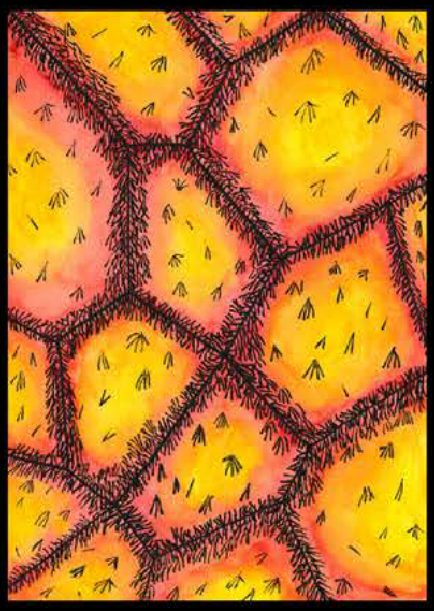
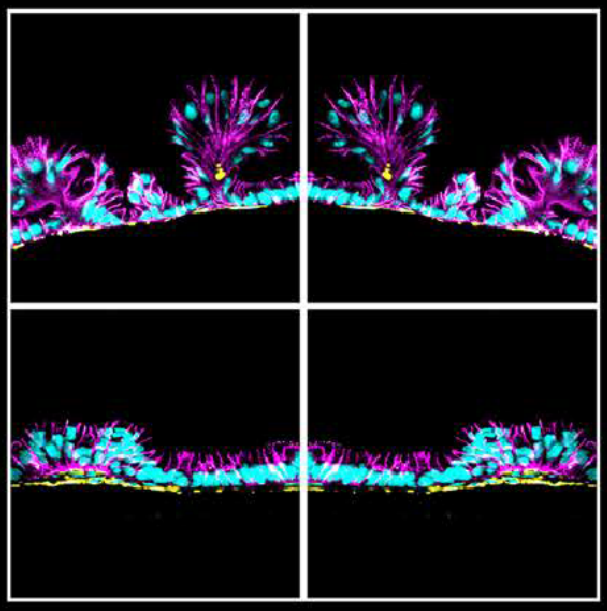
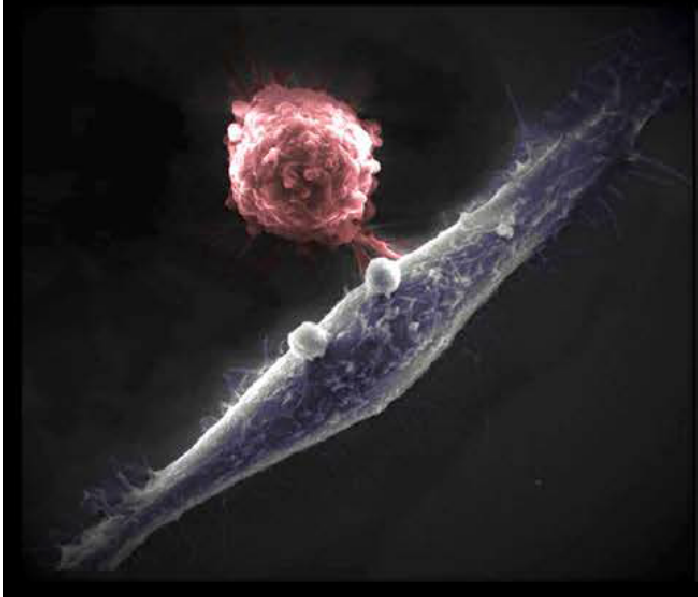
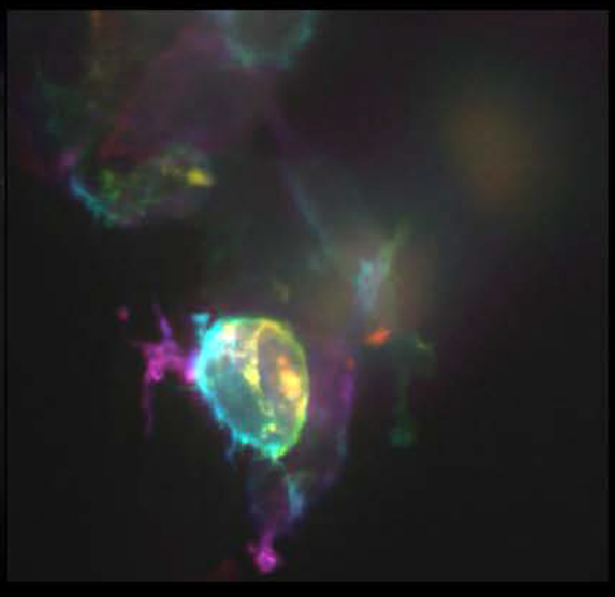
Department of Cell & Developmental Biology

21st Annual Retreat
April 19, 2024









“No matter how counter-intuitive it may seem, basic research has proven over and over to be the lifeline of practical advances in medicine.”

Arthur Kornberg

Discoverer of DNA polymerase and winner of the Nobel Prize in Physiology or Medicine in 1959

JOE C. DAVIS YMCA OUTDOOR CENTER
YMCA CAMP WIDJIWAGAN

YMCA OF MIDDLE TENNESSEE
3088 Smith Springs Road, Nashville, TN 37013
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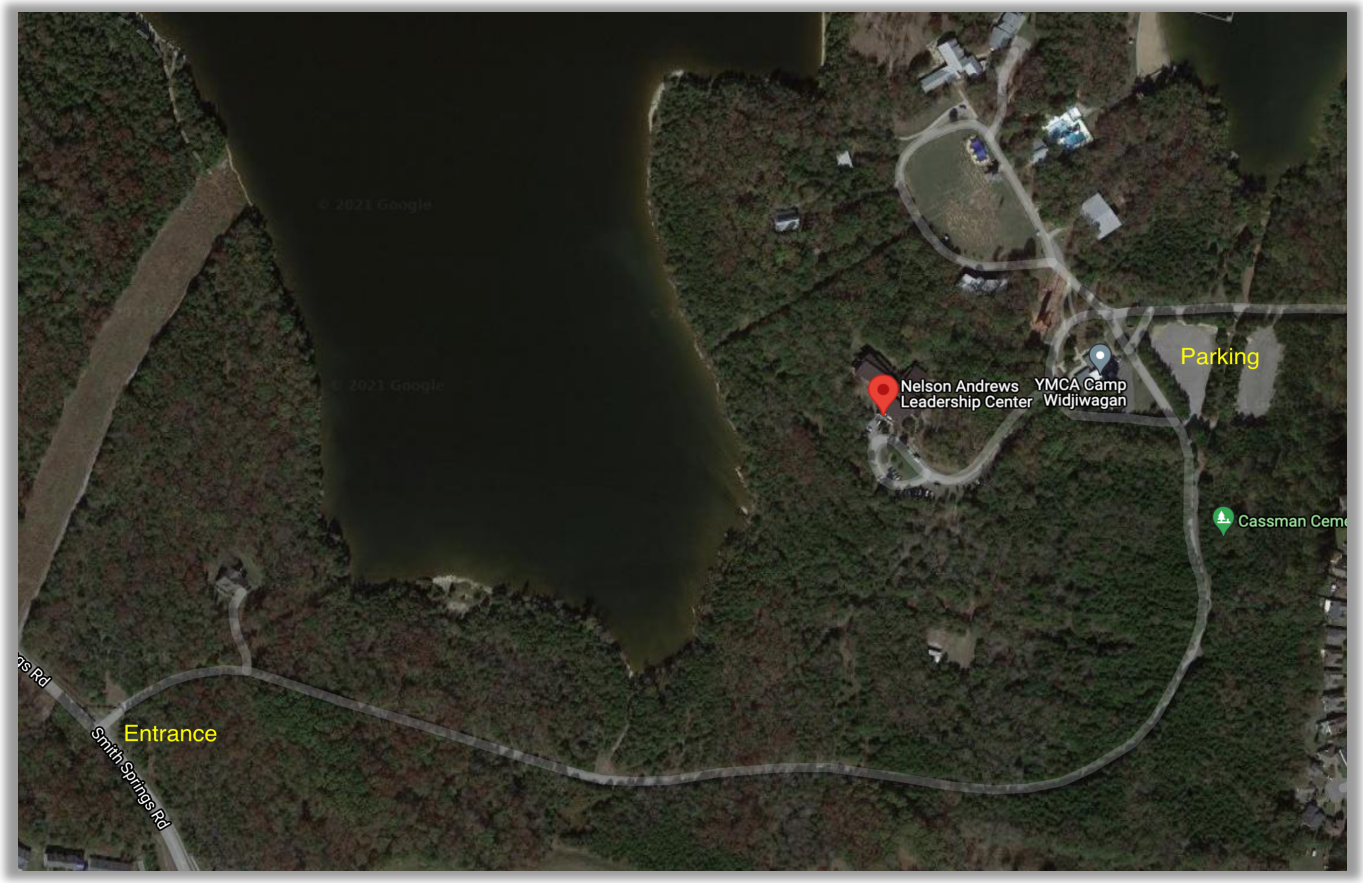
https://www.campwidji.org/retreats-and-events/facilities/nelson-andrews-leadership-lodge-great-room

Street entrance to Camp Widjiwagan

Aerial view of Camp Widjiwagan and the Nelson Andrews Leadership Center



Front Entrance to the Nelson Andrews Leadership Center

Twenty First Annual CDB
Retreat April 19, 2024
Nelson Andrews
Leadership Lodge

8:00-8:30	**BREAKFAST AND POSTER SESSION I SET UP** (Morning speakers- see Aaron Cooper)
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	**First Session**-Moderated by Marija Zanic Stephanie Medina (Irish Lab) Jennifer Silverman (Tyska Lab) Paige Vega (Lau Lab)
9:55-10:55	**Poster Session I** **Breakout Session I** (Students/Postdocs only) Moderated by - Paola Molina (Macara lab): Andrew Dixson (Weaver lab), Adam Ebert (MacGurn Lab), Olivia Perkins (Tyska Lab), Junmin Hua (Page-McCaw Lab), Megan Tigue (Weiss Lab)
10:55-12:45	**1st Annual CDB Olympics**
12:45-2:15	**LUNCH** (Chang Noi Thai-Lao and Urban Cookhouse) **POSTER SESSION I TAKE DOWN AND SESSION II SETUP** (Afternoon speakers- see Aaron Cooper) **ACTIVITIES ON THE YMCA CAMPUS AND FREE TIME**
2:15- 3:00	**Graduate Student/Postdoc Award -** Presented by Ian Macara - Steve Hann Award Winner presentation and Outstanding Postdoc Award winner presentation
3:05- 3:35	**CDB DEI Presentation**
3:40- 4:25	**Second Session**- Moderated by Ben Bratton Matthew Loberg (Weiss Lab) Avishkar Sawant (Kavarina Lab) Aubrie Stricker (Page-McCaw Lab)
4:30-5:30	**Poster Session II and Open bar** **Breakout Session II** (Students/Postdocs only) Moderated by Lauren Schnitkey (Lee lab): Todd Blakely Jr. (Zanic Lab), Monica Brown (Lau Lab), Julissa Burgos (Tyska Lab), Tyler Butsch (Burkewitz Lab), Alisa Cario (Kaverina Lab)
5:30-5:45	**POSTER SESSION II TAKE DOWN**
5:45-8:30	**Reception** **Dream Events & Catering**

"Science is a wonderful thing if one does not have to earn one's living at it."

Albert Einstein

Oral Presentations

First Session- Speaker 1

IL-8 Instructs Macrophage Identity in Lateral Ventricle Contacting Glioblastoma

Stephanie Medina, Asa A. Brockman, Claire. E. Cross, Madeline J. Hayes, Rebecca A. Ihrie, and 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 benefitted from immunotherapies. Microglial and hematopoietic macrophages are the most abundant immune cells in GBM and play contrasting roles in the disease. Our prior studies revealed a signature of CD32+ HLA-DRhi macrophages and STAT3 signaling in hematopoietic macrophages associated with poor patient survival in lateral ventricle contacting GBMs (PMC10371245). Identifying the origin of these macrophages and dissecting their immunosuppressive roles is critical for improving GBM Immunotherapies. We hypothesized that GBM cells may secrete factors to remodel the cellular microenvironment and generate this signature macrophage subset from myeloid cells. Results: IL-8 was identified as the main cytokine secreted ex vivo in primary GBM tumor conditioned media (N=7), and in vivo by human GBM tumors that contact the lateral ventricle (N=73, P=<0.001). To test the role of IL-8 in macrophage polarization, healthy primary macrophages were polarized with tumor conditioned media, IL-8, and with or without the addition of an IL-8 blocking antibody. Protein expression analysis of the polarized macrophages revealed that IL-8 was sufficient to instruct healthy monocytes to adopt the CD32+ signature of in vivo GBM macrophages. Additionally, IL-8 was required for primary GBM TCM to polarize healthy macrophages into CD163+ CD32+ cells. Conclusions: Overall, the findings here indicate that IL-8 is a factor secreted by GBM tumor cells, especially those that contact the lateral ventricle, and that it is sufficient and necessary to instruct healthy monocytes into suppressive macrophages in the GBM tumor microenvironment. These findings reveal a novel role for IL-8 in the instruction of macrophage identity and highlight IL-8 and CD32+ macrophages as potential therapeutic targets, especially for patients whose tumors contact the lateral ventricles. This ex vivo system will now be used to test the function of the GBM signature macrophages, to explore the targeting of IL-8 to improve GBM therapy, and finally to better understand the role of healthy IL-8 signaling in macrophages.

First Session- Speaker 2

Intestinal Tuft Cells Assemble a Cytoskeletal Superstructure Composed of Co-aligned Actin Bundles and Microtubules

Jennifer Silverman, Evan Krystofiak, Leah Caplan, Ken Lau, and Matthew Tyska

Vanderbilt University, Cell & Developmental Biology

Within the small intestinal epithelium, tuft cells are a rare chemosensory cell type (less than 1% of cells) that are morphologically defined by an array of large apical protrusions. Tuft cells have been implicated in sensing intestinal parasites and activating an immune response and are now recognized for their importance in maintaining the health of the intestinal epithelium. Tuft cell morphology is defined by a unique cytoskeleton, consisting of an array of giant actin filament bundles. As some of the longest actin bundles observed in nature, these structures support ~2 μm of apical membrane protrusion and extend over 7 μm down through the cytoplasm to the cell's perinuclear region. Despite their role maintaining a healthy intestinal epithelium, tuft cells remain understudied due to their rarity. As a result, we understand little about the architecture of the tuft cell cytoskeleton, the molecular components involved in building and maintaining tuft cell core actin bundles, or how these cytoskeletal structures support tuft cell biology. To fill these gaps in our knowledge, we have begun the first comprehensive quantitative analysis of tuft cell morphology and cytoskeletal structure. We combined super-resolution light microscopy and computer-based segmentation approaches to build 3D models and perform quantitative morphometry of the tuft. Our initial analysis identified several key differences between tuft cells and enterocytes in F-actin bundling mechanisms and regionalization of actin-binding proteins including a tuft cell specific co-alignment and interdigitation with microtubules, creating a cytoskeletal superstructure. To further investigate the mechanisms by which tuft cell enriched actin binding proteins contribute to bundle architecture, we used both cell culture models to quantify changes to actin bundling in response to expression of LIM domain and Actin Binding 1 (LIMA1), which is restricted to tuft cell rootlets. Insights derived from this work will provide a foundation for understanding of how giant core actin bundles contribute to tuft cell function and the maintenance of intestinal homeostasis.

First Session- Speaker 3

Chlamydia Induces Innate Immune Function of Intestinal Epithelial Cells to Drive Crohn's-like Colonic Inflammation

Paige Vega, Jiawei Wang, Alan Simmons, Yanwen Xu, Yilin Yang, Nicholas Markham, Julia Drewes, Erin Smith, Luisella Spiga, Monica Brown, James Ro, William Kim, Amrita Banerjee, Mariah Harned, Joseph Roland, M. Kay Washington, Raphael Valdivia, Wenhan Zhu, Qi Liu, Lori Coburn, Keith Wilson, and Ken Lau

Department of Cell and Developmental Biology and Program in Developmental Biology, Vanderbilt University; Epithelial Biology Center, Vanderbilt University Medical Center; Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, Vanderbilt University Medical Center; Department of Biostatistics and Center for Quantitative Sciences, Vanderbilt University Medical Center; Department of Surgery, Vanderbilt University Medical Center; Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center; Department of Molecular Genetics and Microbiology, Duke University Medical Center; Department of Veterans Affairs, Tennessee Valley Healthcare System; Center for Mucosal Inflammation and Cancer, Vanderbilt University Medical Center; Johns Hopkins University School of Medicine

The majority of Crohn's disease patients present with ileal or colonic inflammation, however, the factors that drive the development of inflammation in different sites of the gastrointestinal tract remain elusive. To-date, the TnfΔARE model is published as a spontaneous intestinal inflammation model that is specific to the ileum, however, we observe severe colonic inflammation that is dependent on the murine housing facility. We used co-housing methods to expose non-colitic, "restrictive" facility TnfΔARE mice to those with colitis in the "permissive" facility and found these mice developed colonic inflammation, suggesting colitis is transmissible through the microbiota. Shotgun metagenomics was performed to assess intestinal microbiota differences between the two housing facilities. Chlamydia muridarum was unique to permissive facility mice and using immunofluorescence imaging, we found infection was localized to the colonic epithelium. We administered doxycycline to treat C. muridarum infection and found infection clearance was associated with a reduction in colonic inflammation. Moreover, restrictive facility TnfΔARE mice treated with C. muridarum isolate developed colonic inflammation, demonstrating this single microbe is sufficient to drive inflammation in the colon. We used single-cell RNA-sequencing to examine epithelial cells in the context of C. muridarum infection and identified upregulation of indoleamine 2,3-dioxygenase (IDO1), an immune regulator, in intestinal epithelial goblet cells. Doxycycline-treated mice downregulated IDO1 expression and perturbation of the IDO1 pathway resulted in a reduction of colonic inflammation in TnfΔARE mice. Analysis of Crohn's disease patient samples revealed a specific subpopulation of IDO1-expressing epithelial cells in the ascending colon and the presence of intracellular and epithelia-associated microbes. Here, we provide the field with the first model of Crohn's-like inflammation that is specific to the ascending colon and demonstrate the capacity of a specific microbe, C. muridarum, to drive Crohn's-like colonic inflammation by inducing the expression of IDO1 in intestinal epithelial goblet cells.

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

Albert Einstein

Second Session- Speaker 1

Cancer-Associated Fibroblasts Correlate with Aggressive Thyroid Cancer Behavior: Insights from Four Large Patient Cohorts

Matthew A. Loberg, George J. Xu, Courtney J. Phifer, Eric C. Huang, Allan Golding, David Mimston, Mohammed Alshalalfa, Yangyang Hao, Joshua P. Klopper, Richard T. Kloos, and Vivian L. Weiss

Vanderbilt University Medical Center, Nashville, TN, 37232

Thyroid tumor molecular analysis often utilizes fine-needle aspirate (FNA) samples to predict malignancy with high sensitivity. An outstanding question is if molecular sequencing can provide prognostic information to predict disease aggression, potentially informing management. Recently, the composition of the tumor microenvironment, in particular the presence of tumor-promoting fibroblasts, has been associated with aggressive thyroid cancer. However, fibroblast gene expression has not previously been analyzed in thyroid FNA samples. In this work, we detect cancer-associated fibroblast (CAF) gene signatures across 4 distinct bulk RNA sequencing cohorts and confirm detection in FNA samples. Associations between CAF gene signatures and tumor characteristics were explored in: Afirma Genomic Sequencing Classifier (GSC) cohort from FNA (n=47,695), a Memorial Healthcare System (MHS) retrospective FNA cohort with pathology outcome data (n=318), TCGA papillary thyroid cancers (n=496), and a Vanderbilt University Medical Center-University of Washington (VUMC-UW) resection cohort (n=312). Published breast CAF gene sets were used to generate normal fibroblast and CAF subtype scores. Novel thyroid cancer specific CAF gene sets were generated from thyroid cancer single-cell RNA sequencing data. Scores for each gene set were calculated as the average Z-score. Across all cohorts, multiple CAF subsets were enriched in samples with BRAFV600E and papillary histology. The strongest enrichment was in an SFRP2+ CAF subset identified in thyroid cancer single-cell sequencing data. In Afirma GSC FNA samples, SFRP2+ CAFs were enriched in GSC-suspicious and Bethesda V/VI relative to GSC-benign nodules (Wilcoxon $p < 2e\text{-}16$ for all). In TCGA, SFRP2+ CAFs were associated with extrathyroidal extension, advanced disease stage, and aggressive histology. In the MHS cohort of 318 malignant FNA samples, SFRP2+ CAFs were significantly higher in patients with vascular invasion or lymph node metastasis (LNM) (OR:2.75 [95% CI 1.63-4.75], p=4e- 5). In the VUMC-UW cohort, SFRP2+ CAFs were associated with shorter progression-free survival (PFS) ($p=0.0033$). In summary we created a novel thyroid cancer- specific CAF gene signature that is elevated in BRAFV600E and aggressive thyroid cancers across multiple large patient cohorts. We also demonstrated the ability to detect these CAF signatures in thyroid FNAs using the Afirma GSC molecular testing platform, with enrichment in suspicious and malignant samples. Amongst the CAF scores tested, thyroid-specific CAF subsets had the highest prognostic potential, and SFRP2+ CAFs, specifically, were associated with shorter PFS, tumor invasion and LNM. Together, these results highlight the potential of interrogating the thyroid tumor stroma in FNA using RNA-based assays to inform prognosis and possibly management.

Second Session- Speaker 2

Cell Cycle-regulated Tug-of-war Between Microtubule Motors Positions Major Trafficking Organelles

Avishkar V. Sawant and Irina Kaverina

Vanderbilt University, Nashville, TN

Rapidly dividing cell populations must maintain efficient membrane trafficking while constantly remodeling their interior in preparation for cell division. Efficient protein processing and sorting in the mammalian Golgi apparatus relies on the integrity of this organelle. The integral Golgi is assembled around the centrosome by microtubule minus end-directed molecular motor cytoplasmic dynein. However, the Golgi must dissociate from the centrosome to allow for unperturbed centrosome separation in mitosis, which we have previously shown to occur as early as the G1/S transition. In addition, the Golgi exists in a constant membrane exchange with the Endoplasmic Reticulum (ER) through ER exit sites (ERES), which are also transported by microtubule molecular motors. Cell cycle signaling and molecular mechanisms that coordinate Golgi-centrosome and Golgi-ERES association still need to be understood. Here, we apply live cell imaging and loss-of-function approaches to show that cell cycle signaling tunes tug-of-war between the plus-end and minus-end-directed molecular motors, resulting in differential positioning of Golgi and ERES in the interphase sub-stages. Specifically, we find that in G1, the Golgi and ERES are brought to the centrosome by the minus-end-directed action of dynein and KIFC3, respectively. On the onset of the S-phase, kinesin-1-dependent activity at both the Golgi and ERES overpowers minus-end directed motors, driving the Golgi away from the centrosomes and spreading ERES throughout the cytoplasm. Out of known kinesin-1 motors (KIF5s) and kinesin light chains (KLCs), we have identified KIF5B and KLC1 as drivers for Golgi translocation in S/G2. In contrast, our preliminary data suggest that kinesin-dependent ERES transport in S/G2 is driven by KIF5C and KLC3 rather than KIF5B and KLC1. Interestingly, CDK1 inhibition in S-phase reverses the ERES and Golgi transport toward the minus end-directed motor activity, leading to a compact ERES/Golgi configuration around the centrosome, similar to G1. An acute kinesin-1 inhibition at this stage causes similar retrograde repositioning of the Golgi and ERES. This suggests that CDK1 activity in the S phase rises sufficiently to facilitate the switch of molecular motors favoring kinesin-1-dependent transport of these organelles. Our data indicate that CDK1 likely regulates KLCs, enhancing recruitment of respective kinesin-1 variants to the Golgi and ERES in S/G2. Overall, we conclude that CDK1 signaling regulates Golgi and ERES positioning via kinesin-1 recruitment to the membranes and that the differential positioning of these two organelles reflects the association of these organelles with different sets of molecular motors.

