a novel tamoxifen-inducible driver mouse allele which expresses CreERT2 only in gastric chief cells, the GIF-CreERT2-mApple (GApple). The GApple mice were crossed with LSL-$Kras^{G12D}$ together with either $Apc^{fl/+}$ or $Smad4^{fl/+}$ allele. At 6 weeks of age, mice were injected intraperitoneally with tamoxifen and the gastric corpus region was investigated 8 weeks after tamoxifen injection. H&E staining and immunostaining were used for histological examination of stomach tissues.

As previously reported, the Kras induced stomachs in the GApple-Kras mice developed severe metaplasia within 2 months after tamoxifen injection. We observed that the GApple-Kras-$Apc^{fl/+}$ mice showed slightly increased tubulopapillary structures in corpus compared to the GApple-Kras mice. In comparison, the GApple-Kras-$Smad4^{fl/+}$displayed more basophilic mucin production compared to both GApple-Kras and GApple-Kras-$Apc^{fl/+}$. A number of Trop2-positive glands were increased approximately 2-fold in GApple-Kras-$Apc^{fl/+}$ and 3-fold GApple-Kras-$Smad4^{fl/+}$mice, compared to GApple-Kras indicating the metaplasia progression to dysplasia. In particular, the expression of Cldn7 was expanded in the GApple-Kras-$Smad4^{fl/+}$ mice, compared to GApple-Kras and GApple-Kras-$Apc^{fl/+}$ mice, and many Cldn7-positive cells were co-positive for Ki67, indicating that the GApple-Kras-$Smad4^{fl/+}$ mice progressed to dysplasia within 2 months.

Therefore, these results suggest that alterations in both Apc and Smad4 genes may accelerate the progression of metaplasia induced by Kras activation.

The End

“If we knew what it was we were doing, it would not be called research, would it?” – Albert Einstein