Second Session- Speaker 3

Piezo Initiates Transient Production of Collagen IV to Repair Damaged Basement Membranes

Aubrie Stricker, Shane Hutson, and Andrea Page-McCaw

Vanderbilt University, Nashville, TN 37232

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. We found there is a portion of intestinal stem cells that contain the mechanosensitive ion channel, Piezo. These cells are in direct contact with the basement membrane and monitor for basement membrane damage. Upon damage, the basement membrane becomes less stiff, activating Piezo. This results in the Piezo containing stem cells giving rise to a short-lived population of collagen IV producing enteroblasts, termed matrix menders. Collagen IV produced by the matrix menders is required for basement membrane repair. Further, we found that decreased gut basement membrane stiffness was sufficient to activate collagen IV synthesis in the matrix menders, suggesting that Piezo specifically works by detecting basement membrane stiffness to respond to damage.

“They thought I was crazy, absolutely mad.”

Barbara McClintock

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

Poster Presentations

Poster Presenters

Name	Session	Number
Achey, Meredith	1	1
Adeniran, Francisca	1	2
Ahn, Suyeon	1	3
Akizuki, Kazutoshi	1	4
Ankenbauer, Katherine	1	5
Anthony, Christin	1	6
Arceneaux, Deronisha	1	7
Aslaner, David	2	1
Barman, Bahnisikha	1	8
Benthal, Joseph	1	9
Biswas, Sushobhan	1	10
Blakely, Todd Jr.	1	45
Bodnya, Caroline	1	11
Brown, Monica	1	12
Burgos-Rivera, Julissa	1	13
Burman, Andreanna	1	14
Butsch, Tyler	1	15
Caplan, Leah	1	16
Cario, Alisa	1	17
Cassidy, Anna	1	18
Cephas, Amelia	1	19
Chalkley, Mary-Bronwen	1	20
Chalkley, Oliver	1	21
Chen, Lei	1	22
Cho, Youngwon	1	23
Clements, Jeremy	1	24
Colley, Madeline	1	25
Cywiak, Carolina	1	26
de Caestecker, Christian	1	27
Diaz, Diana	1	28
Dixson, Andrew	2	2
Donahue Eric	1	29
Dong, Xinyu	1	30
Dooyema, Samantha	1	31
Dufresne, Martin	1	32
Ebert, Adam	2	3
Eleuteri, Nicholas	1	33

Poster Presenters

Name	Session	Number
Elsayed, Nada	1	34
Esselman, Allison	1	35
Finnel, Ryan	1	36
Fitz, Gillian	1	37
Fort, Loic	1	38
Fye, Maggie	1	39
Gailey, Casey	1	40
Guenther, Alexis	1	41
Harmych, Sarah	1	42
Hartmann, Heather	1	43
Hayes, Caleb	2	4
Hepowit, Nathaniel	2	5
Hua, Junmin	2	6
Islam, Mirazul	2	7
Jimenez, Lizandra	2	8
Jung, Youn Jae	2	9
Kaur, Harsimran	2	10
Koshal, Adeel	2	11
Kumar, Sudiksha	2	12
Li, Xiaodong	2	13
Luo, Feng	2	14
McAtee, Caitlin	2	15
McFadden, Robert	2	16
Mikiyas, Daniel	2	17
Molina, Paola	2	18
Molloy, Kameron	2	19
Momoh, Michael	2	20
Nevills, Simone	2	21
Olson, Claire	2	22
Palacios, Claudia	2	23
Peek, Jennifer	2	24
Perkins, Olivia	2	25
Pham, Thai	2	26
Reynolds, Elizabeth	2	27
Richardson, Laura	2	28
Robinson, Kianna	2	29
Ruark, Elizabeth	2	30

Poster Presenters

Name	Session	Number
Schnitkey, Lauren	1	44
Schwarzkopf, Anna	2	31
Scott, Claire	2	32
Scott, Halee	2	33
Sha, Eric	1	46
Shafiq, Adnan	2	35
Stone, Megan	1	45
Sun, Rong	2	37
Sung, Bong Hwan	2	38
Tigue, Megan	2	39
Torres-Martinez, Diego	2	40
Venkataravi, Aishwarya	2	41
Willet, Alaina	2	42
Yagan, Mahircan	2	43
Zahraei, Ali	2	44

Session 1 breakout
Session 2 breakout

Session 1 P1

Neuronal and Glial lineages in the Post-natal Mesentery

Meredith A. Achey and E. Michelle Southard-Smith

Vanderbilt University Medical Center, Nashville, TN, 37223

Introduction: The enteric nervous system (ENS), a vast network of ganglia throughout the intestinal tract, is formed by the migration of vagal neural crest cells from cranial to caudal along the intestine, as well as via trans-mesenteric invasion of the hindgut. Failure of this migration leads to Hirschsprung's disease (HSCR), the congenital absence of ENS in a variable length of intestine. Strategies to regenerate the ENS from autologous cell populations may enable children with HSCR avoid resection. Methods: We are examining post-natal mouse mesentery to identify neuronal progenitors with potential to contribute to the ENS, using immunohistochemistry (IHC) and fluorescence activated cell sorting (FACS). To identify and flow sort putative progenitors, we are utilizing the Phox2b-CFP mouse transgenic line, which expresses CFP under the control of the regulatory regions for Phox2b. Phox2b is a transcription factor active in neural crest derived glial (low expression) and neuronal (high expression) cells. Results: We have identified cells within the mesentery that express high levels of Phox2b (Phox2b-CFP high), a marker of neuronal progenitors, as well as Hu, a pan-neuronal marker, up to postnatal day 22 in mice. These cells can be isolated using FACS. Conclusions: The post-natal mesentery is an incompletely explored source of post-natally generated neurons. Our preliminary results indicate that there may be a population of neuronal progenitors associated with peripheral nerves to the bowel wall. Future experiments will examine the proliferative and migratory capacity of these populations in vitro, and will examine the human mesentery for evidence of analogous populations.

Session 1 P2

Heterogeneity of Monocarboxylate Transporter Function in Gastrointestinal Epithelium

Francisca Adeniran, Cynthia Ramos, Luc Pellerin, Eunyoung Choi, and Izumi Kaji

Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232 University of Poitiers, Poitiers, France, 86000

Short-chain fatty acids and ketone bodies are important energy fuels of GI epithelial cells. Slc16a family encodes proton-coupled monocarboxylate transporters (MCTs) that mediate cellular metabolism of those energy fuels. Increasing evidence demonstrates that adenocarcinomas with overexpression of MCTs are better able to maintain microenvironmental pH and high energy metabolism to grow. However, the precise distribution and function of each MCT subtype in normal epithelial or precancerous cell lineages are mostly unknown. We investigated cell type-specific expressions of MCT subtypes MCT1, MCT2, and MCT4 by immunofluorescence in normal and gastric metaplasia model mice. As total MCT knockout leads to early lethality, we generated inducible, intestine-targeted knockout mouse models for MCT1 and MCT4, respectively. In normal GI tissues, MCT1 was strongly expressed on basolateral membranes of gastric surface cells and enterocytes, with intense staining of proliferating cells and enteroendocrine cells. No staining was observed in Brunner's glands or Paneth cells. The low affinity subtype, MCT4 expression was localized to the first corpus glands and tuft cells in the stomach, suggesting that these cells may monitor the luminal chemical environment. Intestinal MCT4 was localized on basolateral membranes of mature enterocytes and tuft cells. In contrast, MCT2, which has the highest affinity for lactate, was localized reciprocally to MCT1. Strong MCT2 staining was identified on the basolateral membrane of secretory cells, including gastric parietal and chief cells, Brunner's glands, and Paneth cells. The distinct MCT2 expression suggests that those secretory cells possess different energy metabolism and MCT2 may contribute to their intracellular pH homeostasis. In gastric metaplasia tissues of Mist1-Kras(G12D) mice, MCT1+ metaplastic cells were increased, and Ki67+ cells were more dependent on MCT1 than normal tissues. MCT2 expression in chief cells was disappeared during metaplasia development. MCT1ΔIEC mice lost MCT1 expression on day 8 after tamoxifen injection and decreased transcriptions of other subtypes, Mct4, Mct7, and Mct10 in intestinal mucosa. MCT1ΔIEC jejunum showed significant increase in crypt length, indicating that MCT1 loss disrupted proliferative cell function. Contrary, MCT4ΔIEC mice had no change in crypt length or Mct1 expression, but increased Mct7, and Mct10. These observations indicate that different cell types require a different MCT subtype. 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 1 P3

Spasmolytic Polypeptide-expressing Metaplastic (SPEM) Cell Plasticity Contributes Gastric Gland Recovery After Acute Mucosal Injury

Suyeon Ahn, Yoojin Sohn, Nick Barker, James R. Goldenring, and Eunyoung Choi

Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232

Epithelial damage in gastric corpus mucosa results in metaplastic changes to protect against external stimuli and heal damaged mucosa. Upon loss of parietal cells in response to acute or chronic mucosal injuries, zymogen-secreting chief cells transdifferentiate into mucous-secreting, spasmolytic polypeptide-expressing metaplastic (SPEM) cells present at the base of metaplastic glands. While the SPEM cells are also observed during mucosal recovery, it is obscure whether SPEM cells harbor plasticity that contributes recovery process. Aquaporin 5 (AQP5) has been used as a SPEM marker and its expression is upregulated during the early step of transdifferentiation. Therefore, we utilized tamoxifen-inducible transgenic mouse allele, the AQP52A-CreERT2; Rosa26-tdTomatoLSL (AQP5-tdTOM) mouse model, to trace cell lineages arising from injury-responsive SPEM cells. Two different injury strategies, high-dose injection of tamoxifen (HDT) or DMP-777, were used to induce mucosal injury. The AQP5-tdTOM mice were intraperitoneally injected with HDT (5 mg/20 g body weight) for two consecutive days to induce injury and label AQP5-expressing cells simultaneously. DMP-777 (350 mg /kg body weight) was orally administered for 10 days and tamoxifen (1 mg/20 g body weight) was injected intraperitoneally on days 3, 5 and 7 to label AQP5-expressing cells. Stomach tissues were collected immediately after injury and 1 month of recovery. As previously investigated, both HDT and DMP-777 methods promptly induced loss of parietal cells and emergence of SPEM cells expressing AQP5, CD44v9 and GSII-lectin at the base of metaplastic glands. Concurrently, AQP5 was expressed in tdTOM-labeled cells, confirming that the specificity of the AQP5-tdTOM mouse allele to label SPEM cells. After 1 month of recovery, normal gastric epithelium was regenerated in both HDT and DMP-777-induced methods. tdTOM+ cells were found in more than 90% of proximal corpus glands in both methods, indicating lineage reversion of AQP5-expressing cells to normal gastric cell lineages. In HDT treated mouse stomach, tdTOM+ cells were not only co-positive for MIST1 (chief cells, 37.8%), but also detected in mucus neck cells (25.7%), parietal cells (12.0%), isthmus progenitor cells (7.3%), and foveolar cells (7.7%). Similarly in DMP-777 treated mouse stomach, tdTOM+ cells predominantly observed as chief, neck and parietal cells (35.2%, 17.4% and 40.3%, respectively). This indicates that AQP5-linage labeled cells show a consistent tendency that contributes to normal gastric cell differentiation during the mucosal recovery. These results demonstrate that AQP5-lineage labeled cells, arising during acute mucosal injury, do not disappear during mucosal recovery, and can reprogram the cell identity into multiple normal gastric cell lineages.

Session 1 P4

Mitotic Hyperphosphorylation of the Schizosaccharomyces pombe CK1, Hhp2

Kazutoshi Akizuki, Sierra Cullati, Jun-Song Chen, Alyssa E. Johnson, and Kathleen L. Gould

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

CK1 enzymes are a highly conserved Ser/Thr kinase family in eukaryotes and play important roles in many cellular processes such as circadian rhythm and endocytosis. We previously found that two S. pombe CK1s, Hhp1 and Hhp2, contribute to mitotic checkpoint arrest by inhibiting the septation initiation network (SIN) to delay cytokinesis when the mitotic spindle is disrupted. CK1 is well known to be inactivated through autophosphorylation in vitro; however, there have been no reports that investigated how CK1 itself is regulated by phosphorylation through mitosis although we previously showed that human CK1 enzymes are hyperphosphorylated during mitosis. In this study, we found that Hhp2 is hyperphosphorylated in mitotically arrested S. pombe and in cells normally proceeding through mitosis. Because Hhp2 is not an essential protein and we can easily assess the effects of phosphorylation via mutational analysis, we have focused on understanding the effects of mitotic CK1 phosphorylation in yeast. We have so far determined that both wildtype and kinase-dead Hhp2 are phosphorylated, although the wildtype is more highly phosphorylated than the kinase-inactive form. These results indicate that Hhp2 is both auto-phosphorylated and trans-phosphorylated by other kinases during mitosis. By using traditional phosphomapping approaches in vitro, we identified four major autophosphorylation sites of Hhp2 in its C-terminal tail. These have been mutated to non-phosphorylatable amino acids and the contributions of these phosphorylation events to kinase activity, cellular growth, and mitotic checkpoint signaling are being determined.

Session 1 P5

Osteopontin Drives Epithelial Plasticity in Pancreatic Tumorigenesis

Katherine Ankenbauer, Joanna Dennis, Sabrina Newenham, Danyvid Olivares-Villagomez, Bob Chen, Ken Lau, and Kathleen DelGiorno

Vanderbilt University, Nashville, TN, 37212; Vanderbilt University Medical Center, Nashville, TN, 37212

Background: Acinar to ductal metaplasia (ADM) occurs when acinar cells in the adult pancreas transdifferentiate into ductal-like cells as part of a response to injury to facilitate tissue repair. ADM, however, can serve as the first step towards premalignant lesions for pancreatic ductal adenocarcinoma (PDAC) known as PanIN. Using lineage tracing and single cell RNA-sequencing (scRNA-seq) our lab discovered that ADM does not form a homogenous population of ductal cells, but instead results in the formation of other cell types such as chemoseneory tuft cells and hormone-secreting enteroendocrine cells. Using computational approaches, we identified a putative progenitor population in this dataset and found that osteopontin (OPN) is highly expressed. The purpose of this study was to identify a functional role for OPN in driving epithelial plasticity during ADM and PanIN formation.

Methods: Published scRNA-seq datasets of lineage traced acinar cells subjected to chronic injury or KrasG12D expression were analyzed. Lineage trajectory and differential gene expression analyses on ADM and PanIN were performed. Genetically engineered mouse models (GEMMs) were generated with KrasG12D knocked into the pancreas with or with osteopontin (Spp1) knocked out (KC and KCSpp1 mice). Mice were aged up to 6 months and pancreata were collected for histopathological analyses. Epithelial heterogeneity was evaluated using markers of tuft cells (DCLK1), enteroendocrine cells (synaptophysin), pit cells (MUC5AC), and proliferating cells (Ki67). Patient correlates were evaluated for OPN/SPP1 expression.

Results: Lineage trajectory analysis identified a population of cells derived from acinar cells, preceding the formation of distinct lineages, which we termed "uncommitted" cells. Differential gene expression analysis identified high expression of OPN/Spp1 in this population. Ablation of Spp1 in the pancreas (Spp1fl/fl;Ptf1aCre/+) resulted in a loss of protein expression but had no effect on pancreas development. As compared to KC mice, KCSpp1 mice had significantly lower expression of OPN as well as decreased expression of MUC5AC, Ki67, and synaptophysin with low-grade cystic lesions. OPN/SPP1 expression increased in patient samples with disease progression. Discussion: OPN loss during KrasG12D-mediated ADM and PanIN progression inhibits epithelial plasticity and significantly reduces epithelial heterogeneity. A consequence of this loss of plasticity is the formation of benign cystic lesions as opposed to PanIN. Further studies using scRNA-seq and multiplex immunofluorescence in GEMMs and organoid models are required to fully characterize epithelial populations present following OPN loss during both KrasG12D and inflammation-driven ADM. Identifying molecular drivers of epithelial plasticity may inform strategies to intercept premalignant disease before PDAC forms.

Session 1 P6

Investigating the Role of Cadherin in Canonical Wnt Signaling

Christin C Anthony[1], Vivian Weiss[2], David Robbins[3], Yashi Ahmed[4], and Ethan Lee[1]

[1]Vanderbilt University; [2]Vanderbilt University Medical Center; [3]Georgetown University; [4]Dartmouth College

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 at early timepoints but were increased in the NC cells at later timepoints of Wnt3a treatment. Additionally, E-cadherin overexpression can rescue the overactivation of Wnt signaling in the NC cells, and this rescue is dependent on the β-catenin binding of E-cadherin. 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 as well as using live-cell imaging to investigate the localization of β-catenin in conjunction with cadherin in response to Wnt signaling activation.

Session 1 P7

Anti-inflammatory ATOH1-Independent Type 2 Tuft Cells are Derived from Differentiated Cells

Deronisha Arceneaux, Amrita Banerjee, Joey Simmons, Yanwen Xu, and Ken Lau

Vanderbilt University, Nashville, TN 32171

Tuft cells have garnered increased attention within Inflammatory Bowel Disease (IBD). Both human and murine research has indicated a reduction in the number of tuft cells in Crohn's Disease and ulcerative colitis compared to individuals without these conditions. Furthermore, experimental perturbations have demonstrated that augmenting tuft cell numbers can effectively suppress inflammation. These tuft cells represent a diverse population, featuring two distinct subtypes. We know that tuft-2 cells play a role in immune regulation and are associated with anti-parasitic type-2 immune responses. However, the precise mechanism behind the expansion of tuft-2 cells and their origin remains unclear. Through a combination of single-cell RNA sequencing (scRNA-seq) and immunofluorescence microscopy, our research has unveiled that the proliferation of tuft-2 cells is a result of enterocytes undergoing transdifferentiation. These findings hold promise for potential implications in treating IBD, given the similarities between anti-parasitic and anti-inflammatory immune responses.

Session 2 P1

Liraglutide Protects Against Microvascular Dysfunction and Sepsis-mediated Inflammation and Organ Injury

David M. Aslaner[1,2], Brandon Baer[1], Jamie E. Meegan[1], Lorraine B. Ware[1,3], Julie A. Bastarache[1,2,3]

[1]Department of Medicine, [2]Department of Cell and Developmental Biology, [3]Department of Pathology, Microbiology, and Immunology; Vanderbilt University Medical Center, Nashville, Tennessee, 37203.

Introduction: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection characterized by increased systemic inflammation and microvascular injury. Recently, commonly used diabetes and obesity medications, glucagon-like peptide-1 (GLP-1) receptor agonists, have demonstrated unexpected anti-inflammatory effects. We tested GLP-1 receptor agonist liraglutide in a clinically relevant two-hit murine model of polymicrobial abdominal sepsis and hyperoxia and microvascular endothelial injury in vitro, hypothesizing that GLP-1 receptor agonists exert protective effects during sepsis, limiting microvascular permeability, inflammation, organ injury and death. Methods: Sepsis was induced in male and female mice by intraperitoneal (IP) injection of cecal contents (CS;1.8 mg/g body weight) collected from euthanized donor mice. Mice were also exposed to hyperoxia (FiO2 90-96%) for 24 hours. Six and 18 hours after CS injection, mice were administered saline or liraglutide (0.1 mg/kg). At 12 hours, mice were given fluids and antibiotics. At 24 hours, mice were euthanized for plasma and bronchoalveolar lavage (BAL; right lung) collection. Plasma cytokines, organ injury markers, and BAL immune cells and protein were measured. The left lung was tied off and excised to measure wet-to-dry lung weight ratios. Additionally, for in vitro studies, primary human lung microvascular endothelial cells (HLMVECs) were treated with saline or liraglutide for 24 hours prior to saline (control) or LPS (100 ng/mL). HLMVEC barrier dysfunction was quantified using express permeability testing (XPerT) assay or Electric Cell-Substrate Impedance Sensing (ECIS) to measure transendothelial electrical resistance (TER). Results: In murine sepsis, illness severity scores and lung injury were improved in mice pretreated with liraglutide (N=10). Plasma blood urea nitrogen (BUN; P=0.0581), alanine transaminase (ALT; P=0.0311) and aspartate aminotransferase (AST; P=0.0263), cytokine IL-6 (P=0.0650), and chemokine MCP-1 (P=0.0172) in plasma were reduced in mice treated with liraglutide. In HLMVECs, liraglutide (1.5 nM) significantly reduced LPS-induced barrier dysfunction measured by XPerT assay (P=0.0030) and ECIS (P=0.0075). Conclusion: Liraglutide reduces illness severity, inflammation, and organ injury in a two-hit model of sepsis-induced ALI. Liraglutide has direct effects in the microvascular endothelium, limiting LPS-mediated barrier dysfunction. These findings support a protective role for GLP-1 receptor agonism in sepsis, mediated through the microvasculature.

Session 1 P8

Cholesterol Levels Regulate the Biogenesis of RNA-containing Extracellular Vesicles via ORP1L

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

Vanderbilt University, Nashville, Tennessee 37203, USA

Cell-to-cell communication via extracellular RNA can influence cell and tissue phenotypes; however, the biogenesis of RNA-containing extracellular vesicles (EVs) is poorly understood. Previously, we identified the conserved endoplasmic reticulum membrane contact site (ER MCS) linker protein VAP-A as a significant regulator of the RNA content of a subpopulation of small EVs. As a major function of ER MCS is to transport cholesterol and other lipids, we hypothesized that cholesterol uptake, synthesis, and/or transfer might regulate the biogenesis of RNA-containing EVs. Inhibition of either dietary (via LDL in the serum) or endogenously synthesized (in the ER) cholesterol altered EVs' number and RNA content secreted from colon cancer cells. Interestingly, we observe the most significant decrease in RNA-containing EVs (EV-RNA) secretion in dietary cholesterol-depleted cells. ORP1L is a key protein at the ER MCS that senses and transports cholesterol. We find that the knockdown of ORP1L led to a substantial defect in the biogenesis of RNA-containing EVs. Here, we dissect and propose a mechanism for how cholesterol transfer via ORP1L MCS affects the biogenesis of the RNA-containing EV population.

Session 1 P9

Identification of Disrupted Gene Regulatory Networks in the Murine Sox10Dom Enteric Nervous System via Single Cell Multomics

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

Vanderbilt University Medical Center, Department of Medicine, Division of Genetic Medicine, Nashville, TN, 37232

Mammalian enteric nervous system (ENS) development requires migration of neural crest cells into the foregut and then along the developing intestine to the end of the gastrointestinal tract. During this process, enteric neural crest cells differentiate into neuronal and glial fates, whose maturation is still ongoing after the cells reach their terminal locations in the gut wall. Sox10, a transcription factor that has been shown to interact with factors that influence chromatin accessibility, plays a key role in the development of the ENS, as exemplified by the Sox10Dom mouse model of Hirschsprung's disease. Sox10Dom mutants exhibit deficits of the bowel colonization and subsequent enteric neuron differentiation. Generation of mature ENS cell types requires remodeling of chromatin to properly regulate gene expression of terminal cell fates. Single cell sequencing analyses have been published characterizing both the adult and developing ENS, annotating cell states through gene expression and accessibility of chromatin. However, there are few studies that have examined chromatin accessibility as a contributing factor in the development of enteric neurons and glia. We utilized single cell RNA-sequencing and single-nucleus ATAC-sequencing, methods that assay expressed genes and transposase-accessible chromatin, to further characterize the developing ENS in both wild type and Sox10Dom fetal intestine. By comparing wild type and Sox10Dom expression profiles and chromatin states, we have identified cell type-specific perturbed gene regulatory networks in the developing ENS. Our findings further expand the gene regulatory network that participates in ENS development and identifies candidate genes that may be perturbed in human gastrointestinal motility disorders.

Session 1 P10

Validation of Intercellular Interactions between Epithelial and Mesenchymal Cells in the Postnatal Mouse Utricle by Single Cell RNAseq and In Situ Hybridization

Sushobhan Biswas[1], Abel David[1,2], Macey Soltis[1], Yasmin Eltawil[3], Stefan Heller[4], Alan Cheng[4], and Taha A. Jan[1]

[1]Vanderbilt University Medical Center, Nashville, TN, 37232; [2]Harvard University, Boston, MA, 02138; [3]UCSF, San Francisco, CA, 94115; [4]Stanford University, Stanford, CA, 94305

Background: The utricle, an inner ear vestibular sensory organ, relies on mechanosensory hair cells for detecting motion. In newly born mice, hair cells are made from non-sensory supporting cells and continue to be added for the first ~7 days as part of the normal postnatal developmental process. We hypothesize that there is communication between the mesenchymal and epithelial layers which could be the driving factor behind postnatal proliferation and homeostasis. Identification and validation of these interactive cell populations will provide more comprehensive detail of utricular developmental pathways. Methods: We used published single cell RNAseq (scRNAseq) data of the utricular sensory epithelium (Jan et al., 2021) from P4 and P6 utricles. Non-sensory cells were collected at these same time points and single cell suspension was created. Following flow cytometry for cell isolation, the Smartseq2 protocol was used to generate scRNAseq data. We previously performed computational analyses using CellChat to determine what compartments of the inner ear communicate with each other. Here we validated our findings by in situ hybridization and immunohistochemistry. Results: We were able to annotate 12 cell populations including: mesenchymal cells, type I and II hair cells, transitional epithelial cells, supporting cells, glia, roof cells, pericytes, Schwann cells, endothelial cells, macrophages, and melanocytes. CellChat analysis identified mesenchymal cells as the dominant signal sender with statistically significant pathways including WNT, pleiotrophin (PTN), and midkine (MK). To validate classified genes, we examined whole mount and fixed frozen wild type utricles. Our analysis revealed five highly interactive ligand-receptor pairs between mesenchymal and epithelial compartments. We designed probes against Lgal9/Ighm, Bdnf/Ntrk2, Itga8/Spp1, Tgf-beta2/ Tgf-betar, and Ptprz1/Ptn. Our on-going validation experiments demonstrate strong cross-talk between the epithelial and mesenchymal compartments. Conclusions: CellChat analysis revealed communication pathways among 9 transcriptionally unique cell populations at single cell resolution. The dominant signal senders in the postnatal developing utricle are the mesenchymal cells. Our validation experiments support the computational analyses and open the door for further in vitro and in vivo cell-cell interaction studies in previously unexplored pathways.

Session 1 P45

Elucidating the molecular mechanisms of SSNA1's activity on microtubules

Todd Blakely Jr. and Dr. Marija Zanic

Vanderbilt University, Nashville, TN, 37212

Regulation of the microtubule cytoskeleton is essential for proper microtubule function in vital cellular processes, including neuronal development, cell division and intracellular transport. Microtubules are dynamic polymers, which rapidly undergo changes between phases of growth and shrinkage and compose the main structural component of neurons. Microtubule dynamics are regulated by a range of microtubule-associated proteins, one of which is Sjögren's Syndrome nuclear autoantigen 1 (SSNA1). Overexpression of SSNA1 in rat cerebral cortical neurons caused accelerated neuronal development, in addition to increased axon length and branching complexity. In vitro, SSNA1 was reported to directly induce microtubule branching. Additionally, previous work from our lab has shown that SSNA1 stabilizes microtubule dynamics and localizes at distinct microtubule lattice sites, such as incomplete tubules and growing microtubule ends. It has been suggested that SSNA1's activity may be regulated via tubulin posttranslational modifications (PTMs) and the ability of the protein to oligomerize. However, this regulation has not been directly investigated to date. Here, I propose to use biochemical in vitro reconstitution approaches to systematically determine how SSNA1's microtubule stabilization and damage recognition activity are regulated by microtubule PTMs and SSNA1's oligomerization. Precise characterization of SSNA1's regulation and effects on the dynamic behavior of microtubules will provide fundamental insight into how neurons regulate their microtubule network, and how this system is tuned for proper cellular function.

Session 1 P11

Examining the Impact of Peroxisomal Dynamics on Cell Fate Decisions During Neurodevelopment

Caroline Bodnya[1], Vivian Gama[1,2.]

[1]Cell and Developmental Biology, Vanderbilt University. [2]Vanderbilt Center for Stem Cell Biology, Vanderbilt University.

Zellweger spectrum disorders (ZSDs), which affect 1:50,000 individuals in the U.S., are characterized by dysfunction in any one of 13 peroxisomal biogenesis proteins (peroxins) and result in severe neurological phenotypes, including seizures and developmental delay. Life expectancy for patients rarely surpasses a few years. The peroxisomal biogenesis factor 11 beta (PEX11β), which is essential for peroxisomal fission, is among the peroxins mutated in ZSDs. However, the exact mechanisms by which peroxisomal morphology may contribute to disease pathophysiology remain unclear. To directly manipulate peroxisomal morphology during neurogenesis, I have generated PEX11β knockout human induced pluripotent stem cells (hiPSCs), differentiated them into neural progenitors (NPCs), and begun characterizing how knocking out PEX11β affects peroxisomal morphology and expression of key early neural identity markers. Consistent with previous findings in other models, we found that PEX11β KO NPCs have elongated peroxisomes, but also a reduced number of peroxisomes compared to control. Therefore, I have also generated PEX3 KO hiPSCs. PEX3 is crucial for peroxisomal biogenesis, and PEX3 deletion leads to loss of functional peroxisomes, providing a system to interrogate whether consequences of PEX11β KO are a result of changes in peroxisome morphology or number. Both PEX11β and PEX3 KO hiPSCs are pluripotent and can differentiate into NPCs. I plan to further evaluate the effects of knocking out PEX11β and PEX3 during NPC differentiation, examining peroxisomal morphology and expression of key neural identity markers. Furthermore, I will evaluate the effects of knocking out PEX11β and PEX3 on peroxisomal-mediated metabolic functions, including β-oxidation of long-chain, branched-chain, and very-long chain fatty acids. The successful completion of this project will provide insight into the mechanisms by which peroxisomal morphology, biogenesis, and metabolic functions influence neurodevelopment. Additionally, it will help determine how disruption of peroxisomal dynamics underlies rare peroxisomal diseases.

Session 1 P12

Tuft cell Cytospinules May Act as a Synapse-like Communication Method Between Epithelial Cells

Monica E. Brown and Ken S. Lau

Cell & Developmental Biology, Vanderbilt University, Nashville, TN, 37232 ; Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, TN, 37232

Tuft cells are a major chemosensory epithelial cell found in the small intestine and colon that respond to luminal contents and activate the immune system. Tuft cells contain unique structural architecture including lateral cytoplasmic projections, termed cytospinules, that insert into adjacent epithelial cells to touch their nuclei. In a previous study, these cytospinules were depicted as containing no cytoskeletal structure and could only be readily identified through electron microscopy. Little is currently known about the structure or function of these cytospinules. Utilizing spinning disk confocal microscopy, we have found that cytospinules contain the microtubule-associated protein DCLK1. Tuft cells found within the crypt of the crypt-villus axis have an increase in frequency of these DCLK1+ cytospinules and more distal regions of the intestine seem to be associated with an increase in these same structures. Given the proximity of cytospinules to adjacent cell nuclei, a rapid communication mechanism is likely associated with this structure. Utilizing single-cell RNA sequencing, gene set enrichment analysis was performed to look at potential communication mechanisms associated with tuft cells. We found a significant representation of gene ontology pathways associated with synapses and axons and calcium signaling. Together these pathways in addition to DCLK1+ cytospinule structures, a protein also associated with dendritic filopodia growth and maturation into synapses, suggest that cytospinules may act as a synapse-like communication between tuft cells and adjacent cell nuclei. To further investigate this, 3D surface renderings of tuft cell cytospinules and surround epithelial cell nuclei are generated to allow for 3D characterization of cytospinules and to measure cytospinule-to-nuclei distances as compared to known synaptic cleft distances. Future work aims to further characterize cytospinule-associated proteins and develop potential mechanisms to better understand the functional role of cytospinules when associated with adjacent cell nuclei.

Session 1 P13

Investigating the Role of IRTKS in Enterohemorrhagic E. coli Pathogenesis

Julissa Burgos-Rivera and Matthew J. Tyska
Vanderbilt University, Nashville, TN, 37212

Enterohemorrhagic Escherichia coli (EHEC) is a foodborne pathogen that breaches the intestinal epithelium and causes frequent outbreaks of bloody diarrhea and hemolytic uremic syndrome. Once inside the intestinal lumen, EHEC attaches intimately to the apical surface of enterocyte epithelial cells by secreting Translocated Intimin Receptor (Tir) a bacterial effector, into the host cytoplasm. Tir is inserted into the plasma membrane and binds to intimin, an EHEC surface protein, adhering the bacteria to the cell thereby triggering the signaling pathway for cytoskeletal host protein recruitment. EHEC then reorganizes the host's cytoskeleton to form dynamic actin-rich structures known as "pedestals," which facilitate bacterial cell-to-cell spread and colonization of the intestine. Despite the threat EHEC poses to public health, several gaps remain in our understanding of its unique infectious mechanism, particularly how Tir gets inserted into the membrane to initiate pedestal assembly. Interestingly, Tir also binds to Insulin Receptor Tyrosine Kinase Substrate (IRTKS) which contains actin binding and inverse BAR (I-BAR) domains and was previously implicated in microvillus formation. IRTKS localizes to EHEC pedestals and recruits additional proteins for pedestal assembly. My preliminary data shows that loss of IRTKS in the Caco2BBE cell line, an intestinal epithelial culture model, reduces the amount of Tir at the host-pathogen interface, suggesting that IRTKS may assist in localizing Tir at the apical membrane. We have also performed live imaging of pedestals and will further investigate recruitment of IRTKS to bacterial attachment sites, to ultimately develop a spatiotemporal framework of EHEC infection. Overall, this research will define how IRTKS promotes bacterial attachment and lead to a more comprehensive model of EHEC pathogenesis.

Session 1 P14

Small Intestinal Progenitor Cells Require MYO5B for Proper Stem Cell Identity and Function

Andreanna Burman, Monica Brown, Micheal Momoh, Cynthia Ramos, and Izumi Kaji

Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232

Microvillus inclusion disease (MVID) is a congenital disorder characterized by severe diarrhea. MVID is caused by inactivating mutations in myosin Vb (MYO5B), a motor protein. Mice lacking MYO5B in their intestinal epithelium have demonstrated the importance of MYO5B in brush border formation and cell differentiation. The proliferative zone of the intestinal crypt of MYO5B-deficient mice is elongated compared to controls. We utilized an intestinal progenitor cell-specific Cre driver under Lrig1 to investigate the crypt-specific functions of MYO5B. Lrig1-CreERT2;R26R-YFP and Myo5bfl/fl mice were crossbred to generate Lrig1-CreERT2;R26R-YFP;MYO5Bfl/fl (Lrig1ΔMYO5B) mice. Adult Lrig1ΔMYO5B mice and control littermates received tamoxifen injection at day 0. Lrig1ΔMYO5B mice began experiencing significant weight loss 4 days after tamoxifen injection and lost 20% of their starting body weight on day 5. Villus enterocytes of Lrig1ΔMYO5B mice on day 5 contained abnormal cytoplasmic accumulation of PAS staining and lost apical expression of SGLT1. The PCNA+ crypt region was expanded in Lrig1ΔMYO5B mice beginning at Day 3. The time course of the PCNA+ region expansion corresponded to the wave of MYO5B-loss beginning at the crypt base. MYO5B expression was diminished in the crypts at day 3 and nearly absent from the epithelium by day 5. Differentiation of epithelial cells derived from MYO5B-lacking progenitor cells was altered, noted by a decrease in tuft cells, abnormal goblet cell shape and contents, and altered Paneth cell localization throughout the crypt. SEM on the Day 3 jejunum revealed a disruption in progenitor cell mitochondrial morphology. Compared to the dark, electron-dense mitochondria of control crypt cells, the mitochondria of Lrig1ΔMYO5B crypt cells had a swelled appearance with disorganized cristae, suggesting a depolarized mitochondrial phenotype. A similar disruption in progenitor cell metabolism was evident as Day 3 Lrig1ΔMYO5B crypt, but not villus, cells demonstrated significantly less fatty acid oxidation compared to controls. Single cell RNA seq data revealed a lack of stem cell identity in progenitor cells of the Day 3 and 5 Lrig1ΔMYO5B jejunum and an expansion of secretory progenitor cells. Lrig1ΔMYO5B fibroblasts had altered Wnt and R-spondin pathway expression, which are critical for proper epithelial progenitor cell function. We established a novel mouse model that demonstrates progressive MYO5B loss in the intestinal epithelium, starting at the base of the crypt and reaching villi tips over the course of 5 days following Cre induction. This Lrig1ΔMYO5B strain allows for a better understand the impact of MYO5B loss on intestinal epithelial differentiation and progenitor cell function.

Multimodal Imaging to Identify Differential Biomass Distribution and Turnover in *C. elegans*

Tyler J. Butsch, Rafael Arrojo e Drigo, and Kristopher Burkewitz

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

Metabolic homeostasis is crucial for maintaining cell and organismal health. Notably, metabolic adaptation is a major factor in various cellular stress responses. The advent of analytical techniques such as mass spectrometry has enabled researchers to determine how particular stressors alter metabolism by measuring the abundance of specific proteins, lipids, and metabolites. Additionally, the use of labeled isotopes (i.e. 13C and 15N) in pulse-chase experiments permits the analysis of macromolecule turnover at the cellular and organismal level. However, mass spectrometry requires destruction of the input material (cells or tissue), and thus cannot readily provide spatial information. Recently, multi-isotope mass spectrometry with electron microscopy
(MIMS-EM) was developed to track biomass distribution and turnover in mouse tissue. MIMS permits the measurement of isotope ratios at high resolution, which can then be overlaid on EM images to assess biomass distribution in tissues and cells. While this technique is useful in mice, it would be experimentally powerful to be able to image multiple tissues at once. The small size of the nematode, C. elegans, would thus be an excellent model for MIMS-EM, as all tissues are visible within a single cross section. We are currently creating a pulse-chase protocol to track biomass distribution and turnover in C. elegans. This protocol delivers labeled carbon and nitrogen to worms via feeding them bacteria that were grown in media that lacks unlabeled carbon and nitrogen sources. Therefore, we will be able to control the saturation level. Preliminary data suggest this delivery method has minimal effects on animal physiology, suggesting that this method will provide a reliable readout of basal biomass distribution and turnover. Soon, we will execute this protocol in worms lacking a germline (major source of biomass loss) and worms under dietary restriction conditions (reduced reproduction and biomass loss) to validate this technique in C. elegans. In addition to data from bacteria growth and worm physiology experiments, I will present technical workflows and future uses for MIMS-EM in C. elegans.

Session 1 P16

Investigating the Role of NM2C and Cofilin in Regulating Microvillar Dynamics

Leah R. Caplan and Matthew J. Tyska

Vanderbilt University, Nashville, TN, 37240

Transporting epithelial cells, such as intestinal enterocytes, form apically localized, membrane wrapped, actin-based protrusions called microvilli. These structures are integral to the cellular function of absorption and pathogen protection, and therefore proper microvillar development and maintenance is critical. The core actin bundle of each microvillus is composed of parallel actin filaments oriented with their barbed ends at the tip of the microvillus and pointed ends in the cytoplasmic, terminal web - a meshwork of actin and intermediate filaments held together by various cross-linking proteins located in the apical actin cortex. One protein that localizes to the terminal web of transporting epithelial is non-muscle myosin 2C (NM2C). NM2C forms bipolar filaments which crosslink actin and maintain tension, as well as exert pN-scale force in the terminal web. NM2C can bind microvillar actin rootlets, suggesting that NM2C may affect microvilli dynamics by exerting tension at the base of microvilli in the terminal web. Previous work from our lab demonstrated that inhibiting NM2C contractility caused microvilli to lengthen, while increasing NM2C contractility caused microvilli to shorten. This suggests that actin filament dynamics must be impacted, however the mechanism underlying this phenotype is unknown. Another actin-binding protein that alters actin dynamics is cofilin, which has an affinity for ADP-rich curved actin, and severs actin by causing rotational strain of the actin filament. Previous studies in neuronal growth cones suggest that NM2 drives contractility-dependent actin turnover via cofilin. Given that NM2C can bind and exert tension on actin filaments, and that cofilin preferentially binds curved actin, I hypothesize that NM2C contractility drives actin filament turnover, and consequently microvillar motility, via cofilin recruitment. Preliminary staining in cell culture and mouse intestinal tissue confirmed that cofilin localizes primarily just below NM2C in the terminal web, as well as along ADP-rich actin in the actin core bundle. Future experiments will focus on perturbing cofilin and NM2 function to observe the effects on microvillar dynamics.

Session 1 P17

CLASP2 Regulates Secretory Cargo from the Golgi

Alisa Cario[1], Briahnah Streeter[1,2], Anna AWM Sanders[1,3], and Irina Kaverina[1]

[1]Vanderbilt University, Nashville, TN 37235; [2]University of Maryland, College Park, MD 20742; [3]University of Iowa, Iowa City, IA 52242

CLIP-associated protein-2 (CLASP2) is a microtubule associated protein (MAP) with known roles in cell division, cell motility, and microtubule stabilization. Due to its localization on the plus ends of microtubules, CLASP2 is categorized as a plus-end tracking protein (+TIP). Interestingly, CLASP2 also strongly localizes to the Golgi with the only known function is to promote nucleation of Golgi-derived microtubules. In this study, we study the role of CLASP2 in regulation of Golgi cargo export through use of the Erv29/Surf4-dependent secretory cargo (ESCargo) assay using a CLASP2 knockout cell line. We find that knockout of CLASP2 impedes cargo export which can be rescued through expression of full length CLASP2. Additionally, our data indicate the Golgi-binding C-terminal CLIP-ID domain of CLASP2, which has no microtubule binding capacity, is sufficient to rescue the CLASP2 KO phenotype. Interestingly, it was recently discovered that the CLASP2-CLIP-ID interacts with the Golgin GOLGA4, a protein with known roles in cargo export. Similar to the CLASP2 KO phenotype, KD of GOLGA4 also impedes cargo export. We are currently investigating the model where CLASP2 acts in complex with GOLGA4 to regulate export from the Golgi. Overall, our work reveals a novel microtubule binding independent function of CLASP2 in regulation of export from the Golgi.

Session 1 P18

Investigating the Role of the GTP-Cap in the Regulation of Microtubule Dynamics in Mammalian Cells

Anna Cassidy, Veronica Farmer, Göker Arpağ, and Marija Zanic

Vanderbilt University, Nashville, TN, 37232; Kadir Has University, Istanbul, Turkey

Microtubules are dynamic cytoskeletal polymers essential for cell division, cell motility, and intracellular transport. 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. Although the GTP-cap is thought to protect the growing microtubule end, whether the GTP-cap size affects microtubule stability in cells is not known. Notably, a family of microtubule end binding proteins, EBs, recognize the nucleotide state of tubulin, and can thus be used as a proxy for the GTP-cap. Previous work from our lab demonstrated that EB comet size increases with increasing microtubule growth rates, achieved in vitro using microtubule polymerase XMAP215. Surprisingly, the rate of microtubule catastrophe also increased, despite 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 dynamics in interphase 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 EB comet intensity at the onset of catastrophe in cells and find that, in contrast to our in vitro work, EB comet intensity is not predictive of microtubule catastrophe in cells. Taken together, our data reveal that the GTP-cap size is similarly modulated in response to microtubule growth rate in the two investigated systems. Furthermore, our results emphasize the necessity to investigate the role of the GTP-cap in modulating microtubule stability in cells.

Session 1 P19

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

Amelia Cephas, Vincent Q Trinh, Marcus C.B. Tan, and Kathleen DelGiorno

Vanderbilt University, Nashville, TN, 32712

BACKGROUND & AIMS: Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer-related deaths in the United States. Intraductal papillary mucinous neoplasms (IPMNs) are neoplastic lesions of ductal origin that are typically benign, however ~20-30% progress to PDAC. There are currently no markers to distinguish between IPMN that will remain benign and those that will progress to cancer. Recently, we published that a heterogenous population of enteroendocrine cells (EECs) form during metaplasia and neoplastic progression in the pancreas. The aims of this study were to characterize EEC heterogeneity and abundance during IPMN progression. METHODS: 71 IPMN patient tissue samples spanning low grade, high grade, and invasive disease underwent immunostaining for pan-endocrine marker ChromograninA (ChgA). Further staining was conducted on a subset of 20 IPMN for EEC subtype hormones serotonin (5-HT), gastrin (GAST), insulin (INS), pancreatic polypeptide (PPY), glucagon (GCG), somatostatin (SST) and ghrelin (GHRL). Four EEC-high IPMN underwent spatial transcriptomic analysis using the Nanostring GeoMx platform. RESULTS: ChgA expression did not differentiate between benign and invasive disease; however, it was enriched in a subset of gastric folveolar (GF) and pancreatobiliary (PB), but not intestinal (INT)-type IPMN (>2% epithelium). These EEC-high IPMN were also highly enriched for synaptophysin (SYP). SYP+/5-HT+ and SYP+/GAST+ co-positive cells were identified in 90% of samples. Of the 10 EEC-high IPMN, 70% contained lesions that were SYP+/5-HT+ and 20% SYP+/Hormone-. Spatial transcriptome profiling of EEC-high IPMN using the NanoString GeoMx platform identified expression of additional neuronal genes not identified in our initial histological screen. Furthermore, we observed that genes distinguishing between high-grade and low-grade regions encompass mucin genes (MUC2, MUC13, MUC17), Claudins (CLDN3, 4, 7), and cancer-associated genes (CEACAM1/5, MYC, CLU, GABRP). In ChgA-Hi regions, there was notable expression of actin filament-associated genes (ITPRID2, BRK1) and regeneration-related genes (REG3A), alongside various other neuropetide-related genes(PCSK1N,MS4A8). CONCLUSIONS: We have identified an EEC-high subtype of IPMN enriched for expression of endocrine hormones. SYP+/5-HT+ and SYP+/hormone- IPMN suggest possible 'functional' and 'non-functional' categories. The abundance of hormone expression in GF, but not INT-type IPMN suggests a role in disease progression for this lineage. Further studies are required to identify a functional role for these hormones in IPMN to PDAC progression.

Patient TSC2 Mutant Cells Exhibit Aberrations in Early Neurodevelopment that are Accompanied by Changes in the DNA Methylome

Mary-Bronwen L. Chalkley, Lindsey N. Guerin, Tenhir Iyer, Samantha Mallahan, Sydney Nelson, Emily Hodges, Kevin C. Ess*, and Rebecca A. Ihrie*

Department of Cell & Developmental Biology, School of Medicine Basic Sciences, Vanderbilt University, Nashville, Tennessee, 37240

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 isogenic induced pluripotent stem cells (iPSCs). 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 misexpression of key transcription factors associated with lineage commitment and premature electrical activity. Collectively, these data suggest that mutation or loss of TSC2 has early effects on gene expression in proper neural development. Indeed, we have found that DNA methylation is changed with some key genes in neurodevelopment in the wild type cells compared to 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 1 P21

Developing a Novel Cryo-Electron Tomography Tag

Oliver Chalkley, Feng Luo, Kevin McCarty, Robert McFadden, and Qiangjun Zhou

Vanderbilt University, Nashville, TN 37235

A cryo-electron Tomography(cryo-ET) tag would give insight into the nanoscale's disease mechanisms and cellular functions. A novel effort is made here using a smaller ferritin-like protein by building on prior attempts to make a cryo-ET tag that uses ferritin and ferritin-like proteins. This design creates a "one protein of interest to one tag" ratio not found in other ferritin-based tags, reduces the distance between the tag and the protein of interest, and creates a high-contrast iron granule for quick and easy identification. To accomplish this, first, a nano-ferritin, composed of twelve protein monomers, was engineered to reduce monomers and to create one Iron Granule Forming (IGF) subunit that can make a high-contrast iron granule and one "inert subunit" that can't generate an iron granule. The plan is to genetically encode the IGF subunit as a fusion protein to the protein of interest and have the inactive subunit co-expressed so iron granules only form in mini-ferritin cages attached to the protein of interest. The in vitro data from the bioengineered nano-ferritin subunits confirm that engineered subunits can form a cage. Furthermore, the inert subunit-only cages do not produce an EM-visible iron granule, 11% of the IGF subunit-only cages form an EM-visible iron granule, and interestingly, mini-ferritin cages that are a mixture of inactive and IGF subunits, similar to how these cages will function as a tag in the cell, formed an iron granule 37 % of the time. These data provide evidence that this design may be a viable candidate as a cryo-ET tag for cellular studies. In HeLa cells, the IGF subunit was introduced with a lentivirus as a fusion protein to a 1) mitochondrial targeting sequence 2) a GFP, and the inert subunit was introduced with a lentivirus to be co-expressed with a mScarlet as a soluble protein so that a nano-ferritin cage may form in the cell. These two fluorophores enable flow cytometry sorting into pure cell lines and fluorescent localization data. Creating a pure cell line and confirming proper localization with a confocal microscope is essential to streamlining cryo-ET data to locate the characteristic iron granule of this tag.

Session 1 P22

Insm1 Regulates Cerebellar Granule Cell Lineage Development

Lei Chen and Chin Chiang

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, 4114 MRB III, Nashville, TN 37232

Cerebellar granule cells are crucial for motor coordination and learning. As the most abundant neurons in the central nervous system, their vast numbers are attributed to the rapid and transient proliferation of granule cell precursors (GCPs) within the external granular layer (EGL) of the developing cerebellum. Dysregulation in this developmental process can lead to cerebellar hypoplasia or medulloblastoma, the most common pediatric brain tumor. The zinc-finger protein Insulinoma-associated 1 (Insm1), a transcription factor widely expressed in the developing brain, acts as a transcriptional repressor essential for regulating neuronal proliferation and differentiation. In human, INSM1 is strongly expressed in the EGL and marks predominantly differentiated neurons of medulloblastoma. However, the specific functions of Insm1 in the development of GC lineage and its potential linkage to tumorigenesis have not been fully explored. Here, we report that Insm1 is dynamically expressed in the EGL of mouse cerebellum, with the strongest expression detected in differentiating GCP and rapidly downregulated as the granule cells mature. Global ablation of Insm1 resulted in mice with reduced cerebellar volume and a diminished granule cell lineage population. However, the majority of cells in the EGL are proliferative with fewer differentiated cells. Notably, Ccnd1 is transcriptionally up-regulated in Insm1 mutant GCPs, accompanied by a reduced rate of EdU incorporation into DNA, suggesting slower DNA synthesis. Similar findings were obtained with GCP-specific knockout of the Insm1 gene. We are currently investigating the role of Insm1 in cell cycle regulation of granule cell lineage development.

Session 1 P23

Apc and Smad4 Deletion Forces Neoplastic Progression in Active Kras-induced Dysplastic Organoids

Youngwon Cho, James R. Goldenring, and Eunyoung Choi

Department of Surgery and Epithelial Biology Center, Vanderbilt University Medical Center, Nashville, Tennessee, 37232

Background: Ras pathway activation in gastric chief cells induces a sequential cascade from normal to metaplasia and dysplasia, a key stage of developing gastric cancer. Despite of various gene mutations in cancer, it is unclear impacts of additional gene mutations in precancerous dysplasia induced by Kras activation. Apc and Smad4 are known as one of the most frequently mutated gene in WNT or TGF-β pathway in gastric cancer. We therefore performed a genetic manipulation of the Apc or Smad4 gene using the CRISPR/Cas9 technology in mouse dysplastic cells induced by Kras activation in gastric chief cells and determined whether the loss of Apc or Smad4 can lead to dysplasia evolution to cancer cells. Methods: Previously, we established dysplastic organoid lines (Meta4) from active Kras induced mouse stomachs. We utilized CRISPR/Cas9 to target the Apc or Smad4 deletion and established isogenic lines (Meta4-ApcKO or Meta4-Smad4KO). Both 3D and Air-Liquid Interface (ALI) cultures and RNA-sequencing were conducted to compare altered cellular behaviors and characteristics. Results: In 3D cultures, Meta4-ApcKO and Meta4-Smad4KO organoids showed increases in organoid size and budding formation, indicating enhanced proliferation activity and cell behavior changes. While the Meta4 cells displayed a single layer of mucinous cells positive for MUC5AC or TFF3 in ALI condition, Meta4-ApcKO cells showed increased TROP2 and Ki67 positive cells without mucinous cells. Additionally, Meta4-Smad4 cells displayed multilayering cells with polypoid structure. Transcriptomic profiling identified global changes in gene expression. In particular, gene-sets related to epithelial-mesenchymal transition and dysplastic stem cell were enriched in Meta4-Apc KO, and MYC-target related gene-set was enriched in Meta4-Smad4KO. Conclusion: Loss of Apc or Smad4 in Kras induced dysplastic cells promoted structural change and cell lineage evolution to cancerous cells. Also, RNA sequencing revealed global transcriptional change in Meta4-Apc and Meta4-Smad4. These results suggest that Apc and Smad4 are responsible for dysplasia evolution to cancer during gastric carcinogenesis.

Mechanisms Underlying Mesoderm Induction in 2D and 3D Models using Human Pluripotent Stem Cells

Jeremy Clements, Loic Fort, and Ian Macara

Vanderbilt University, Nashville, TN, 37235

Pluripotent stem cells are crucial for the proper development of many tissues and organisms. Pluripotent stem cells can commit down various lineages to differentiate into multiple types of tissue. This process involves a complex array of signaling events to occur so that stem cells properly differentiate. For stem cells to differentiate into cardiomyocytes the activation of WNT signaling alongside the activation of the purinergic G-protein coupled receptor (GPCR), P2Y2 must occur. P2Y2 binds extracellular ATP and is believed to couple with the downstream signaling protein GNAQ. However, the effects downstream of this signaling protein in cardiomyocyte differentiation and whether this system is translatable to other more physiologically relevant models are unknown. To answer these questions, a CRISPR knockout of GNAQ in stem cells coupled with pharmaceutical screens using various small molecule inhibitors was used. Furthermore, a gastruloid model was adapted to H9 human embryonic stem cells to test the effects of the apoptosis inhibitor Q-VD on gastruloid formation. Results suggest the importance of apoptotic signaling in gastruloids and that GNAQ and other signaling elements may play important roles in cardiomyocyte differentiation.

Session 1 P25

Structural Elucidation of Lipids and Metabolites for Imaging Mass Spectrometry by Innovative Instrumentation and Chromatography Techniques

Madeline E Colley, Lukasz G. Migas, Martin Dufresne, Alina Theisen, Cody R Marshall, Allison B Esselman, Melissa A. Farrow, Christopher A Wootton, Arne Fuetterer, Raf Van de Plas, and Jeffrey M. Spraggins

Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN;; Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN ; Bruker Daltonik GmbH, Fahrenheitstraße 4, 28359 Bremen, Germany; Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands; Chemical Physical Biology Program, Vanderbilt University School of Medicine, Nashville, TN; Department of Cell & Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN; Department of Chemistry, Vanderbilt University, Nashville, TN

The complexity of human tissues requires both high spatial and spectral resolution imaging mass spectrometry (IMS) to confidently associate specific molecules to cell types and their neighborhoods. Elucidating the structure of these specific molecules is critical to understanding the biological pathways associated with the structures. Here, we outline our strategies to annotate lipids and metabolites from human tissues using a variety of techniques and collaborations. One tool we employ is untargeted lipid and metabolomics by LC-MS/MS which gives a comprehensive view of both species and structure in four dimensions: 1) mass accuracy, 2) fragmentation, 3) collision cross section (ion mobility), and 4) retention time. Our methods for untargeted lipidomics take advantage of PASEF on the timsTOF mass spectrometers as well as MS/MS stepping to increase the mass range of detectable species. This technology provides a tissue-specific database of species present within the sample though the spatial context is lost. A second tool we employ is prm-PASEF IMS which enables highly multiplexed in situ fragmentation to confirm up to 100 lipid species within a 30 x 30 um pixel. This analytical technique can be used to mine the LC-MS/MS database for both tandem MS spectral comparisons and collisional cross sections. A third technology we are developing in collaboration with Bruker Daltonics is the use of a custom developed hybrid ion mobility Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer which offers ultra-high spectral resolution that enables highly confident molecular annotation on tissue through sub-ppm mass accuracy and fine isotope analysis. We utilize this system to study the human kidney at 10 um spatial resolution and with a resolving power of 1.1 million at m/z 400. The result is highly confident chemical formulae which are given deeper molecular annotation by comparison with the custom LC-MS/MS database. The combination of technologies with custom sample preparation strategies and in-house developed software provide a unique matrix of tools to confirm the identity of species we visualize with IMS.

Session 1 P26

Receptor-Dependent Wnt Activation in APC-deficient Cells

Carolina Cywiak[1], Anna Schwarzkopf[1], Starina D'souza[1], Jingjing Li[1], Jamal Bryant[1], Mary Rockouski[1], David Robbins[2], Yashi Ahmed[3], and Ethan Lee[1]

[1]Department of Cell and Developmental Biology, Vanderbilt University Medical Center, [2]Department of Molecular and Systems Biology and the Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth College, [3]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University

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 □-catenin signaling immediately after LRP6 CRISPR-Cas9 treatment. Our preliminary studies indicate loss of LRP6 initially results in significantly decreased □-catenin levels, followed by a gradual increase in □-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 1 P27

A Size Filter Regulates Apical Protein Sorting

Christian de Caestecker and Ian G. Macara

Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37232

Despite decades of research, apical sorting of epithelial membrane proteins remains incompletely understood. We noted that apical cytoplasmic domains are smaller than those of basolateral proteins; however, the reason for this discrepancy is unknown. We investigated whether a size barrier at the trans-Golgi network (TGN) might hinder apical sorting of proteins with large cytoplasmic tails. We focused on Crb3 and Ace2 as representative apical proteins with short cytoplasmic tails. By incorporating a streptavidin-binding peptide, these proteins can be trapped in the endoplasmic reticulum (ER) until addition of biotin, which triggers synchronous release to the Golgi and subsequent transport to the apical cortex (RUSH system). Strikingly, departure from the Golgi could be significantly delayed simply by increasing cytoplasmic bulk. Moreover, large and small Crb3 segregated into spatially distinct Golgi regions as detected by super resolution imaging. Biologically, Crb3 forms a complex through its cytoplasmic tail with the Pals1 protein, which could also delay departure, but although associated at the ER and Golgi, we found that Pals1 disassociates prior to Crb3 departure. Notably, a non-dissociable mutant Pals1 hampers the exit of Crb3. We conclude that an unexpected mechanism involving a size filter at the TGN facilitates apical sorting of proteins with small cytoplasmic domains and that timely release of Pals1, to reduce cytoplasmic domain size, is essential for the normal kinetics of Crb3 sorting.

Session 1 P28

Novel Use of Proteolysis-targeting Chimera (PROTACs) as a Therapeutic Approach for Colorectal Cancer

Diana Diaz, Alex G. Waterson, Stephen W. Fesik, Ethan Lee, and Vivian L. Weiss

Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, 37232 ; Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37235 ; Department of Pharmacology, Vanderbilt University, Nashville, TN, 37235 ; Department of Biochemistry, Vanderbilt University, Nashville, TN, 37235 ; Department of Chemistry, Vanderbilt University, Nashville, TN, 37235

Colorectal cancer (CRC) is currently the third leading cause of cancer death worldwide. Around 80% of CRC is caused by an APC mutation, leading to activated Wnt/β-catenin signaling. Activation of the Wnt/ β-catenin pathway leads to translocation of β-catenin to the nucleus and binding of TCF/LEF transcription factors to promote the expression of Wnt target genes. This pathway activation can cause cell division and increased cancer proliferation. Researchers have sought ways to inhibit Wnt signaling in CRC with limited success. Proteolysis-targeting chimeras (PROTACs) that bind to both β-catenin and an E3 ligase have been discovered as part of an ongoing project at Vanderbilt. These PROTACs induce the ubiquitination of β-catenin, leading to proteasomal degradation, and downregulation of Wnt target gene expression. To test the effect of β-catenin knock-down using these novel PROTACs, we utilized a Wnt reporter cell line to examine three different PROTACs (VU0945633, VU0948882, and VU0948590). To test the efficacy of these novel PROTACs in cells, we used colorectal cancer spheroid lines HCT116 and DLD1. We found that VU0948882 effectively decreased spheroid growth and altered the morphology of the HCT116 ($p<0.05$) line. The DLD1 spheroid line was responsive to VU0945633, VU0948882, and VU0948590 ($p<0.0001$). These findings are promising, but more experiments are needed in additional cell lines and mouse models to examine and validate efficacy in vitro and in vivo.

Session 2 P2

Cumate-inducible Overexpression of the Ceramide Transfer Protein (CERT) in DLD-1 Cells

Andrew Dixson and Alissa Weaver

Vanderbilt University, Nashville, TN, 37232

Transfer of proteins, lipids, and RNAs from one cell to another via extracellular vesicles (EVs) is emerging as a widespread mechanism of cell-cell communication across species and organ systems. However, it remains unclear how some cargoes are packaged into EVs while others are excluded. Our lab previously identified the ER resident protein VAP-A as an important regulator of a subpopulation of EVs characterized by their small size, high density, and abundant RNA content. Disruption of the VAP-A binding partner CERT also decreased the secretion of microRNAs via EVs. The major known function of VAP-A:CERT complexes is to transfer ceramide out of the endoplasmic reticulum (ER) and into other organelles at ER membrane contact sites (MCSs). I am developing colorectal cancer cell lines that inducibly overexpress either of the two major spicing isoforms of CERT, with and without an N-terminal V5 tag, when cumate is added to the culture medium. I will present data showing the fold-change in CERT protein expression at various timepoints after cumate addition in these cell lines. I plan to use these lines to test the effect of CERT overexpression on EV abundance using nanoparticle tracking analysis. I will also determine whether CERT overexpression affects EV cargo content using RNA sequencing and proteomics. These experiments are expected to identify one or more RNAs or RNA-binding proteins that enter EVs in response to CERT overexpression.

Session 1 P29

ER-phagy Drives Remodeling of the ER During Early Aging

Donahue EKF, Hepowit NL, Mulligan AG, MacGurn JA, and Burkewitz KJ

Vanderbilt University School of Medicine, Nashville, TN, 37232

Functional outputs of the endoplasmic reticulum (ER) correspond closely with specific ER morphologies and subdomains. While altered ER stress and dysfunction are commonly linked to pathogenesis during aging, whether shifts in ER morphology underlie these functional changes is unknown. Here, we combine electron microscopy and live imaging of natively labeled ER proteins enriched in rough ER cisternae and smooth ER tubules to demonstrate that the ER undergoes dramatic structural remodeling as an early step in the aging process. We observe a substantial decline in overall ER mass beginning in the first days of adulthood across virtually all C. elegans tissues. Distinct from the intestinal atrophy previously observed in aging C. elegans, ER remodeling is independent of sex and conserved in S. cerevisiae. We find that this remodeling is mechanistically an active, ER-phagy dependent process that tends to selectively target rough ER, thereby promoting shifts toward more tubular ER networks in aged animals. The IRE-1/XBP-1, but not ATF-6 or PERK, branch of the ER stress response is required for age-related ER-phagy, and we find that increasing secretory protein flux through the ER accelerates the age-dependent loss and shifts in morphology. Together, these results are consistent with a model where ER protein-folding burdens arise surprisingly early in the aging process and trigger ER stress-driven turnover of the rough ER. To determine whether these ER morphological shifts trigger functional consequences in aged animals, we counter ER tubulation by ablating the conserved YOP-1 and Reticulon membrane-shaping proteins and observe protection against age-onset mitochondrial fragmentation, suggesting that ER remodeling alters inter-organelle interactions and modifies behaviors in distal organelles. Intriguingly, mechanistically diverse lifespan-extending paradigms promote pronounced autophagic remodeling of ER even in young animals, suggesting that long-lived animals pre-emptively adopt an ER network morphology optimized for avoiding the accumulation of protein damage. Altogether, these results reveal ER remodeling as an understudied mechanism of aging and longevity.

Session 1 P30

α-Parvin, an Integrin-related Scaffold Protein, Regulates Actin Dynamics to Facilitate Kidney Ureteric Bud Branching Morphogenesis

Xinyu Dong[1], Fabian Bock[1], Nada Bulus[1], Ali Hashmi[2], Reinhard Fassler[3], Ambra Pozzi[1], Sara Wickström[2,3], and Roy Zent[1]

[1]Cell and Developmental Biology, Vanderbilt University Nephrology, Vanderbilt University Medical Center; [2]University of Helsinki, Finland; [3]Max Planck Institute of Biochemistry, Germany

The kidney collecting system is essential for water and electrolyte homeostasis. It develops from the ureteric bud, which undergoes branching and tubule elongation via cell division and movement. Growth factor- and integrin-dependent signaling, as well as an intact actin cytoskeleton in the UB cells, is required for this process to occur. 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. In this study we show that α-parvin, an integrin associating and actin-binding protein, plays a critical role in UB development by regulating ureteric bud cell movement. 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. Direct ex vivo imaging and cell tracking using membrane-tethered GFP (mTmG mice) demonstrated that α-parvin regulates neighbor exchanges (cell intercalation) and motion persistence, which are essential for tubule narrowing. Isolated α-parvin-null CD cells had increased cell adhesion and spreading but impaired migration. Surprisingly, α-parvin mediated these effects only by regulating Rho family GTPase-dependent depolymerization of F-actin via cofilin and not by affecting integrin function. Thus, we conclude that □-parvin regulates collecting system development by controlling actin cytoskeleton turnover, which is required for ureteric bud cell movement.

Session 1 P31

Investigation of Activin Receptor/Smad2/3 Requirement During Embryonic RPE Development

Samantha Dooyema, Maria Elias, and Sabine Fuhrmann

Ophthalmology and Visual Sciences, Vanderbilt University Medical Center, Nashville, TN, 37232; Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, 37232

Development of the retinal pigmented epithelium (RPE) occurs in a stepwise manner; it initiates with specification, followed by differentiation and then maturation. Our past work showed that Activin is sufficient to stimulate early RPE development in chick explants. Here we investigated the requirement of the Activin pathway components Activin receptor type II and Smad2/3 during RPE specification and differentiation in chick and mouse. Chick optic vesicle explants and chick embryos were treated with soluble Activin type II receptors or control Activin type I receptors in culture media or by bead implantation and examined for mRNA expression of the RPE marker Mitf. Mouse embryos with simultaneous disruption of germline Smad3 and conditional Smad2 were generated using constitutively active Rx3-Cre and two tamoxifen-inducible Cre lines, activated between E6.5 and E8.75. Double mutants were analyzed by immunolabeling at E8.75, E9.5, E10.5, E11.5, E12.5 and E17.5. Soluble Activin type II receptors prevented Mitf expression in 65% of the explants (n=18) and caused partial downregulation of Mitf in 38% of bead-implanted chick embryos (n=13). Smad2/3 disruption at E8.75 by Rx3-Cre showed loss of phospho-(p)Smad2/3 expression first in the ventral optic vesicle at E9.5 (n=3), and then in most of the optic cup including the RPE at E12.5 (n=3), without obvious changes in RPE and retina morphology. At E17.5, loss of pSmad2/3 expression was widespread, while Pax6 and Pou4f2 were normally present (n=3). Upon tamoxifen treatment at E6.5, loss of total Smad2 was evident at E11.5 (n=2), and Mitf was normally expressed. Results from chick explants and embryos demonstrate a role for the Activin signaling pathway in chick RPE development. In mice, we observe that it takes approximately 3 days for pSmad2/3 to be downregulated in the RPE layer. However, no other changes in protein expression or RPE and retina morphology were observed. This suggests that the timing of Smad2/3 expression may need to be further refined or that there are other factors involved in RPE specification.

Session 1 P32

Sublimated/Annealed Aminated Cinnamic Acid Analogues for High Sensitivity 3 µm Spatial Resolution MALDI IMS of Lipids in Human Kidneys

Martin Dufresne[1], Lukasz G Migas[2], Katerina Djambazova[1], Richard M Caprioli[1], Raf Van De Plas[2], and Jeffrey M Spraggins[1]

[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; [2]Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands

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 target selected molecular classes through a wide variety of sample preparations. Recent advancements in instrumentation led to the acquisition of sub 10 µm MALDI IMS datasets being more common. While these have resulted in high-quality IMS images, smaller pixel sizes can lead to a decrease in sensitivity. Here we propose the use of sublimated aminated cinnamic acid analogues (ACAA) followed by matrix annealing for up to 3 µm spatial resolution dual polarity MALDI IMS of lipids with high sensitivity. ACAA compounds have been shown to be great candidates to replace common MALDI matrices for dual polarity MALDI IMS of lipids due to their reduced laser power requirement, their low toxicity, high sensitivity, and their near-perfect vacuum stability. This gives ACAA compounds an edge for both high spatial resolution MALDI IMS and high specificity imaging experiments (e.g., ion mobility or FTMS) that require longer acquisition times, often at elevated source temperatures (150ºC) that can lead to loss of signal for commonly used matrices that degrade or sublime from the tissue surface over time. A new sublimation protocol for high spatial resolution MALDI IMS was developed by combining an ultra-thin amorphous matrix layer followed by an annealing step to induce crystallization increasing signal by 3-4 fold without inducing detectable analyte delocalization. Previously, we demonstrated the use of 4-(dimethylamino)cinnamic acid for dual polarity 5 µm spatial resolution phospholipid MALDI IMS of human kidney and eye. In the case of the human kidney, we were able to annotate through exact mass ~120 lipids in positive mode and ~175 lipids in negative mode. From this point, we have synthesized a 2nd generation of ACAA compounds to expand both the molecular coverage and sensitivity by tailoring their absorption band to the wavelength of our MALDI laser (355 nm). This fine-tuning of the absorption band through the addition of selected functional groups allows an additional reduction in laser power leading to a further reduction of the spot size while increasing affinity for lipid MALDI IMS. This 2nd generation of ACAA compounds was applied to human kidney sample from the HuBMAP consortium at 10, 5, and 3 um spatial resolution using MALDI IMS.

Session 2 P3

Understanding how Sphingolipid Metabolism Regulates Mitochondrial Maintenance in Aging Cells

Adam Ebert, Nathaniel Hepowit, and Jason MacGurn

Vanderbilt University Nashville TN 37232

Aging is the prime risk factor for maladies responsible for most human death including diabetes, cardiovascular disorders, neurological dysfunctions, and cancers. One of the pillars of aging is mitochondrial function, which declines as organisms and cells age. This decline in mitochondrial function correlates with changes in morphology. In healthy cells and tissues, functional mitochondria exhibit a tubular morphology undergoes dynamic fission and fusion. In conditions of stress, mitochondria tend to fragment and become swollen. For example, mitochondrial swelling is reported in human cardiomyocytes following ischemic injury, in motor neurons of aged rats, and in isolated mitochondria of aged Caenorhabditis elegans (C. elegans). Despite reports of stress-associated swelling of mitochondria in multiple model organisms the underlying mechanisms are not known. Accumulating evidence in multiple aging models reveals that depletion of sphingolipids confers longevity and benefits health span, although the cellular pathways that mediate these benefits are not well-defined. I am interested to understand the mechanisms linking SL metabolism and aging. Recently, I found that SL depletion prevents mitochondrial swelling and preserves mitochondrial function in aging yeast.

Session 1 P33

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 both the histone demethylase LSD1 and the epigenetic reader BPTF have emerged as compelling MYC recruiters. If WDR5, LSD1, and BPTF 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 act to dictate target gene selection by MYC proteins. Using the "degron tag" (dTAG) system, I will acutely deplete WDR5, LSD1, and BPTF 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 1 P34

Mapping the Cellular Landscape of Cortical Development in Tuberous Sclerosis Complex

Nada A Elsayed, Mary-Bronwen S Chalkley, Justine Sinnaeve, Rebecca A Ihrie, and wKevin C Ess

Medical Scientist Training Program, Vanderbilt University School of Medicine, Nashville, TN, 37232

Tuberous Sclerosis Complex (TSC) is a genetic neurodevelopmental disorder caused by mutations in the TSC1/TSC2 genes, resulting in upregulation of mammalian target of rapamycin (mTOR) signaling and unregulated cell growth. Within the brain, dysplastic cortical lesions called tubers emerge during fetal development. Although tuber growth appears to stop postnatally, repercussions do not. TSC patients suffer debilitating neurological sequelae from these tubers, including epilepsy, autism, and neuropsychiatric disorders. The molecular mechanisms underlying tuber development remain unclear. In this study, we sought to understand changes in cellular identity during cortical development of TSC2 mutant cells and their association with tuber formation. Using CRISPR-Cas9 technology, we generated isogenic lines of TSC patient-derived induced pluripotent stem cells (iPSCs) with pathogenic mutations in the TSC2 gene. iPSCs were differentiated into neural lineages and collected at several timepoints for analysis. Immunoblots revealed an inverse relationship between TSC2 (tuberin) and EGFR expression throughout neural differentiation in TSC2 mutant neurons. Mass cytometry analysis revealed unique populations in both early TSC2 mutant neural precursor cells and mature excitatory neurons with upregulated mTOR-specific and mTOR-independent markers. Comparatively, mass cytometry analysis of patient-resected tubers also showed distinct cellular identities with unique populations exhibiting differential expression of mTOR downstream targets and stem cell/astrocytic markers. Taken together, these results indicate that an interplay of EGFR and mTOR signaling with cell-specific changes during cortical development may play a role in tuber formation and these changes can be effectively modeled using human iPSCs.

Session 1 P35

Discovery of Glomerular Cell Type Lipidomic Profiles With High Spatial Resolution MALDI IMS and Multiplexed Immunofluorescence

Allison B. Esselman, Felipe A. Moser, Léonore Tideman, Lukasz G. Migas, Katerina V. Djambazova, Madeline E. Colley, Ellie L. Pingry, Nathan Heath Patterson, Melissa A. Farrow, Haichun Yang, Agnes B. Fogo, Mark deCaestecker, Raf Van de Plas, and Jeffrey M. Spraggins

Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN 37235, USA; Department of Chemistry, Vanderbilt University, Nashville, TN 37235, USA; Delft Center for Systems and Control, Delft University of Technology, 2628 Delft; The Netherlands; Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232, USA; Department of Biochemistry, Vanderbilt University, Nashville, TN 37205, USA; Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37205, USA; Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232, USA; Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Glomeruli are spherical structures in the kidney that filter blood with unique cell types including podocytes, mesangial cells, and fenestrated endothelial cells. Kidney diseases can alter the cellular composition of glomeruli. For example, podocyte loss and thickening of the basement membrane occur in glomeruli impacted by diabetic kidney disease. Insight into the degree of molecular variation among healthy humans and during aging can lead to a better understanding of cellular changes in diseased states. One technology that is suited to uncover the distribution of biomolecules in tissue sections is matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS); however additional imaging modalities must be combined with MALDI IMS to associate these biomolecular distributions to specific cell types. To investigate molecular changes of glomeruli in the human kidney on a cellular level, spatially targeted MALDI IMS at 5 μm spatial resolution was used to map the lipid distributions. Multiplexed immunofluorescence (MxIF) using antibodies that stain for glomerular cell types was performed on the same tissue sections after MALDI IMS to connect inter-glomerular lipid heterogeneity to specific cell types. Segmentations of glomerular cell types were uncovered with k-means clustering, and these segmentations were then used to train a classification model. Shapley additive explanations was used to calculate the most important ions in each cluster for the model to make its predictive decisions. This allowed us to link distinct lipidomic profiles to glomerular cell types. Overall, the work presented herein established a workflow using high spatial resolution MALDI IMS and MxIF to map lipid heterogeneity among glomerular cell types.

Session 1 P36

Identification of Sox17 Distal Regulatory Elements that Regulate Hepato-pancreato-biliary Development

Ryan R. Finnel, Anna B. Osipovich, Jessica Musselman, Leesa Sampson, Thomas Mehaffey, Linh T. Trinh, and Mark A. Magnuson

Vanderbilt University, Nashville, TN, 37232

Sry-box 17 (Sox17) is a master transcription factor that performs many undefined functions within the extra-embryonic and definitive endoderm, as well as hemogenic endothelium. Understanding the function and complex regulation of Sox17 across various lineages requires the identification and characterization of specific distal regulatory elements (DREs) that enable precise spatial and temporal regulation of Sox17 during development. DREs can be located over one million base pairs (bps) away from their target gene, therefore identification of such elements is challenging. Topological associating domains (TADs) are chromatin regions whose boundaries are denoted by the binding of CTCF. TADs bring linearly distant DNA sequences into close spatial proximity to promote interactions between widely separated cis-regulatory elements while limiting interactions with genes outside the TAD. Therefore, we sought to identify the topological associating domain (TAD) for Sox17 since it would constrain our search for such DREs. Using publicly available ChIP-seq for CTCF, we performed a meta-analysis that suggests a Sox17-specific TAD of ~351 kbps of DNA. The two CTCF binding sites that serve as boundaries for the putative Sox17 TAD are highly conserved and present in mESCs and organs derived from all three germ layers. Next, using gene conservation tracks displayed by the UCSC genome browser, we examined the putative Sox17 TAD for distinct conserved regions. Additionally, we surveyed ATAC-seq and ChIP-Seq data for permissive epigenetic histone modifications. This data suggests that up to 39 different evolutionarily conserved regions lie within the Sox17 TAD and are differentially permissive between lineages, suggesting they are DREs that regulate the developmental and/or cell-specific expression of Sox17. Given our interest in definitive endoderm-derived hepato-pancreato-biliary development, we focused our efforts on two sites that display permissive endodermal chromatin and binding of FOXA2 and GATA4, two critically important endodermal transcription factors. These two sites, which we refer to as conserved region 3 (CR3) and 4 (CR4), are located 10 kbps and 231 kbps upstream of Sox17 and are 391 bps and 315 bps in length, respectively. To ascertain the function of CR3 and CR4 in endoderm development, we used CRISPR/Cas9 to delete these regions in mice. Our findings indicate that CR3 temporally reduces endodermal Sox17 expression as well as known pancreatic markers, suggesting CR3 is an endoderm-specific enhancer that regulates the temporal differentiation of pancreato-biliary progenitor cells. In contrast, the deletion of CR4 causes an increase endodermal Sox17 expression, suggesting that this region functions as a silencer.

Session 1 P37

Molecular Counting of Myosin Force Generators in Growing Filopodia

Gillian N. Fitz and Matthew J. Tyska

Vanderbilt University School of Medicine, Nashville, TN, 27232

Actin-based surface protrusions are defining features of animal cells that support a range of biological activities ranging from cell motility to mechanosensation to solute uptake. Long-standing models of protrusion growth suggest that actin filament assembly provides the primary mechanical force for "pushing" the plasma membrane outward. Expanding on these actin-centric models, our recent studies used the chemically inducible system to establish that plasma membrane-bound myosin motors, which are normally abundant in protrusions, can also power robust filopodial growth. How protrusion resident myosins coordinate with actin polymerization to drive elongation remains unclear, in part because the number of force generators and thus, the scale of their mechanical contribution remains undefined. To address this gap, we leveraged the SunTag system to count membrane-bound myosin motor domains in actively growing filopodia. Using this approach, we found that the number of myosins was log normally distributed with an average of 12.4 ± 2.2 myosin motors [GeoMean ± GeoSD]. Together with unitary force values derived from biophysical studies on single myosin molecules, and duty ratio estimates for the myosin motor used in these experiments, we calculate that a population of membrane-bound myosins can generate an average force of ~8.4 pN (calculated with an average duty ratio of 0.7) to elongate filopodia. Thus, myosins can generate a level of force that is comparable to actin (~10-30 filaments per core bundle x ~1 pN per filament = 10-30 pN), a point that may constrain future physical models of protrusion growth.

Intracellular Cell Tension Regulates Stem Cell Conversion to the Mesoderm Lineage Downstream of the Apoptotic Pathway

L. Fort, W. Wang, C. Reinhart-King, and I. G. Macara

Vanderbilt University, Cell and Developmental Biology department, Nashville, TN, 37240
Vanderbilt University, Department of Biomedical Engineering, Nashville, TN 37235

The heart is the first functional organ to form during morphogenesis and failure to specify cardiac progenitors can result in congenital heart defects, affecting ~1% of all US newborns. Activation of the WNT pathway in human induced pluripotent stem cells (hiPSCs) recapitulates the initial stages of cardiac lineage commitment, driving sequential expression of primitive streak, mesoderm, and cardiac mesoderm markers. In parallel, cells undergo an epithelial-to-mesenchymal transition (EMT), similar to gastrulation in vivo, during which drastic morphological changes occur, including increase of cell number in a constrained space, active contractile forces and tension-dependent apical constriction, together driving morphogenesis. The acto-myosin cytoskeleton, a highly dynamic network of proteins that maintain cell morphology, is a crucial interface to translate physical signals into internal biochemical responses. While the relations between EMT and lineage specification have been widely studied, our understanding of the connection between cell shape and lineage identity remains poor. Moreover, it remains unclear how, mechanistically, cells communicate and integrate the state of their environment to coordinate developmental responses at the tissue level. We hypothesize that mechanical forces, such as cell tension, regulate mesodermal identity in hiPSCs. Using Quantitative Polarization Microscopy and immunofluorescence, we show that intracellular tension increases during cell differentiation. Surprisingly, pharmaceutical and genetical inhibition of cell contractility increases mesoderm genes expression. On the other hand, overexpression of a constitutively active ROCK2 to increase tension totally blocks cardiac mesoderm identity. These cells also maintain their epithelial features, suggesting a blockage of the EMT, a crucial step during lineage specification. Probing for a mechanism, we investigated the effect of the apoptosis pathway. We previously published a crucial role for apoptosis during stem cell commitment to the mesoderm lineage. Surprisingly, while cells treated with apoptosis inhibitor fail to differentiate, adding ROCK inhibitor overcomes this blockage. This epistasis experiment suggests that contractility acts dominantly, downstream of the apoptotic pathway. Together, our data suggest that apoptosis and contractility work together in balancing mesodermal identity. We are currently investigating the molecular mechanisms linking these two pathways to the genetic reprogramming occurring during mesoderm differentiation.

Session 1 P39

Subcellular Microtubule Configuration is Associated with Insulin Secreting Adhesion Activity

Maggie Fye, Pi'ilani Noguchi, Guoqiang Gu, and Irina Kaverina

Vanderbilt University, Nashville, TN, 37232

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, a process dependent upon proteins common to the presynaptic active zone in neurons. Among these proteins are the scaffolding protein ELKS and the cortical MT-anchoring protein LL5β. However, the mechanisms underlying hot spot formation and the role that the cytoskeleton plays in their regulation remain unknown. We have defined these regions of directed secretion as "insulin secreting adhesions" (ISAs): mechanosensitive subcellular domains which we propose use cytoskeletal regulation to accomplish directed and clustered secretion. We further propose that there are dormant ISAs, which are capable of directed secretion but are not observed to secrete, and active ISAs, which are observed to secrete. Here we seek to determine if and how MTs influence ISAs to become active or dormant. Studies using TIRF microscopy of mouse islets expressing ELKS to identify ISAs and addition of the Zn2+-binding dye FluoZin-3 to identify secretion events indicate that not all ELKS nor LL5β patches secrete; these are active and dormant ISAs. These differences are further elucidated by imaging Halo-tubulin and GFP-ELKS together, revealing that the majority of active ISAs are adjacent to, but not overlapping, MTs. Finally, we propose the MT-binding protein CLASP2, which can change MT binding position from end to lattice depending on phosphorylation state, as a mechanism by which MTs exert influence over the ISA's secretory status. These results point to a role for MTs in influencing ISA activity status, and potentially for MT configuration to act as a mechanism by which ISA activity changes from dormant to active.

Session 1 P40

NCAM (Neural Cell Adhesion Molecule) Promotes Synaptic Remodeling in Developing GABAergic Neurons

Casey Gailey[1,2], Robert Held[4], Leah Flautt[1], Andrea Cuentas-Condori[1], John Tipps[1], Siqi Chen[1], Eleanor Rodgers[5], Seth R. Taylor[1], Engin Ozkan[6], and David M. Miller, III[1,2,3]

Department of Cell and Developmental Biology[1], Program in Developmental Biology[2], Program in Neuroscience[3], Vanderbilt University[4], Nashville, TN, USA. Saint Cecilia Academy[5], Nashville, TN, USA. Department of Biochemistry and Molecular Biology[6], University of Chicago, Chicago, IL, USA.

Neural circuits are actively restructured during development as synapses are dismantled in some locations and assembled in others. To investigate the underlying mechanism, we are exploiting the DD-type GABAergic motor neurons which undergo synaptic remodeling during early development in C. elegans. In newly hatched larva, DD presynaptic boutons are initially positioned on ventral muscles but are then relocated over a ~5 hr period to innervate dorsal muscles. This DD remodeling event can be readily observed with live cell markers for presynaptic proteins (e.g., GFP::RAB-3). The conserved homeodomain protein, IRX-1/Iroquois orchestrates DD remodeling. IRX-1 activates expression of the sodium epithelial channel (ENaC), UNC-8, to trigger a Ca2+-dependent endocytic mechanism that promotes presynaptic disassembly and recycling to dorsal synapses. Other downstream effectors are required, however, because UNC-8 removes a subset of presynaptic components whereas IRX-1 also acts in parallel to target the complete presynaptic apparatus. We used single cell RNA-Seq (scRNA-Seq) to profile D-class GABAergic neurons and identified additional transcripts upregulated during DD remodeling. A mutant of one of these genes, ncam-1, impairs both the removal of ventral GFP::RAB-3 and its reassembly at dorsal DD neurites. Strikingly, ncam-1 mutants enhance RAB-3 retention in an unc-8 mutant, suggesting that NCAM-1 functions in parallel to UNC-8. NCAM-1 is a conserved member of the Neural Cell Adhesion Molecule (NCAM) protein family. A biochemical screen detected strong interaction of NCAM-1 with RIG-3, a DIP family Ig-domain cell adhesion protein. A rig-3 mutant impairs delays relocation of GFP::RAB-3, in DD neurons suggesting that a NCAM-1-RIG-3 complex may mediate synaptic remodeling. Because NCAM can function as a key regulator of synaptic plasticity in mammalian neurons, we suggest that NCAM-1 may drive synaptic remodeling in C. elegans in a conserved mechanism that also governs circuit refinement in the developing brain. NIH Funding: T32HD007502 (CG), F31NS134292 (CG), R01NS10695 (DMM).

Session 1 P41

SOX9 is essential for metaplastic progression during gastric carcinogenesis

Alexis Guenther and Eunyoung Choi

Vanderbilt University, Nashville, TN, 37240 Vanderbilt University Medical Center, Nashville, TN, 37240

Gastric cancer develops through a series of glandular precancerous lesions in the stomach mucosa marked by the presence of spasmolytic polypeptide-expressing metaplastic (SPEM) cells. SPEM cells arise during infection or injury via transdifferentiation from gastric chief cells, a normal, enzyme-secreting cell type in the gastric epithelium. While it is currently unclear what role SPEM cells play in the progression of gastric carcinogenesis, we hypothesize that they promote the succession of precancerous stages towards adenocarcinoma. We observed high expression of the transcription factor SOX9 in SPEM cell nuclei in the precancerous stages of both human and murine stomach tissue, leading me to further hypothesize that SOX9 acts to control chief cell transdifferentiation, and progression of gastric carcinogenesis. To investigate this question, I developed a mouse model with Sox9 knock-out to determine if loss of Sox9 effects carcinogenesis. I found that, upon SOX9 loss, chief cell transdifferentiation following acute injury is reduced and that carcinogenesis following oncogene activation is severely disrupted. Following oncogene activation, early-stage time points show the number of glands containing metaplasia are reduced by nearly half in the Sox9 knock-out, and at later stage time points around 80% of the glands show a phenotype similar to wild-type. 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. Currently, I am investigating the role of SOX9 in microenvironment recruitment during carcinogenesis, as well as the fate of oncogene-induced SOX9 knock-out chief cells. I hypothesize that SOX9 loss reduces microenvironment recruitment, and that chief cells with SOX9 loss and oncogene induction die before becoming fully SPEM.

Session 1 P42

Regulation of HB-EGF in Polarized Epithelial Cells

Sarah Harmych, Claiborne Tydings, Neeraj Joshi, James Higgenbottom, Jens Meiler, and Bhuminder Singh

Vanderbilt University, Nashville, Tennessee; 37203

The development of cancer is facilitated by the dysregulation of proteins, leading to the development of key features of cancer such as hyperproliferation and loss of cell polarity. One-way proteins become dysregulated in cancer is through altered subcellular localization. Polarized epithelial cells, from which more than 90% of cancers arise, maintain separate apical and basolateral membranes with distinct lipid and protein composition. Delivery to the proper membrane in polarized epithelial cells is essential for the proper maturation, and function, of proteins. Epidermal growth factor receptor (EGFR) and its ligands have been shown to induce increased cell proliferation and loss of cell polarity when their basolateral localization is altered in polarized epithelial cells. Preferential trafficking of proteins relies on specific post-translational modifications and sequences within the cargo protein and adaptor proteins, which interact with these modification and sequences to facilitate the movement of the cargo protein within the cell. While the trafficking of some EGFR ligands has been studied this process has not been investigated for one EGFR ligand, heparin-binding EGF-like ligand (HB-EGF). HB-EGF has been shown to be a potent driver of tumor growth and angiogenesis and is overexpressed in a variety of cancers. Despite the evidence for HB-EGF being a potential driver of tumor development, studies have not been done to understand the trafficking of this protein in polarized epithelial cells, a critical upstream regulatory mechanism. I hypothesize that altered localization of HB-EGF leads to disruption of epithelial polarity and aberrant cellular transformation. To address this hypothesis, I will pursue two different aims. First, I will identify the sorting motif directing the basolateral localization of HB-EGF in polarized epithelial cells and investigate the impact of localization on the function of HB-EGF Second, I will identify the proteins interacting with the cytoplasmic domain of HB-EGF and characterize their role in HB-EGF localization and function. Completion of these studies will elucidate a new upstream regulatory mechanism for HB-EGF and provide critical insight into its role in the

Session 1 P43

The Role of Tenascin-C in the Wnt Signalosome

Heather Hartmann, Matthew Loberg, Courtney Phifer, Ethan Lee, and Vivian Weiss

Vanderbilt University Medical Center, Nashville, TN, 37232

Tenascin-C (TNC) is a hexametric, multimodular extracellular matrix protein that is highly expressed during embryonic development but is normally silenced in adults. In pathological conditions, however, TNC is strongly re-expressed in adults during tissue injury, wound healing, inflammation, and tumor growth. Preliminary studies from our lab indicate that Wnt ligands play an important role in the genesis and progression of anaplastic thyroid cancer (ATC), a highly lethal cancer with an abysmal ~3-5 month median survival. Using a thyroid cancer Wnt reporter line (TOPFlash K1 Papillary Thyroid cancer cells) in co-culture with a fibroblast line (WPMY), we show that overexpression of Wnt-2 ligands by fibroblasts leads to upregulation of tumor cell Wnt reporter activity. Recently, we found a dramatic increase in TNC production along the invasive edge of ATC tumors a single cell-wide. Previous studies suggest that the expression of TNC enhances Wnt signaling and may play an important role in cancer cell proliferation and invasion. Thus, we hypothesize that TNC production stimulates/enhances/potentiates? WNT signaling along the tumor's leading edge to promote increased cellular invasion. We found that overexpression of TNC in a tumor cell line leads to a modest increase in Wnt reporter activity. However, co-culture of TNC-expressing tumor cells with Wnt-2-expressing fibroblasts resulted in a dramatic 6-fold increase in Wnt reporter activity. Understanding the role of TNC and its interaction with Wnt-2 could lead to the development of targeted therapeutics for this highly aggressive thyroid cancer. Additionally, understanding how TNC binds to the Wnt signalosome will fundamentally alter our understanding of the Wnt signaling pathway and have broad implications for many Wnt-reliant diseases.

Session 2 P4

Interrogating the mechanisms of mitochondrial cristae remodeling during neurogenesis

Caleb S. Hayes, Vincent Ling, Caroline Bodnya, & Vivian Gama

Vanderbilt University, Nashville, Tennessee 37203, USA

As neural stem cells differentiate into neural progenitor cells and eventually mature neurons, they undergo a metabolic shift from glycolysis towards oxidative phosphorylation (OXPHOS). This new metabolic context, along with myriad biochemical and signaling requirements, relies on dynamic changes to mitochondrial morphology. In addition to changes in overall morphology, the mitochondrial cristae, invaginations of the inner mitochondrial membrane, undergo remodeling that results in increased density to facilitate increased OXPHOS. The major regulator of cristae formation and maintenance is the mitochondrial contact site and cristae organizing system (MICOS) complex, comprised of the MIC60 and MIC10 subcomplexes which are bridged by MIC13. Unsurprisingly, when MICOS genes are experimentally knocked out, cristae architecture and mitochondrial function is severely impacted. Mutations identified within the genes encoding for MICOS proteins have been associated with devastating developmental and degenerative diseases. Previous work interrogating MICOS and its role in cristae architecture in a human context has been done using non-neuronal systems, despite many disease-associated mutations resulting in neurological phenotypes. My study aims to investigate the role of MICOS and cristae remodeling within neurogenesis by using the induced pluripotent stem cell (iPSC) model, which can be readily differentiated into neural progenitor cells and mature neurons. Within this model, key MICOS genes encoding for MIC60 and MIC13 will be knocked out or mutagenized to ablate or modulate cristae architecture. This powerful cell model coupled with advanced microscopy techniques to visualize cristae morphology in real-time will grant novel insight into how cristae remodeling impacts neural cell identity throughout neurogenesis. This work aims to show how critical cristae morphology is for neurogenesis while also elucidating how increased metabolism signals for such complex morphological and function mitochondrial changes.

Session 2 P5

Nutrient-Induced Autophagy and its Regulation in Yeast

Nathaniel L. Hepowit, Alexa N. DelBene, and Jason A. MacGurn

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

Caloric restriction reproducibly promotes healthy lifespan in various model organisms by stimulating autophagy. However, its applicability to humans remains highly challenging as reduction of food consumption or complete dietary abstinence is a severe intervention that results in both beneficial and detrimental effects. Although amino acids are known inhibitors of autophagy, here we find that excess amounts of Ile, Phe or Met reversibly stimulate lysosomal degradation of organelles and clearance of disease-implicated protein aggregates in yeast. Supplementation with Arg suppresses the autophagy-inducing effect of Ile and Met, but not Phe, which is indicative of a divergent nutrient sensing mechanism. Met-induced vacuolar sequestration and degradation of mitochondria requires Dnm1 and the Atg11/Atg32 complex, which therefore indicates that mitochondrial fission and selective mito-tethering precedes mitophagy. Absence of the highly conserved Gcn2/Gcn4 completely abrogates the mitophagy-inducing effects of excess Met, which suggests signaling mimicry activating the unfolded protein response during amino acid starvation but in an unstarved culture condition. In addition, mitophagy suppression by deletion of arrestin-like Art1, deubiquitylation of autophagy receptor Ede1, and inhibition of K63-linked ubiquitin polymerization collectively indicate ubiquitin-dependent mechanisms modulating Met-induced mitophagy. Interestingly, the gcn2 knockout and Ede1-DUB fusion cells have abnormal swollen mitochondria, which are reminiscent of dysfunctional mitochondria in aging cells. Overall, our data in the yeast model system suggest that excess amino acid treatment points to new approaches in developing healthspan-promoting interventions that do not necessarily require dietary restriction.

Session 2 P6

After Tissue Injury, Plasma Membrane Damage Primes Epithelial Cells to Fuse, a Process that Requires High Tension

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

Vanderbilt University, Nashville, TN 37232

Cell-cell fusion is essential for tissue development, repair, and regeneration. While abnormal fusion during development causes diseases like cancer, osteopetrosis, and myopathies, after tissue injury, fusion between cells that do not typically fuse has been repeatedly shown to facilitate repair and regeneration. How is cell fusion regulated after damage to promote repair? Further, cell-cell fusion is energetically demanding, and thus developmentally regulated fusion requires pre-programmed machineries such as fusogenic proteins (fusogens). However, many cell types that undergo ectopic fusion after damage, such as epithelial cells, appear to lack such fusion machinery. How does injury allow non-fusogenic cells to overcome the energy barrier? My data show that plasma membrane damage is necessary to trigger fusion among epithelial cells after injury. Although membrane damage initiates cytoplasmic sharing spontaneously, it is not sufficient to drive complete cell-cell fusion, which requires fusion pores to form and expand between cells. These two steps of fusion are regulated after injury because fusion pores can not only form hours after the damaged plasma membrane is expected to be repaired, but also change their size dynamically. How are adjacent membranes brought into proximity against repulsive forces to form fusion pores after damage? Autophagy mediates wound-induced fusion according to my data and may facilitate the forming of fusion pore. What regulates the change in the size of fusion pores? I show that dynamin, a membrane remodeling GTPase, is essential for wound-induced fusion. Fluorescently tagged dynamin is recruited to fusion cell borders after injury, and the binding partner of dynamin, endophilin, also has mild regulatory effect over fusion. Inhibition of either dynamin or endophilin decreases post-wound cortical tension, which encompasses membrane and cytoskeletal tension. Published mathematical models demonstrate that high membrane tension is required for fusion pore expansion. Hence, we propose a novel framework of wound-induced cell fusion primed by plasma membrane damage and mediated by dynamin together with endophilin that functions to maintain high tension.

Temporal Recording of Mammalian Development and Precancer

Mirazul Islam, Yilin Yang, Alan J. Simmons, 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

Key to understanding many biological phenomena is knowing the temporal ordering of cellular events, which often require continuous direct observations [1, 2]. An alternative solution involves the utilization of irreversible genetic changes, such as naturally occurring mutations, to create indelible markers that enables retrospective temporal ordering [3-8]. Using NSC-seq, a newly designed and validated multi-purpose single-cell CRISPR platform, we developed a molecular clock approach to record the timing of cellular events and clonality in vivo, while incorporating assigned cell state and lineage information. Using this approach, we uncovered precise timing of tissue-specific cell expansion during murine embryonic development and identified new intestinal epithelial progenitor states by their unique genetic histories. NSC-seq analysis of murine adenomas and single-cell multi-omic profiling of human precancers as part of the Human Tumor Atlas Network (HTAN), including 116 scRNA-seq datasets and clonal analysis of 418 human polyps, demonstrated the occurrence of polyancestral initiation in 15-30% of colonic precancers, revealing their origins from multiple normal founders. Thus, our multimodal framework augments existing single-cell analyses and lays the foundation for in vivo multimodal recording, enabling the tracking of lineage and temporal events during development and tumorigenesis.

Session 2 P8

Evaluation of Impact of Palmitic Acid on Induction of RNA-enriched Small Extracellular Vesicles

Lizandra Jimenez, Bahnisikha Barman, and Alissa Weaver

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

Extracellular vesicle (EV)-cargoes, including RNAs and proteins, secreted from cancer cells regulate gene expression and phenotypic properties of recipient cells. A previous paper from our laboratory found that the endoplasmic reticulum (ER)-endosome membrane contact site (MCS) linker proteins VAP-A and ceramide transporter protein CERT are important for the biogenesis of RNA-containing EVs. However, the upstream regulatory process by which the RNA-enriched EVs are generated is unclear. Palmitic acid (PA) is a fatty acid that is upregulated in the circulation in metabolic dysfunction syndromes, including obesity, and involved in ceramide synthesis. We hypothesize that PA acts upstream of VAP-A and CERT to induce the formation of RNA-enriched EVs (EVs). Using colorectal cancer cells as a model system, we observed that PA treatment leads to a marked increase in the number of small EVs. PA treatment also increased the total RNA content and selected miRNA levels of CRC small EVs, without changing the levels in cells. Western blot analysis revealed that PA treatment alters the levels of some RNA-binding proteins and select EV markers in small EVs. These data suggest that PA boosts EV-RNA biogenesis. Future studies will determine the role of ceramide and CERT in PA-driven EV-RNA biogenesis.

VAP-A Regulates the Secretion of Small Extracellular Vesicles and Tumor Progression in Immunocompetent Models of Colorectal Cancer

Youn Jae Jung[1,2,3], Barman Bahnisikha[2,3], Cara Becas[2,3], Lauren Cocozza[2,3], John T. Wilson[1,3], and Alissa M. Weaver[2,3]

[1]Department of Chemical and Biomolecular Engineering, Vanderbilt University School of Engineering, Nashville, Tennessee.
[2]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee.
[3]Center for Extracellular Vesicle Research, Vanderbilt University School of Medicine, Nashville, Tennessee.

Extracellular vesicles (EVs) have emerged as a promising strategy to deliver effector molecules for intercellular signaling. EVs secreted from tumor cells can promote cancer progression and increase invasive and metastatic activity. Our laboratory recently identified a novel biogenesis mechanism for RNA-containing EVs, in which ribonucleoprotein complexes are sorted into endoplasmic reticulum membrane contact sites (ER MCS) dependent on the ER MCS linker protein VAP-A.

We previously showed that knockdown (KD) of the VAP-A decreased EV secretion from human colorectal cancer cell lines and inhibited tumor progression in immunocompromised mouse models (Barman B. et al., Dev Cell. (2022). We hypothesized that KD of the VAP-A could decrease EV secretion in murine tumor cell line MC38 and limit its tumor progression. Here we purify EVs from MC38 cells cultured in 2% EV-depleted FBS in DMEM by cushion density ultracentrifugation and characterize them by nanoparticle tracking analysis and Western blots. We show that VAP-A KD significantly decreased small EV secretion and sorting RNA into EVs, even though VAP-A KD doesn't have the effect of in vitro MC38 cell growth rate. We further show that the VAP-A KD in MC38 cells suppressed tumor growth in C57BL/6 mice, and EV injection intratumorally rescued tumor growth rates. VAP-A KD promotes the migration of T cells into tumors while inhibiting the recruitment of macrophages and endothelial cells into the tumor microenvironment. The majority of EVs from MC38 cells exhibit uptake by macrophages in the tumor microenvironment, followed by dendritic cells, granulocytes, T cells and B cells. These findings suggest that VAP-A regulate small EV secretion and tumor growth *in vivo* by remodeling the tumor microenvironment.

Session 2 P10

Predicting Histo-pathology Annotations from Gene Expression in Spot-based Transcriptomics Data

Harsimran Kaur, Jeremy Philip D'Silva, Aaron Matthew Newman, and Ken S. Lau

Chemical Physical Biology Program, Vanderbilt University, Nashville, TN, 37235 Cancer Biology, Stanford University, CA, 93405 Department of Biomedical Data Science, Institute for Stem Cell Biology and Regenerative Medicine, Stanford Cancer Institute, Stanford University, CA, 93405 Cell and Development Biology, Epithelial Biology Center, Vanderbilt University, Nashville, TN, 37235

Colorectal cancer (CRC) is an ailment of the colon and rectum which originates in benign overgrown structures called polyps. As the disease progresses, distinct morphological changes occur in the colonic mucosa, which are commonly used for clinical diagnosis. While the molecular pathways associated with CRC progression are well known, the specific cellular niches underlying cancer associated patho-morphologies are still not well understood. With the advent of spatially resolved assays such as spatial transcriptomics, opportunities to investigate spatial biology of tumors has emerged which has created avenues to elucidate the changes in tumor microenvironment related to these morphologies. In this study, we utilized deep learned techniques to explore the relationship between morphological changes and gene expression. We analyzed a meticulously annotated visium spatial transcriptomics dataset and trained a Graph-Neural Network using this data to predict spot-based pathology annotations based on gene expression profiles in each spot. Subsequently, we employed this trained model to predict spot-based pathology annotations in an unlabeled dataset. Finally, we conducted post-hoc analysis to discern the molecular features associated with various CRC histology annotations.

Session 2 P11

A Systems Biology Approach to Analyzing Trans-endocytosis

Adeel Koshal, Christian de Caestecker, and Ian Macara

Dept of Cell & Developmental Biology Vanderbilt University School of Medicine Nashville TN 37240

There are multiple ways by which cells can share materials and information, including through transfer of vesicles and ligands. One understudied process is transendocytosis, in which membrane proteins on a sender cell are transferred directly to a receiver cell and internalized by endocytosis. Examples include transfer of Ephrins to cells that express Eph receptors, transfer of nectin-4 by cells expressing nectin-1, transfer of claudins, and of CD47 into SHPS-1 expressing cells and of CD80 and CD86 by CTLA-4. Some of these transfers involve clathrin mediated endocytosis by the receiver cells but others do not; the kinetics of transfer has not been investigated, the structure of the internalized endocytic vesicle is for the most part unclear, and the ultimate fate of internalized proteins from the sender cells remains largely unknown. To provide a quantitative and manipulable approach to the analysis of transendocytosis, we have created artificial sender and receiver proteins that can be easily modified and are subject to temporal control. The sender protein is sequestered in the endoplasmic reticulum through the Retention Using Selective Hooks (RUSH) system, until synchronous release by biotin addition initiates trafficking to the plasma membrane, when transendocytosis to adjacent receiver cells can then begin. We show that transfer occurs over a period of 3 hrs after the sender protein arrives at the plasma membrane and, remarkably, that almost 50% of the total sender protein is transferred to the receiver. The transendocytosis process is predominantly clathrin-mediated. Co-labeling of sender cells with an mCherry-CAAX construct demonstrated that plasma membrane from the sender cell is transferred to the receiver together with sender protein. Attachment of SNAPtags to the C-terminus of the sender protein, using a chemically inducible dimerization system, slowed delivery to the plasma membrane but did not reduce accumulation in the receiver cell. Overall, this new approach provides an adaptable, quantitative, time-resolved approach to investigating the molecular mechanism of transendocytosis.

Session 2 P12

Fighting the Fire: HIKESHI-associated Leukodystrophy

Sudiksha Rathan Kumar, Grant Westlake, and Kevin C Ess

Vanderbilt University, Nashville, TN- 37235

HIKESHI-associated leukodystrophy (HAL) is a lethal genetic condition with most patients dying in childhood. Patients display white matter hypomyelination, resulting in developmental delay, microcephaly, paraparesis, and high rates of fatality following febrile illness and infections. The disease is caused by homozygous HIKESHI missense mutations, resulting in loss of protein. HIKESHI is an evolutionary conserved and ubiquitous protein whose primary role appears to be during heat shock responses. When cells are exposed to physiological stressors such as heat or toxins, proteins misfold and aggregate resulting in cellular dysfunction and apoptosis. To prevent this, cells initiate a heat shock response where chaperone proteins refold damaged proteins and maintain homeostasis. The HIKESHI protein serves as an import carrier to the chaperone protein, HSP70, shuttling it into the nucleus as part of the heat shock response. As the primary symptoms are seen in the white matter, we hypothesize that lack of HIKESHI impairs heat shock response resulting in abnormal oligodendrocyte development and function. Samples were collected from an affected homozygous patient, their non-symptomatic heterozygous parents, and healthy individuals and fibroblasts were generated. The fibroblasts were reprogrammed into iPSCs which were differentiated into oligodendrocytes and neurons. The cells were exposed to physiological stressors. Immunofluorescent staining and western blots for various protein markers were conducted. The cells were also allowed to myelinate nanofiber plates to measure myelination at baseline as well as after exposure to physiological stressors. Cells were further treated with proteosome inhibitors to attempt restoration of HIKESHI protein and cellular function. Cell analysis indicates significant functional differences in homozygous cells compared to heterozygous and control cells. Immunofluorescent staining indicated that compared to control and heterozygous phenotypes, Hsp70 and other heat-shock proteins displayed altered localization in the homozygous phenotype. Homozygous oligodendrocytes are enlarged and display aberrant myelination. We expect these changes to be further exacerbated by exposure to physiological stressors. Proteosome inhibitor treatment restores some HIKESHI protein with functional assays being conducted. Thus, loss of HIKESHI results in heat shock mechanism changes and potential oligodendrocyte changes. Due to the immediate patient need, further understanding of this disease and mechanism would allow us to develop therapeutics to improve the quality of our patients' lives.

Session 2 P13

The Requirements of the Homeodomain Transcription Factor Vsx2 in Regulating Early Retinal Development Change Rapidly

Xiaodong Li, Francesca Napoli, Alan Hurtado, Edward Levine

Department of Ophthalmology and Visual Science
Program in Chemical and Physical Biology
Department of Cell and Developmental Biology

The multipotent progenitor cells of the early retina must progressively proliferate and make cell fate decisions through temporal intra- and extracellular cues. Vsx2 is a central orchestrator of retinal development that functions to preserve retinal identity, promote proliferation, influence neurogenesis, and later induce bipolar interneuron cell fate. While there is evidence that the role of Vsx2 in bipolar cell fate specification is distinct from its other functions, it remains unclear whether the roles of Vsx2 in regulating retinal identity, proliferation, and neurogenesis are mechanistically distinct or change over time. To address this, we created a tamoxifen-inducible Cre conditional knock-out to inactivate Vsx2 expression at crucial neurodevelopmental timepoints. We hypothesized the roles of Vsx2 in lineage fidelity and neurogenesis initiation to be temporally succinct, while its role in proliferation spans development. Vsx2 inactivation immediately following retinal domain specification emulates the germline mutant, resulting in a microphthalmic eye with centrally restricted proliferation and neurogenesis. When inactivated during neurogenesis initiation at E11.5, the extent of proliferation and neurogenesis are comparable to the heterozygous control. Surprisingly, Vsx2 inactivation during neurogenesis at E14.5 resulted in the overproduction of photoreceptors to the apparent detriment of proliferating RPCs. While quantification and cell cycle assays are necessary to articulately describe the resultant phenotypes, our current findings suggest Vsx2 to have a continued, active role in neurogenesis. Lastly, efforts are underway to determine if Vsx2's role in suppressing non-retinal gene expression is temporally restricted.

Session 2 P14

Design and Application of Genetically Encoded EM-visible V-tag for cryoET

Feng Luo and Qiangjun Zhou

Department of Cell and Developmental Biology, Vanderbilt Brain Institute, Center for Structural Biology, Vanderbilt University, Nashville, TN, 37240, USA

Cryo-Electron tomography (cryoET) has revolutionized our understanding of biological function by revealing the native molecular details of cells, membranes, and organelles. Despite the advances, the exact localization of biomolecules of interest within the tomographic volumes remains a formidable challenge due to the crowding biological sample environment and low signal-to-noise ratios. To address this challenge, we are developing several novel tags with distinctive shapes and low molecular weights. Among these, the V-shapped tag, validated through high-precision structural prediction software and protein purification techniques, enables us to visualize the proteins or organelles we've labeled within the cellular context.

Session 2 P15

Exosomes in Filopodia Formation

Caitlin McAtee (1), Daisuke Hoshino (2), Nan Hyung Hong (3), Bong Hwan Sung (1), Anthony Maldonado (1), Ariana Von Lersner (4), Evan Krystofiak (1), Andries Zijlstra (5), and Alissa M. Weaver (1,4)

(1) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37232; (2) Cancer Cell Biology Division, Kanagawa Cancer Center Research Institute, Japan; (3) ESSA Pharma, San Francisco, CA, 94080; (4) Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, 37240; (5) Genentech, San Francisco, CA, 94080

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

Session 2 P16

Bioengineering Nano-Ferritin Proteins as Cryo-ET Tags: Controlling Protein Association for Enhanced Visualization

Robert McFadden, Oliver Chalkley, and Qiangjun Zhou

Vanderbilt University, Nashville, TN 37235

Cryo-electron tomography (cryo-ET) is a rapidly developing technology that enables nanometer-resolution three-dimensional imaging of biological materials in their near-native state. However, despite the many benefits of the cryo-ET imaging technique, an effective tag has not yet been developed to identify proteins accurately in cryo-ET images. Graduate student Oliver Chalkley has investigated the utility of a bioengineered nano-ferritin protein as a proposed novel cryo-ET tag. The nano-ferritin protein oxidizes iron and sequesters it into an electron-dense iron granule within a heteropolymer cage. When a subunit of the nano-ferritin cage is fused to a protein of interest, the iron-rich cage serves as a tag that aids in cryo-ET visualization of the protein of interest by producing a black circle easily identified on a tomogram. Exactly one nano-ferritin subunit (NFS) fused to a protein of interest must be included in each nano-ferritin heteropolymer cage. In other words, only a single protein of interest should be associated with each tag. Thus, NFSs fused to a protein of interest should not form a cage with themselves but only with NFSs. We have successfully achieved this goal by fusing the protein of interest (in this case, GFP) to the N-terminal of the NSF (GFP-NFS) using linkers of varying lengths. By employing size-exclusion chromatography and single particle cryo-EM, we have shown that altering the length of the linker region significantly impacts the rate of cage formation. Removing one or two amino acids from the disordered region between GFP and the nano-ferritin subunit substantially diminishes the ability of GFP-NFSs to form cages with each other. While the GFP-NFS is still able to form cages with NFSs. This approach effectively regulates the number of proteins of interest incorporated into each tag.

Session 2 P17

Apical Intercalation of Mammary Epithelial Cells

Mikiyas Daniel and Ian Macara

Vanderbilt University , 2201 West End Ave, Nashville, TN 37235

Cell intercalation is a fundamental process that plays important roles in embryonic development, wound healing, and tissue morphogenesis. Apical intercalation involves the insertion of cells into a confluent epithelial monolayer and involves a complicated series of processes including re-organization of intercellular junctions and cell polarity. This study investigates regulatory mechanisms influencing intercalation within mammary epithelium. Previous studies found that knockout of a tight junction protein, ZO-1 blocks apical intercalation (Pfannenstein, Dev Cell 2023). Intercalation often occurs between cells, especially at tricellular junctions. Therefore, I am testing the role in this process of LSR (Angulin-1), a transmembrane protein required for normal tricellular tight junction formation. I successfully deleted the Angulin-1 gene in Eph4 mammary epithelial cells. We use an in vitro intercalation assay in which fluorescently tagged cells are plated onto the top of a confluent monolayer and the progress of intercalation is tracked over time. Preliminary observations did not detect any major effect of Angulin-1 KO on the efficiency of intercalation, but the reproducibility of the assay needs to be further improved. In a second project, I am exploring the role of actomyosin contractility in apical intercalation. The myosin II inhibitor blebbistatin blocks intercalation (Pfannenstein 2023) but this will impact all cells in the dish, so a genetic tool is required to dissect the requirement for actomyosin contractility in the monolayer versus intercalating cells. Dr. Loic Fort in the Macara lab created such a tool using a constitutively active fragment of MYPT1, a regulatory subunit of myosin phosphatase, which potently inhibits contractility by dephosphorylating myosin light chain (MLC). He created a Dox-inducible lentivirus for CA-MYPT1, and I have made a stable Eph4 cell line using this virus. With this system, my goal is to test if the induction of CA-MYPT1 blocks apical intercalation when expression is limited to the monolayer cells, or the incoming cells, or both.

Session 2 P18

EGFR Signaling Drives Transformed Cell Extrusion

Paola Molina amd Ian Macara

Dept of Cell and Developmental Biology, Vanderbilt University School of Medicine
Nashville TN 37240

Maintaining homeostasis during development and repair requires precise, organized, and well-regulated signaling. The loss of tissue homeostasis is a hallmark of cancer. Hence, cells have developed various mechanisms to maintain homeostasis and prevent cancer. Carcinomas are epithelial in origin and account for as many as 90% of all human cancers. They arise from a single mutant cell. During early stages of cancer oncogenic cells are sparse in tissues. In epithelial monolayers, transformed cells that are surrounded by WT cells are apically extruded. Apical extrusion of these cells may be a mechanism to maintain homeostasis. Previous work has shown that this process is not cell autonomous and depends on the presence of WT and transformed cells. Differences in protein localization/expression, metabolism and actin rearrangement have been observed in WT cells that surround Ras cells and Ras cells surrounded by WT cells. To further understand the mechanism of extrusion we use a Dox-inducible, constitutively active H-RasQ61L expressed in mammary epithelial cells. In our model, within 15hrs of Ras induction, Ras cells are preferentially extruded from a WT cell monolayer when mixed 1:50. Initial experiments using a MEK inhibitor confirmed previous results that the ERK pathway is required for cell extrusion. Strikingly, EGFR inhibitor Erlotinib (Erlot) also efficiently blocked cell extrusion. However, Erlot did not reduce the level of p-ERK in cultures of cells expressing mutant Ras. Likewise, p-ERK levels in WT cell cultures (in the presence of serum) were not affected by Erlot treatment . These data show that EGFR inhibition of cell extrusion is not mediated by blocking ERK activation and that p-ERK is required but not sufficient to drive cell extrusion. To distinguish between EGFR activity in the Ras mutant cells vs WT cells we used CRISPR to knock out EGFR in each cell line. We found that EGFR knockout in Ras cells was sufficient to interrupt extrusion, while EGFR knockout in WT cells had no effect. These findings suggest that EGFR plays a role independent of ERK signaling to regulate extrusion. We are actively seeking the EGFR pathway that promotes Ras cell extrusion.

Session 2 P19

Enhancing Sensitivity and Specificity of Neutral Lipids via Sample Preparation Techniques and MALDI-2

Kameron R. Molloy, Madeline E. Colley, Martin Dufresne, Lukasz G. Migas, Raf Van de Plas, and Jeffrey M. Spraggins

Department of Chemistry, Vanderbilt University, Nashville, TN, 37235; Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, 37235; Department of Biochemistry, Vanderbilt University, Nashville, TN, 37235; Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands; Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, 37235

Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) is a powerful tool for mapping the spatial distribution of biomolecules in tissue. However, advancements are still needed to improve sensitivity and molecular coverage for molecular classes that are difficult to ionize by MALDI. Two strategies that have been shown to enhance the detection of neutral lipids in a sample are: 1) salt deposition onto the sample and 2) the integration of laser post-ionization (MALDI-2). Previous studies have reported improved sensitivity with both techniques for various classes of neutral lipids and carbohydrates. Here, we assessed the performance of MALDI-2 and salt doping on tissues such as human and mouse kidney and brain. A systematic comparison between MALDI and MALDI-2 on various organs revealed that MALDI-2 increased the sensitivity for a few classes of neutral lipids. The MALDI matrix DHA contributed most to the efficiency of the MALDI-2 process. Cholesterol, hexosylceramides, and di- and tri-acylglycerols were annotated more frequently with MALDI-2. M/z 425.377 and m/z 810.682, tentatively identified as a sterol (ST (30:3; O)) and a hexosylceramide (HexCer (42:2; O2)), respectively, are two examples of lipids only detected using MALDI-2. To maximize sensitivity and specificity, signal intensity and matrix performance were optimized for MALDI-2. In all cases, MALDI-2 performed best using few laser shots (~5 shots per pixel) and laser energy high enough to puncture the sample. This is atypical from traditional MALDI experiments that used lower laser energy with a greater number of laser shots (50-200 shots per pixel). However, more advancements are needed to further reduce the laser power required for MALDI-2 to be used in multimodal studies where maintaining the structural integrity of the sample is a necessity. A previously studied alternative to MALDI-2 is salt doping during sample preparation to charge carry neutral lipids as potassium and sodium cations. Findings in murine brain tissue suggest that the selectivity toward neutral lipids is similar to that of MALDI-2.

Session 2 P20

Acute Tuft Cell Ablation Induces Malabsorption and Alterations in Secretory and Immune Cell Lineages in a Mouse's Small Intestine

Michael Momoh, Cynthia Ramos, and Izumi Kaji

Vanderbilt University Medical Center, Nashville, TN, 37232

Intestinal tuft cells have been recently investigated thoroughly due to their roles in type 2 immunity and chemosensing. They have also been implicated in extensively interacting with other cell types to maintain intestinal immunity. However, the effects of tuft cell deletion on intestinal physiological functions have not been fully understood. In the present study, we investigated the impact of acute tuft cell loss on the absorptive process, secretory cell lineage differentiation, and immune cell population in the mouse intestine. Tuft cell deletion was induced in DCLK1-IRES-GFP-CreERT2/+;Rosa-DTA (DCLK1-DTA) mice by a single tamoxifen injection along with littermate controls. Intestinal tissues were sampled 2-, 4-, or 7-days post tamoxifen injection. DCLK1-DTA mice showed significantly shortened small intestinal length and crypt depth on day 4 compared to controls. Immunostaining of mouse tissues revealed a transient deletion of intestinal tuft cells, which was abolished on day 4 and recovered on day 7. Impaired SGLT1 and CFTR activities were observed in Üssing chamber experiments, although the brush border structure and transporter localizations were intact. Correlated with the tuft cell reduction, the frequency of mislocalized Paneth cells was increased in the middle through upper part of the villi. In the intestinal mucosa, fewer mast cells and leukocytes were found in the day 4-DCLK1-DTA mice than in control tissues. Ablation of intestinal tuft cells may cause nutrient malabsorption through alterations in epithelial cell proliferation and differentiation and changes in mucosal defense response. These observations elucidate their roles as regulators involved in intestinal absorption and mucosal regeneration processes.

Session 2 P21

Epigenetic Determination of Beta Cell Fitness

Simone Nevills, Guoqiang Gu, and Ken Lau

Vanderbilt University, Nashville, TN, 37212

Pancreatic beta cells are heterogeneous, but the origin and stability of this heterogeneity remains poorly characterized. It is also known that intrauterine nutrient levels impact the risk of diabetes in offspring, but causative mechanisms are largely unclear as well. We have identified pancreatic endocrine cell subtypes defined by differential methylation signatures that give rise to differentially-fit beta cells. I hypothesize that maternal malnutrition may disrupt epigenetic programming in the developing embryo, resulting in insufficient functional beta cell mass postnatally. Our findings suggest that the beta cells of mice exposed to maternal high-fat diet during gestation demonstrate altered expression of genes relevant to beta cell stress and survival. Ongoing studies examine the role of DNA methylation specifically in regulating these differences.

Session 2 P22

Single-cell Gene Expression Profiling of a *C.elegans* Neuronal Lineage

Claire Olson[1], Rebecca McWhirter[1], Seth Taylor[1,2], and David Miller[1]

[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37212; [2]Department of Cell Biology and Physiology, Brigham Young University, Provo, UT, 84602, USA

In a striking feature of development, individual progenitor cells undergo multiple rounds of division to generate multiple types of cells. With its well-defined cell lineage, C. elegans is particularly useful for investigating the dynamic changes in gene expression that drive cellular differentiation. During larval development, 13 progenitor or "P-cells" undergo stereotypical rounds of division to produce specific classes of cholinergic (VA, VB, VC, AS) and GABAergic (VD) motor neurons. To profile gene expression across the P-cell lineage, we generated single cell RNA-Seq (scRNA-Seq) profiles of P-lineage cells isolated by FACS from three successive developmental windows. We report transcriptomes of >30,000 differentiated neurons and their progenitors, with an average sequencing depth of 3000 UMIs and 1000 genes per cell. Each of nine predicted neuronal progenitor types and six neuron classes are represented with a minimum of five hundred cells per cell type. Notably, the distribution of clusters in UMAP space matches the branching patterns of known cell divisions thereby revealing graded changes in gene expression that correlate with the cell lineage and terminal differentiation. We detected many instances of differential gene expression between progenitor classes, notably in functional categories such as transcription factors, providing candidate genes that might regulate cell fate decisions. Ultimately, this dataset provides a powerful resource for elucidating genetic mechanisms that drive differentiation of neuron types throughout development.

Session 2 P23

NCAM-1 Promotes Remodeling of the Active Zone Protein CLA-1

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

Cayetano Heredia University[1], Lima, Peru. Department of Cell and Developmental Biology[2], Program in Developmental Biology[3], Program in Neuroscience4, Vanderbilt University, Nashville, TN, USA. Saint Cecilia Academy5, Nashville, TN, USA

Synaptic remodeling (SR) in the human brain is crucial for development and learning. This process is characterized by the dynamic modification of synaptic connections, including formationincluding the assembly of new synapses as others are removed, elimination, and. To investigate the mechanism of synaptic remodelingunderstand SR mechanisms, I study the DD-type GABAergic motor neurons of the animal modelin Caenorhabditis C. elegans. Initially, in the embryo, DD neurons innervate ventral body muscles. Later, during early larval development, ventral DD synapses are removed and reassembled on the dorsal side. We have previously shown that These neurons exhibit presynaptic domain removal during early larval development, driven by the homeodomain transcription factor IRX-1. IRX-1 controls the expression of UNC-8, the epithelial sodium channel, UNC-8, is upregulated in remodeling DD neurons to promote a Ca++-dependent mechanism for the removal and recycling of synaptic vesicle associated proteins (e.g., RAB-3). UNC-8 is not required, however, for the removal of the active zone protein CLA-1/Clarinet which suggests that another remodeling pathway functions in parallel to unc-8. which triggers an activity-dependent mechanism that facilitates the removal of presynaptic proteins like synaptic vesicles (e.g., RAB-3), but not the active zone protein CLA-1, which is important for the clustering and releasing of synaptic vesicles during the synapsis. Single cell RNA-Seq of remodeling DD neurons detected transient expression of The Nneural Ccell adhesion Adhesion Mmolecule (NCAM-1), in remodeling DD neurons. which is essential for synapse building, is upregulated during SR, and its relationship with UNC-8 is unknown. For experiments toTo determine if ncam-1 NCAM functions with or independently ofis required for the UNC-8 in promoting the removal of CLA-1, we used CRISPR to label endogenous CLA-1 with a C-terminal split-GFP tag. Imaging during the DD remodeling period detected a significant delay in the removal of ventral CLA-1::GFP and its assembly at nascent dorsal synapses. , Our findings are consistent with the hypothesis thata mutant worm strain was generated lacking NCAM, and they were analyzed during the mid/late remodeling (17 hours post hatch). Analysis showed that the deletion of NCAM impairs the cla-1 remodeling, suggesting that NCAM-1 functions in parallel to UNC-8 to promote the synaptic disassembly of presynaptic componentssynaptic remodeling. This observation is important because the vertebrate NCAM homolog is highly expressed in the brain where it regulates synaptic plasticity and function.

Session 2 P24

Proximal Tubule Targeted Gene Therapy for Cystinuria

Jennifer Peek, Alan Rosales, Lauren Woodard, Aravind Asokan, and 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 novel adeno-associated viruses AAV.cc47 and AAV.K20, we have shown efficient viral delivery to the proximal tubule in vivo. We have also used these kidney-targeted AAVs to induce rBAT expression in vitro, demonstrating the potential for cystinuria treatment. Finally, we are currently developing an iPSC-derived human kidney organoid model of cystinuria to assess transduction and functional cystine transport. 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 provide hope for overcoming the barriers to kidney gene therapy.

Session 2 P25

Clathrin-Mediated Endocytosis Locally Restrains the Dimensions of Growing Microvilli

Olivia L. Perkins, Bryan A. Millis, and Matthew J. Tyska

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

Intestinal epithelial function and homeostasis depend on the assembly of apical microvilli: actin bundle-supported membrane protrusions that amplify apical surface area. Although many of the molecules which comprise microvilli have been identified through proteomic and biochemical studies, mechanisms that regulate and promote protrusion growth are poorly understood. Recent studies point to lipid availability as a key regulator of protrusion elongation from non-polarized cells, suggesting the possibility that membrane trafficking might regulate microvillar growth and maintenance. Consistent with this, previous studies from our lab found that Pacsin2-mediated endocytic pits that at the inward curving membrane between microvilli play a crucial role in maintaining intestinal brush border morphology. However, it remains unclear whether endocytosis is also involved in microvillar assembly. To explore this idea further, we leveraged a live imaging assay that enables observation of individual microvilli and allows for measurements of protrusion length, thickness, and growth rates. Using this approach, we found that inhibiting clathrin-mediated endocytosis with Chlorpromazine (CPZ) lead to dramatic reorganization of apical microvilli that contain more actin and microvilli-associated proteins than endogenous microvilli, suggesting that these exaggerated structures on CPZ-treated cells represent canonical microvilli. Furthermore, these oversized microvilli also demonstrated more persistent active motility than endogenous microvilli. Remarkably, we also observed that microvilli grow specifically at sites of clathrin-mediated endocytosis, as demonstrated by the apical localization of clathrin light chain A (LCA), AP-2, and dynamin2, which could indicate the direct involvement of endocytosis in microvillar growth beyond global regulation of membrane tension. Consistent with these observations, preliminary data show that inhibition of endocytosis via CPZ prevents brush border packing in epithelial cell culture, suggesting regulation of microvillar growth and dimensions by endocytosis is critical to brush border maturation. These data suggest that endocytosis plays a crucial and direct role in microvillar growth by locally restraining protrusion dimensions and actin content.

Session 2 P26

Mapping Molecular Profiles to Cell Types and Neighborhoods through the Integration of MALDI IMS and CODEX Multiplexed IF Microscopy

Thai Pham[1,2], Ellie L. Pingry[1,2], Martin Dufresne[1,3], Lukasz Migas[1,4], Filipe A. Moser[4], Angela R. S. Kruse[1,2], Melissa A. Farrow[1,3], Raf Van de Plas[1,4], and Jeffrey M. Spraggins[1,2,3,5,6]

[1]Mass Spectrometry Research Center, Vanderbilt University, Nashville, Tennessee, USA; [2]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee, USA; [3]Department of Biochemistry, Vanderbilt University, Nashville, Tennessee, USA; 4 Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands; [5]Department of Chemistry, Vanderbilt University, Nashville, Tennessee, USA; [6]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee, USA.

Imaging mass spectrometry (IMS) enables untargeted mapping of a wide range of molecular classes at high spatial resolution. However, assigning molecular profiles to specific tissue features requires integration with complementary imaging technologies. Highly multiplexed immunofluorescence microscopy (MxIF) is rapidly developing, providing comprehensive imaging of specific cell types, and enabling cellular neighborhoods to be defined at single-cell resolution. Herein, we describe a multimodal workflow integrating MALDI-IMS with co-detection by indexing (CODEX) MxIF to allow lipidomic signatures to be correlated to cell types in situ. Fresh frozen human kidney tissues were cryosectioned at 10 micrometer thickness and thaw mounted. Autofluorescence (AF) images were collected prior to MALDI IMS and CODEX MxIF image acquisition. IMS data was acquired on a MALDI timsTOF Flex (Bruker Daltonics) system from m/z 400-2000 in positive and negative modes. Ion image data was visualized using SCiLS Lab version 2023. MxIF was collected using the Phenocycler Fusion (Akoya Biosciences). The AF images were utilized to co-register MALDI IMS and MxIF images to generate a multimodal dataset. Individual cells are segmented based on DAPI signal with code adapted from the Cellpose method and cellular intensities calculated in Qupath. SCANPY was used for preprocessing, transformation, dimensional reduction, and cell-type clustering. Co-registration of IMS and CODEX MxIF links molecular and cellular insights of the same tissue section together. Molecular profiling by MALDI IMS imaging allowed us to map several lipid classes. Single-cell segmentation and analysis of MxIF data yielded the identification of more than 15 distinct cell clusters. These clusters are further put into a spatial context with other clusters, forming cellular neighborhoods that represent the functional tissue units of the kidney, including proximal tubules, distal convoluted tubules, glomeruli, collecting ducts, and loop of Henle, as well as clusters of injured cells and areas of immune cell enrichment. Upon combining these two sets of data, we observed that several defined lipids are concentrated in particular regions. For instance, SM(42:1; 2O) is predominantly found in the descending limb of the loop of Henle, SHex2Cer(d42:1) and PC(34:2) are localized in the proximal tubules, while PE(O-38:5) and SM (34:1;2O) reside mainly in the glomeruli. This integration of data provides a deeper understanding of the cellular and molecular composition of the kidney, paving the way for further research into renal function and pathology.

Session 2 P27

Targeting MYC with WDR5 WIN Site Inhibitors in Colorectal Cancer

Brian C. Grieb, Hiroaki Niitsu, Elizabeth Reynolds, Michelle Leon Salazar, Soumita Goswami, Macey J. Slota, Kay Thein, Jing Wang, Andrea C. Florian, Joy Crieghton, Shelly L. Lorey, Kristie L. Rose, Brittany K. Matlock, Qi Liu, Gregory C. Howard, Stephen W Fesik, Robert J Coffey, and William P. Tansey

Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, 37232 ; Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN, 37232 ; Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, 37232 ; Mass Spectrometry Research Center, Nashville, TN, 37232 ; Flow Cytometry Shared Resource, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN, 37232 ; Department of Biochemistry, Vanderbilt University, Nashville, TN, 37232

Colorectal cancer (CRC) is the third most common cancer in the United States and the third leading cause of cancer related mortality. The oncogenic transcription factor c-MYC (MYC) is overactive in the majority of human cancers. In CRC specifically, MYC is activated by aberrant Beta-catenin and MAP kinase pathway signaling, secondary to common mutations in APC and RAS family members, respectively. Experimental inhibition of MYC in mouse models of CRC leads to tumor regression; however, a pharmacologic means to directly inhibit MYC in the clinic does not exist, and many consider MYC undruggable. We recently discovered WDR5 is a novel cofactor for MYC, recruiting it to chromatin at genes encoding a conserved subset of ribosomal protein subunits. Early tool compounds binding the WIN site of WDR5 displace WDR5 from chromatin, prevent MYC recruitment to chromatin by WDR5, decrease transcription of MYC-WDR5 coregulated genes and induce apoptosis in leukemia cell lines. Now, we have generated potent, orally bioavailable WDR5 WIN site inhibitors that are moving rapidly toward first-in-class phase I clinical trials, but the full breadth of their antineoplastic activity and their practical path to clinical application remains undefined. Here, we demonstrate disruption of the MYC-WDR5 nexus has high therapeutic potential in CRC. Using a thorough multi-omics approach, we are developing an interconnected core mechanism of action for WDR5 inhibitors in CRC. We conducted a CRISPR screen and identified genes that confer sensitivity and resistance. We are actively investigating high-value targets using unique sgRNA sequences in Cas9 expressing CRC cell lines. These analyses have informed intelligent selection of novel drug-drug combinations with WDR5 inhibitors the produce robust synergy. The data also provide candidate patient selection biomarkers for future clinical trials. Thus, WDR5 WIN site inhibitors represent a viable therapeutic option for CRC and our data provide foundational information that will aid early clinical design.

Session 2 P28

SSNA1 Regulates Microtubule Stability via Interactions with the Microtubule Lattice

Laura B. Richardson, Elizabeth J. Lawrence, and Marija Zanic

Vanderbilt University, Nashville, TN, 37228

SSNA1 (Sjögren's Syndrome Nuclear Autoantigen 1) is a microtubule-associated protein implicated in Sjögren's Syndrome, a highly prevalent autoimmune disease. In cells, SSNA1 plays critical roles in cell division, intraflagellar transport, and axonal branching. However, SSNA1's direct regulation of microtubules in these processes is not well understood. Our previous work using in vitro reconstitution approaches illustrated SSNA1's striking affinity for sites of damage along the microtubule lattice and SSNA1's ability to inhibit severing by its binding partner and microtubule severing enzyme, spastin. However, we have not fully uncovered the mechanisms by which SSNA1 interacts with the microtubule lattice or the effects this lattice regulation has on microtubule stability. Here, we utilize total internal reflection microscopy to determine the effects of SSNA1 on microtubule self-repair and microtubule mechanics. We show that SSNA1 localization at microtubule damage sites lowers the efficiency of microtubule self-repair both by limiting the area and density of tubulin incorporation at damage sites. Additionally, we utilize microfluidics to determine the influence of SSNA1 binding on microtubule mechanics. Intriguingly, our preliminary results suggest that SSNA1 increases the rigidity of the microtubule lattice and its resistance to breakage against hydrodynamic flow. Together, this work establishes SSNA1 as a member of a novel class of microtubule associated proteins that regulate microtubules through the microtubule lattice damage-and-repair paradigm.

Session 2 P29

Defining the Molecular Interactions Required for MYO7B Motility

Kianna L. Robinson and Matthew J. Tyska

Vanderbilt University, Nashville, TN, 37232

The intestinal epithelium is responsible for both nutrient absorption and pathogen protection via the brush border, composed of thousands of actin-supported finger-like protrusions, known as microvilli. The intermicrovillar adhesion complex (IMAC) drives tight packing of microvilli to maximize the number of microvilli on the apical surface. The IMAC localizes to the distal tips of microvilli and consists of two modules: extracellular adhesion links and cytoplasmic proteins. The adhesion links are composed of the transmembrane protocadherins CDHR2 and CDHR5. The ectodomains of CDHR2 and CDHR5 extend from neighboring microvilli and form a threadlike ~50 nm link that controls microvillar length and packing. CDHR2 and CDHR5 also have cytoplasmic domains that interact with the module of cytoplasmic proteins. IMAC cytoplasmic proteins include the actin-based motor myosin-7B (MYO7B), which contains a C-terminal tail with an SH3 domain flanked by two MYTH4/FERM domains, and scaffolding proteins ankyrin repeat and sterile alpha motif domain containing 4B (ANKS4B) and Usher syndrome 1C (USH1C). ANKS4B and USH1C bind to the first and second MYTH4/FERM domains of MYO7B, respectively. MYO7B was previously shown to interact with other IMAC proteins through its tail domain and promote their enrichment at the distal tips of microvilli. However, MYO7B lacks a clear dimerizing motif like that found in most processive motors (e.g. a coiled coil), and the mechanisms that allow it to move to the distal tips are currently unknown. Our preliminary studies using in-cell interaction assays revealed that the SH3 domain of MYO7B binds to some motif in the C-terminal tail, suggesting a potential novel mode of multimerization. Future experiments will focus on testing whether dimerization of MYO7B through its tail domain, and specifically the SH3 domain, promotes processive movement.

Session 2 P30

InsP3R Calcium Signaling Promotes Mitochondrial Quality Control and Longevity in Response to Electron Transport Chain Perturbation

Elizabeth M Ruark, Gaomin Feng, Alexandra G Mulligan, Eric KF Donahue, Brianne Jacquet-Cribe, and Kristopher Burkewitz

Vanderbilt University, Nashville, TN, 37235

Perturbing mitochondrial function elicits cellular responses that extend lifespan across animal models. Mitochondrial stress can also trigger pathogenesis, however, and limited understanding of the adaptations that distinguish between these potential opposing outcomes precludes therapeutic utility. One underexplored consequence of mitochondrial stress in these contexts is altered cellular calcium handling, given that mitochondria both store and respond to Ca2+ ions. Furthermore, vertebrate studies have revealed that the endoplasmic reticulum InsP3R Ca2+ channel stimulates mitochondrial functions through both cytosolic signaling pathways and direct Ca2+ flux into the mitochondrial matrix. We find that C. elegans InsP3R signaling potently stimulates mitochondrial respiration through mechanisms similar to mammals. Additionally, we reveal that longevity induced by electron transport chain impairment is exquisitely dependent on InsP3R function, focusing on a long-lived Complex I mutant model to define the mechanisms of this requirement. We find that InsP3R's obligate partner for matrix calcium uptake, the mitochondrial calcium uniporter, is dispensable for respiration and lifespan indicating InsP3R signaling promotes stress adaptation independently of matrix Ca2+. We find that reduced InsP3R signaling results in maladaptive hyper-expansion of dysfunctional mitochondrial networks and elucidate parallel Ca2+/calmodulin pathways by which the InsP3R coordinates mitochondrial quality control. One pathway regulates mitochondrial biogenesis transcriptionally, whereas the other promotes remodeling of the actin network in response to mitochondrial damage. Finally, we find that actomyosin recruitment to damaged mitochondria and fission-dependent pruning of the network are sufficient to restore longevity in InsP3R mutants. Overall, these findings reveal an inter-organelle Ca2+ signaling pathway essential for controlling the balance between mitochondrial biogenesis and degradation while highlighting critical new roles for actomyosin in mitochondrial longevity paradigms.

Session 1 P44

Characterization of the Role of STK38 Kinase in Wnt Signaling and Heart Development

Lauren Schnitkey, Christin Anthony, and Ethan Lee

Vanderbilt University, Nashville, TN, 37203

The Wnt pathway plays a critical role in developmental processes such as heart development, and dysregulation of Wnt signaling has been linked to congenital heart defects. We have recently identified a new component of the Wnt pathway, STK38, and preliminary studies in zebrafish indicate that it plays a critical role in heart development. Our preliminary studies suggest that STK38 interacts with Pygopus, a core nuclear Wnt component shown to play a role in vertebrate heart development, providing further evidence for the role of STK38 in Wnt signaling and cardiogenesis. I aim to identify STK38's substrates within the Wnt signaling cascade by employing techniques involving co-immunoprecipitation, mass spectrometry, and cellular labeling. Characterizing these substrates and assessing their function will shed light on STK38's role as a regulator of Wnt signaling. Induced pluripotent stem cells (iPSCs) are a powerful platform for modeling the progression from stem cells to cardiomyocytes. By monitoring endogenous STK38 levels during iPSC cardiac differentiation and correlating them with known periods of Wnt pathway activity, I will elucidate STK38's temporal expression dynamics during cardiac development. Finally, I will utilize iPSCs to chemically induce precise temporal depletion of STK38, enabling me to dissect STK38's influence on Wnt signaling and cardiomyocyte differentiation/proliferation during critical periods of heart development.

Session 2 P31

A Novel Approach to Chemically Target KRAS(G12D)

Anna Schwarzkopf, Laura Friggeri, Kathleen DelGiorno, and Ethan Lee

Vanderbilt University, Nashville, TN, 37272

Activating mutations in the Ras family of small GTPases are major drivers in ~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. There are currently no FDA approved drugs that specifically target non-cysteine G12, G13, or Q61 KRAS mutants; thus, there remains an urgent need for strategies to effectively and specifically target these 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 identified 100 candidate molecules predicted to bind specifically to the DDRP of KRAS(G12D). Screening by surface plasmon resonance (SPR) selected three compounds that bind to KRAS(G12D) in the low micromolar range. Follow-up experiments revealed that two of these compounds inhibit the interaction between KRAS(G12D) and its downstream binding partner Raf. Further, one compound exhibited a significant decrease in ERK phosphorylation, a downstream target of active KRAS, following treatment in a KRAS(G12D)-mutant pancreatic cancer cell line. 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 therapeutic strategies for treating a large percentage of KRAS-driven cancers.

Session 2 P32

Multimodal Imaging of Human Brain Tissue Reveals Cellular and Lipidomic Profiles of White Matter Hyperintensities in Alzheimer's Disease

Claire F Scott, Cody R Marshall, Madeline E. Colley, Elena Solopova, Lukasz G Migas, Angela Kruse, Katerina V Djambazova, Wilber Romero Fernandez, Matthew S Schrag, Raf Van de Plas, and Jeffrey M Spraggins

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN; Chemical Physical Biology Program, Vanderbilt University School of Medicine, Nashville, TN; Department of Neurology, Vanderbilt University, Nashville, TN; Department of Biochemistry, Vanderbilt University, Nashville, TN; Department of Chemistry, Vanderbilt University, Nashville, TN; Delft Center for Systems and Control, Delft University of Technology, Delft, Netherlands

White matter hyperintensities (WMH) in magnetic resonance imaging (MRI) brain scans indicate lesions in white matter. Demyelination and cognitive decline in Alzheimer's disease (AD) have been linked with WMH, and understanding the molecular underpinnings of these features will improve our understanding of AD. Approaching this knowledge gap with multimodal techniques allows for a more comprehensive understanding of both the cellular and molecular alterations present within WMH. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) unveils spatial distributions of lipids within tissue sections, while microscopy techniques provide cell-type information. While existing literature indicates alterations in whole-brain lipid levels in AD, we aim to expand on this knowledge by spatially examining cellular and lipidic changes within WMH of human AD donors. Incorporating MRI into our multimodal workflow provides a clear distinction between WMH and normal-appearing white matter (NAWM), facilitating robust inter- and intra-donor comparisons. Post-mortem T2-FLAIR MRI directs the selection of formalin-fixed AD human brain tissue samples. Microscopy images were acquired on a Zeiss Axioscan.Z1 slide scanner. MALDI IMS data were acquired in triplicate at 20 µm spatial resolution on a Bruker timsTOF FleX mass spectrometer (Bruker Daltonics) following ammonium formate washes and sublimation of 4-Dimethylaminocinnamaldehyde (DMACA). LC-MS/MS lipidomics data were acquired from serial tissue sections to build a reference library for MALDI IMS annotations. Data analysis was performed using SCiLS, ZEN3.5 software, and in-house software. Multimodal imaging data has been acquired from areas of WMH and NAWM brain tissue found in the frontal and occipital lobes of three human donors. In one illustrative case, MALDI IMS data showed 243 annotated lipid peaks common to both WMH and NAWM samples, and 78 and 148 peaks unique to the NAWM and WMH respectively. Five LPAs shown in literature to promote AD-related neurodegeneration were uniquely present in the WMH. Among 243 shared peaks, various lipid species such as [SHexCer (42:1;O2)-H]- (m/z 890.638) were present at lower abundance in the WMH than the NAWM, while other lipid species such as [PA (36:1)-H]- (m/z 701.513) were present at higher abundance in the WMH than the NAWM. Furthermore, we observe oxidized lipids and their unoxidized counterparts at varying levels across tissue samples. Together, integrated multimodal approaches will reveal cellular and molecular characteristics of AD WMH.

Altered DNA Methylation Influences Cell Lineage Evolution During Metaplasia Progression

Halee Scott and Eunyoung Choi

Vanderbilt University, Nashville, Tennessee, 37232

Gastric cancer is commonly preceded by a series of distinct cellular changes within the gastric mucosa. These changes include the trans differentiation of zymogenic chief cells into spasmolytic polypeptide expressing metaplastic (SPEM) cells, the major cell type of pyloric metaplasia. Pyloric metaplasia is commonly followed by the development of intestinal metaplasia (IM) and neoplastic stages such as dysplasia and cancer. While SPEM cells are present during these stages, their exact functions are unknown. One notable phenomenon that occurs upon metaplasia development is the increase of DNA methyltransferase 1 (Dnmt1) expression. Dnmt1 maintains the DNA methylation status in dividing cells and it's dysregulation is observed in many cancer types. In this study, we examine Dnmt1 expression patterns and the effect of altered DNA methylation during SPEM cell evolution using mouse SPEM, IM, and dysplastic organoid lines. Immunofluorescence staining for Dnmt1 in stomach tissue sections from a Kras driven mouse model of metaplasia shows that the location and expression levels of Dnmt1 change upon metaplasia development. Additionally, Dnmt1 expression is increased in SPEM organoids but lost in dysplastic organoids suggesting that DNA methylation plays an important role in metaplastic cell types. An examination of Dnmt1 expression levels in mature SPEM organoids showed a decrease in Dnmt1 expression upon increased expression of mature SPEM markers Muc6 and TFFII. To examine the effect of global alterations in DNA methylation during metaplasia progression, a DNA methylation inhibitor, 5-Aza-2'-deoxycytidine (5-AZA-CdR), was used to treat SPEM and IM organoids for one week. While the SPEM and IM organoids showed a decrease in metaplastic cell markers AQP5 and CD44v9 following DNA methylation inhibition, the SPEM organoids showed an increase in villin and TFF3, markers of intestinal metaplasia. In contrast to this, the IM organoids treated with 5-AZA-CdR depicted common cytological characteristics of dysplastic epithelial cells such as multilayering and formation of budding structures. The 5-AZA-CdR treated IM organoids showed increased expression of dysplastic cell markers Trop2 and Cldn7. Together, these results demonstrate that the global inhibition of DNA methylation leads to distinct changes in cell lineage evolution during metaplasia progression. Additionally, alterations in cell lineage evolution are dependent upon their original cell types, SPEM or IM cells. Therefore, our study provides a new insight on distinct DNA methylation states of pyloric metaplasia and intestinal metaplasia and suggests that changes in DNA methylation can lead to metaplasia progression during gastric carcinogenesis.

Session 1 P46

The Role of Rac1 in Proximal Tubular Repair

Eric Sha, Meiling Melzer, Shensen Li, Xinyu Dong, Roy Zent, and Fabian Bock

Vanderbilt University Medical Center, Nashville, TN, 37203 ; Vanderbilt University, Nashville, TN, 37203

Ischemic reperfusion injury in the kidney is characterized by the restriction of blood supply to an organ followed by restoration of blood flow and re-oxygenation. The kidney proximal tubule (PT) is very susceptible to ischemic injury, and contains large, interconnected networks of mitochondria. Cell structure after injury is defined by the actin cytoskeleton, which is dynamically organized by small Rho guanosine triphosphatases (GTPases). In this study, we identify the Rho GTPase, Rac1, as a driver of proximal tubule repair. We show that after undergoing ischemic acute kidney injury (AKI), Rac1 promotes the recovery of PT morphology and the reconstitution of mitochondrial integrity. We found that Rac1 deficient PTs have reduced mitochondrial function and accumulate damaged mitochondria. Furthermore, we show that Rac1 is required for post-injury mitophagy, the clearance of damaged mitochondria. Rac1 deficient PT cells are unable to form damage-associated actin cytoskeletal cages around mitochondria, which is an essential step in mitochondrial clearance through mitophagy. We propose that Rac1 promotes proximal tubular repair by organizing mitochondrial networks, likely through enhancing the actin-dependent clearance of damaged mitochondria.

Session 2 P35

Comparative Proteomics Analysis of Small EVs-derived from Mouse Oral Carcinoma Cells

Adnan Shafiq, Shinya Sato, and Alissa Weaver

Vanderbilt University, Nashville, TN, 37235

Abstract: Cancer metastasis is intricately influenced by both tumor cell attributes and the microenvironment. Extracellular vesicles (EVs) play a key role in tumor metastasis by both promoting cancer cell migration and invasion and influencing cells in the tumor microenvironment. To identify key cargoes driving metastasis of head and neck squamous cell carcinoma, we performed a quantitative proteomics analysis of EVs purified from matched mouse oral cancer (MOC) cell lines with different metastatic abilities. Methods: small EVs were purified by cushion density gradient method from MOC1 and MOC2 oral cancer cell lines. MOC1 is a nonmetastatic cell line that is "immune-hot", recruiting T-cells into tumors. By contrast, MOC2 is a metastatic cell line that is "immune-cold". EVs were analyzed by isobaric tagging for relative and absolute quantitation (iTRAQ) mass spectrometry. The data were analyzed by STRING and Gene Set Enrichment Analysis (GSEA) methods to identify enriched functional and interacting groups of proteins. Results: iTRAQ proteomics analysis of MOC1- and MOC2 cell-derived exosomes revealed that MOC1 cell-derived small EVs contained many RNA binding proteins, including those involved in ribosome biogenesis, RNA processing, and nonsense mediated decay (NMD) as well as apoptosis. By contrast, MOC2 cell-derived small EVs were enriched with factors related to cell migration, angiogenesis, ECM organization and wound healing. Conclusion: We performed a comparative proteomics analysis of matched oral carcinoma cell lines that have distinct indolent (MOC1) versus metastatic (MOC2) phenotypes. We identified increased expression of RNA binding proteins in MOC1 cell-derived small EVs, suggesting increased RNA content. By contrast, MOC2 cells-derived small EVs were significantly enriched for proteins associated with cell migration, invasion, and tumor angiogenesis. Future work will investigate the RNA content of MOC1 small EVs and its role in modulating the immune microenvironment, along with the role of MOC2 small EV cargoes in promoting tumor cell metastasis and angiogenesis.

Session 1 P45

Potential Role for Electrical Stimulation in Reprogramming of Muller Glia in the Adult Mouse Retina Ex Vivo

Megan Stone, Hannah Lee, and Ed Levine

Dept of Cell & Developmental Biology Vanderbilt University School of Medicine Nashville TN 37240

There is no replicative cell population in the adult mammalian retina able to produce new neurons following injury. Müller glia, the major glial cell type in the retina, have a natural capacity for regeneration in fish and recent studies have shown that mouse Müller glia can be genetically manipulated to transdifferentiate into neurons in an injury context. However, these studies are challenging in that they are done in vivo, making the testing of candidate factors slow and laborious. Ex vivo culture of whole retina potentially addresses this problem because the tissue remains intact, cell-cell interactions are maintained, the retina can be imaged during culture, and delivery of candidate factors, especially small molecules is straightforward. However, gene delivery is challenging because the retina is cultured at the air-liquid and not submerged in the medium. To address this challenge, I have developed a submergence-free electroporation technique that allows for efficient gene delivery to the ex vivo murine retina. In developing the method, I found that electrical stimulation alone increased BrdU incorporation in Müller glia and induced expression of OTX2 in a subset of Müller glia, which is an early indication of Müller glia reprogramming toward a neuronal cell-like state. This finding suggests that electrical stimulation may promote reprogramming of Müller glia and could enhance the effects of genetic manipulation to promote neurogenesis. Ongoing studies are aimed at identifying how far the Muller glia progress toward producing neurons under these conditions and to add in additional candidate factors, such as overexpressing L-Myc and Ascl1, and inactivating Cdk1b, that I predict can make the reprogramming process more efficient.

Session 2 P37

Unveiling Ferrosome Formation by Cryo-electron Tomography

Rong Sun[1,2,3,4], Hualiang Pi[5,6], James R. McBride[7], Eric P. Skaar[5,6], and Qiangjun Zhou[1,2,3,4]

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

Recent research has identified ferrosome organelle as a potential iron storage mechanism in three Gram-negative environmental anaerobes. The exclusivity of ferrosome generation to Gram-negative bacteria, however, remains uncertain. Additionally, the fundamental processes involved in ferrosome formation are not well understood. In this study, we utilized cryo-electron tomography (cryo-ET) to discover ferrosomes in the Gram-positive human pathogen, Clostridioides difficile. These ferrosomes are encapsulated by lipid membranes and are frequently found adjacent to cellular membranes. We further demonstrated that the expression of FezA and FezB from Clostridioides difficile in Escherichia coli, which lacks an intracellular membrane system, leads to the formation of membrane vesicles. We discovered that these vesicles are equivalent in size to ferrosomes in Clostridioides difficile and are generated from the inner membranes of the cell. Notably, overexpression of FezA or FezB alone could not induce vesicle formation in Escherichia coli. Our findings suggest a novel mechanism for vesicle formation within the cell and offer a potential vehicle for drug delivery.

Session 2 P38

Exosomes are Necessary and Sufficient for Fibronectin Assembly

Bong Hwan Sung[1,2,3], Merlyn Emmanuel[4], Metti Gari[5], Jorge Guerrero5, Suzanne M. Ponik[5], and Alissa M. Weaver[1,2,3,6]

[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN. [2]Program of Cancer Biology, Vanderbilt University, Nashville, TN. [3]The Vanderbilt Center for Extracellular Vesicle Research, Vanderbilt University, Nashville, TN. 4Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada. [5]Department of Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI. [6]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN.

Extracellular matrix assembly is critical for tissue integrity and is deregulated in diverse diseases. Fibronectin is a key stromal matrix molecule whose assembly into fibrils is thought to require cells. Here, we identify small exosome-type extracellular vesicles (EVs) as critical initiators of fibronectin assembly. Fibroblasts engineered to be deficient in exosome secretion showed greatly reduced assembly of fibronectin and other stromal matrix molecules in 2D, 3D, and in vivo environments and led to reduced lung metastasis by breast cancer cells. Consistent with a critical role for exosome secretion in stromal matrix assembly, bleomycin-treated Rab27a/b double knockout mice exhibited greatly reduced lung fibrosis. Using a newly developed purified component assay, we find that the addition of purified small EVs to purified soluble cellular fibronectin is sufficient to induce fibronectin assembly - contradicting the dogma that fibronectin assembly requires cells. The EV-induced fibronectin assembly requires the presence of fibronectin-binding integrins and syndecan-1 in the EVs. These data indicate that exosome secretion plays a critical and unsuspected role in the intrinsic ability of stromal cells to assemble and organize fibronectin. We propose a model in which fibronectin-binding integrins and syndecan-1 clustered in exosomes synergistically bind and unfold fibronectin to produce a robust assembly.

Wnt Ligands Support an Immunosuppressive Microenvironment in Anaplastic Thyroid Carcinoma

Megan L Tigue, Matthew A Loberg, Rincon Jagarlamudi, George Xu, Courtney Phifer, and Vivian L Weiss

Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN

Anaplastic thyroid carcinoma (ATC) is an aggressive disease with almost no effective treatment options and a median survival of only 5 months. Trials of immunotherapy in ATC have had some success, but over half of patients show little or no response. ATC is a very rare and highly heterogenous disease, which makes studying the nuances of this treatment response difficult. Our lab has FFPE tissue and bulk RNA sequencing data from a cohort of 351 thyroid lesions, including 34 ATCs. Using this data, we have demonstrated two distinct populations of ATC: one with increased lymphocytes, which is predicted to respond to immune checkpoint inhibitors (ICIs), and one with few lymphocytes but high predicted infiltration of cancer associated fibroblasts (CAFs) and tumor associated macrophages (TAMs). The CAF and TAM-high subset of ATCs also demonstrates increased Wnt signaling and Wnt2 ligand production. Wnt signaling is associated with poor prognosis and more aggressive disease in thyroid cancer. We hypothesize that targeting Wnt ligand production in combination with ICI therapy may be a viable therapeutic option for patients with CAF-rich ATCs. In this study, we demonstrate a role for CAF-secreted Wnt ligands in promoting an immunosuppressive microenvironment through recruitment of TAMs and subsequent exclusion of T cells in a novel orthotopic mouse model of ATC. Future studies aim to target Wnt ligand production to promote a more lymphocyte-rich and immunotherapy responsive tumor microenvironment.

Session 2 P40

Rab27a-dependent Secretion of Small Extracellular Vesicles from Breast Cancer Cells Controls ECM Deposition

Diego Torres Martinez, Bong Hwan Sung, and Alissa Weaver

Vanderbilt University, Nashville, TN, 37235

Exosomes are small extracellular vesicles (SEVs) that are critical for intercellular communication and play known roles in cancer progression and metastasis. Tumor cell-derived SEVs transport distinctive functional cargoes, including adhesion receptors known to mediate cell-extracellular matrix (ECM) interactions. ECM is a complex network of macromolecules critical for cellular function and behavior; however, interactions between cancer-derived SEVs and ECM are poorly understood. Preliminary data from our laboratory revealed that exosome secretion from normal and cancer fibroblasts is important for fibronectin (FN) assembly. Furthermore, we found that purified fibroblast SEVs are sufficient to assemble cellular fibronectin in a cell-free environment, dependent on the presence of integrin α5 on SEVs. It is not known if the ECM assembly function is shared by SEVs from other cell types, such as epithelial cells. To test the capacity of epithelial-derived SEVs to affect ECM assembly, Rab27a was stably knocked down (KD) using shRNA in 4T1 mouse epithelial breast cancer cells. Rab27a controls the docking event of exosome-containing multivesicular endosomes at the plasma membrane and thus exosome secretion. Rab27a KD cells were grown for 3 or 6 days and ECM samples were then collected for biochemical characterization and immunofluorescence (IF). Western Blot analysis of the decellularized ECM deposited by Rab27a KD cells revealed lower levels of perlecan and nidogen compared to control cells. Moreover, IF analysis of collagen IV demonstrated that Rab27a KD cells deposited less ECM compared to the control. Taken together, these studies suggest that release of epithelial cell-derived SEVs can alter ECM assembly. Future studies will determine if SEVs isolated from parental 4T1 cells can rescue ECM assembly defects in Rab27a KD cells. Additionally, 4T1-derived SEVs will be tested functionally using a cell-free ECM assembly assay.

Unraveling the Role of Polarity Remodeling During Cell Intercalation

Aishwarya Venkataravi[1], Alexander Pfannenstein[1, 2], and Ian Macara[1]

[1]Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN 37240, USA
[2]Present affiliation: Department of Cell & Developmental Biology, Weill Cornell Medical College of Cornell University, New York, NY 10065, USA

Morphogenesis involves the constant remodeling of sheets of cells, which expand, elongate, invaginate and fold to form an organ. Deciphering the mechanisms underlying these cellular rearrangements is critical to understanding morphogenesis. The mammary gland has been long appreciated as a model for studying epithelial behaviours during development. The key events of mammary gland development occur postnatally during puberty and throughout repeated cycles of pregnancy. The terminal end bud (TEB) orchestrates the formation of the ductal tree by giving rise to mature cell types. The TEB is a multilayer cluster of body cells that must resolve into a single luminal layer as the duct elongates and invades the surrounding fat pad. The precise mechanism of this process is poorly understood. Previous work from the lab (Pfannenstein & Macara, Dev Cell 2023) identified a unique process of apical cell intercalation as the driving force behind resolving the multilayered TEB structure into the single luminal layer of the duct. An in vitro intercalation assay using either Eph4 mammary epithelial cells or primary luminal epithelial cells found that the tight junction (TJ) protein ZO-1 is critical for intercalation. ZO-1-depleted cells fail to intercalate into a WT epithelial monolayer, a result that was confirmed in vivo by mammary gland intraductal injections. Surprisingly, however, depleting ZO-1 in the monolayer enhances the in vitro intercalation of wild-type cells. Although ZO-1 is an abundant tight junction component, its depletion does not inhibit TJ formation, which suggests another role of ZO-1 during intercalation. Actin dynamics at the interface of the incoming cell and the monolayer was found to be essential for intercalation. We are further investigating the function of ZO-1, other TJ proteins, and actin dynamics. Evidence from previous studies and preliminary data suggest that ZO-1-actin interactions might be crucial for intercalation. We are also addressing another aspect of the mechanism, by examining how apicobasal polarity is remodeled during intercalation. Preliminary data indicate that the apical compartment of the incoming cell faces the apical surface of the monolayer and is reorganized as the incoming cell attaches and begins to penetrate at intercellular junctions

The Core Spindle Pole Body Scaffold Ppc89 Links the Pericentrin Ortholog Pcp1 to the Fission Yeast Spindle Pole Body

Alaina H. Willet, Jun-Song Chen, Maya Igarashi, Liping Ren, Sarah M. Hanna, Lesley A. Turner, Nathan A. McDonald, Janel R. Beckley, and Kathleen L. Gould

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37240

Centrosomes and spindle pole bodies (SPBs) are important for mitotic spindle formation and also serve as cellular signaling platforms. Although centrosomes and SPBs differ in morphology, mechanistic insights into centrosomal function have been gleaned from SPB studies. In the fission yeast Schizosaccharomyces pombe, the coiled-coil protein Ppc89, identified based on its interaction with the septation initiation network scaffold Sid4, comprises the SPB core. High-resolution imaging has suggested that additional SPB proteins assemble on Ppc89 during SPB duplication, but such interactions are undefined. Here, we define a connection between Ppc89 and S. pombe pericentrin Pcp1. Specifically, we found that a predicted third helix within Ppc89 binds the Pcp1-PACT domain complexed with calmodulin. Ppc89 helix 3 contains sequence similarity to PINC motifs found in fly SAS-6 and human Cep57 that are used by these centrosomal proteins to bind pericentrin-calmodulin complexes and also to sequences within S. cerevisiae Spc42 that bind pericentrin Spc110. Further, AlphaFold2 predicts homologous complexes among the four organisms. Finally, mutational analysis supports the importance of the predicted binding interface in vivo. Our studies have provided additional insight regarding the architecture of the S. pombe SPB and suggest an evolutionarily conserved mechanism of scaffolding pericentrin-calmodulin complexes for mitotic spindle formation.

Myelin Transcription Factors Regulate Islet β-Cell Identity and Survival in a Stress Level Dependent Fashion

Mahircan Yagan[2,a], Xin Tong[1,a], Ruiying Hu[2,a], Simone Nevill[2], Teri D. Stevenson[1], Roland Stein[1], Bulamurugan N. Appakalai[3], and Guoqiang Gu[2]

[1]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. [2]Vanderbilt Program in Developmental Biology, Department of Cell and Developmental Biology, and Center for Stem Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. [3]Department of Surgery, Clinical Islet Transplantation Laboratory, Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, 40202, USA.

A defining feature of type 2 diabetes (T2D) is endocrine islet β-cell failure, which can occur via cell dysfunction, loss-of-identity, and/or death. A potential pathway underlying all three β-cell defects is stress response. Myelin transcription factors (Myt1, Myt2, and Myt3) play a key role in regulation of stress response by repressing stress response genes. Inactivation of all three *Myt Tfs* in pancreatic progenitors (*MytPanc*$^{\Delta}$) results in compromised β-cell proliferation, identity, function, and survival. Here we look at the changes in defects of *MytPanc*$^{\Delta}$ β cells under varying glycemic conditions. We show that *MytPanc*$^{\Delta}$ β cells dedifferentiate and die under hyperglycemia and obesity-induced insulin resistance. Yet they dedifferentiate and transdifferentiate under euglycemic conditions. RNAseq along with IF assays show that *MytPanc*$^{\Delta}$ β cells have a propensity to dedifferentiate to progenitor-like cells and transdifferentiate to Ppy+ cells. These findings suggest that an important determinant of endocrine islet β-cell failure is the interplay between metabolic load and Myt TF-regulated stress response.

Session 2 P44

Mapping Metabolites in the Human Eye: Integrating High Spatial Resolution MALDI IMS for Insights into Ocular Health

Ali Zahraei[1], Martin Dufresne[2], David M. Anderson[2] Madeline E. Colley[1], Angela R.S. Kruse[1], Lukasz Migas[3], Jeffrey D Messinger[4], Christine A. Curcio[4], Raf Van de Plas[3], Kevin L. Schey[2], and Jeffrey M. Spraggins[1]

[1]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN USA; [2]Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, TN USA; [3]Delft University of Technology, Delft, Netherlands; [4]Department of Ophthalmology and Visual Sciences, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL USA

Metabolite distributions and their alterations in tissue microenvironments are essential to understanding biology. This extends to the human eye, where diverse cell types play a key role in ocular pathologies such as age-related macular degeneration and cataracts. MALDI imaging mass spectrometry (IMS) faces challenges in analyzing low molecular weight ions due to limitations in sensitivity and spatial resolution, hindering their comprehensive characterization. Here, we present a high spatial resolution MALDI IMS method for mapping the primary metabolites in the human eye. Future integration with multimodal imaging workflows will allow eye metabolism to be defined at cellular resolution. The Human ocular globe was then dissected into anterior and posterior portions. Both regions were sectioned and thaw-mounted onto indium tin oxide-coated glass slides. IMS data were acquired with a 10 µm pixel size in negative ion mode, covering a mass range of m/z 100-900. Autofluorescence microscopy combined with histological stains aided in annotating the tissue. Utilizing high spatial resolution IMS technologies, a diverse range of tissue metabolites were imaged. Data from the posterior segment show that endogenous fatty acids have specific localizations to several anatomical features, including the unmyelinated nerve fibers, the neural retina (m/z 327.23, docosahexaenoic acid), and the myelinated nerve (m/z 281.24, oleic acid). These data revealed metabolite heterogeneity within the layers of the neural retina. Taurine (m/z 124.00) was found to be the most abundant peak in the posterior segment. This metabolite plays a critical role in retinal health, effectively improving stress damage, especially oxidative stress damage, arising in the retina. Hexose monophosphate (m/z 259.02), a product of the carbohydrate metabolism pathway, was also detected. Additionally, spatial localizations of signals in the anterior segment, including ophthalmic acid (m/z 288.11), and glutathione (m/z 306.07); were observed throughout the lens. Fatty acid species, such as linoleic acid (m/z 279.23) were found in the ciliary body, iris, and cornea. Current work provides highly multiplexed spatial distributions for various endogenous compounds in the lens, ciliary body, iris, and cornea, and enables the differentiation between multiple retinal layers and the optic nerve on the same section. The resulting method enables untargeted metabolite imaging with significantly improved spatial resolution creating new possibilities for multimodal data integration and mining. These findings significantly contribute to our understanding of metabolic dynamics within the normal eye. In summary, this study established a reliable methodology for mapping metabolites with a high spatial resolution while preserving their localization in ocular tissue.