JOHNS HOPKINS DEPARTMENT OF CELL BIOLOGY INVITES YOU TO

THE 3RD ANNUAL

RISING STARS IN CELL BIOLOGY SYMPOSIUM

2024

20 APRIL, 2024

A day of science talks, posters, and networking with cell biologists in the mid-Atlantic region

Keynote address from

DR. CHANTELL EVANS

Duke University // Department of Cell Biology

Talk Title: **Investigating the temporal dynamics of mitochondrial turnover in neurons**

Visit: **http://cellbio.jhmi.edu/the-rising-stars-in-cell-biology-symposium/**

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About the Rising Stars Symposium

Organized by the IDEA team, the Rising Stars in Cell Biology Symposium is an opportunity for early career scientists (undergraduates, post baccalaureates, PhD students, and postdoctoral fellows) from diverse backgrounds to present their research and network with peers. This symposium will include talks and poster sessions for trainees to exchange ideas and feedback with peers and senior scientists. We hope you enjoy the symposium.

About the IDEA Team

The IDEA Team is a group of graduate students, postdoctoral fellows, and faculty from the Department of Cell Biology at the Johns Hopkins School of Medicine. We recognize the need to work continually to foster a welcoming and inclusive environment within our department, and it is our mission to develop and implement initiatives that promote diversity, equity, and inclusion at our own institution and in science more broadly.

Participating Institutions

Catholic University

Coppin State University

George Washington University

Johns Hopkins University

Morgan State University

University of Maryland, Baltimore County

University of Delaware

National Institutes of Health

El Paso Community College, TX

American University

University of Pennsylvania

National Cancer Institute

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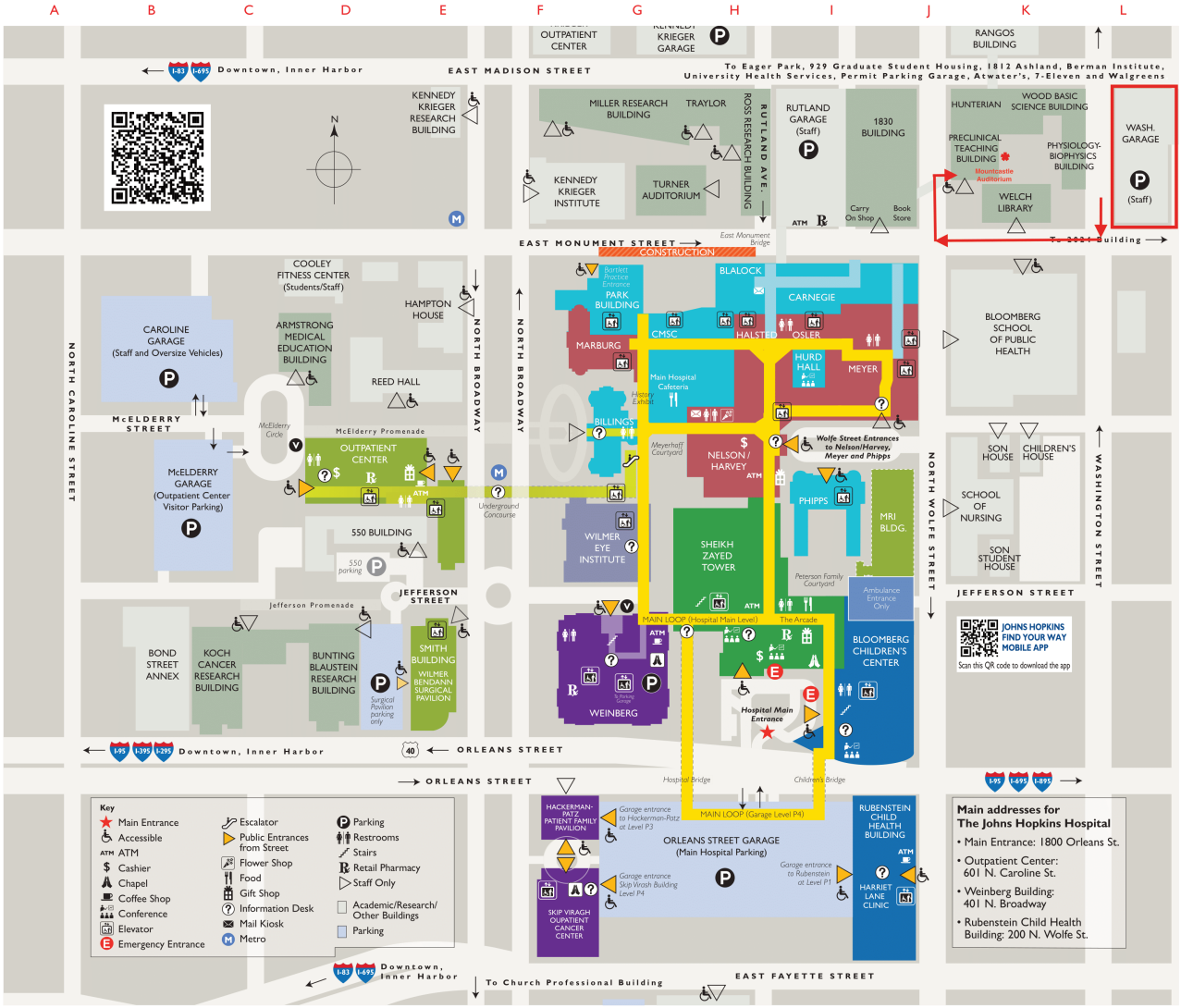
Johns Hopkins University Department of Cell Biology

Map

Registration: Apr 20th, 8AM at Mountcastle Auditorium (left red star, K2 on the map)
Ground floor Preclinical Training Building

Conference Location: 725 N Wolfe St
Baltimore, MD 21205

Parking Location: Washington Street Garage (L2 on the map)
701 N Washington St
Baltimore, MD 21205



Schedule of Events

Time	Event	Location
8:00-9:20AM	Registration, Breakfast Set up for poster session I	PCTB Hallway
9:20-10:30AM	Scientific Session I Moderator: Nelson Yeung Jennifer Viveiros, JHU, PhD student *Assessment of heterogeneity within the Drosophila germline stem cell niche* Ji Hoon Kim, JHU, Research associate *Outspread determines organ dimensions during salivary gland tubulogenesis* Rohan Paraparambil, JHU, PhD student *Targeting lipid metabolism in breast cancer metastasis* Tamara McErlain, NCI, NIH, Post-doctoral fellow *Pericyte derived lipids alter disseminating tumor cell fate decisions*	Mountcastle Auditorium
10:30-11:30AM	Poster Session I	Stile Room
11:30-12:15PM	Lunch and Networking	Greenhouse Cafe

12:15-1:30PM	Career Development Panel	
12:15-12:45AM	Introduction of the Panelists (Jose Catala Torres)	Stile Room
12:45-1:30AM	Discussion	Mountcastle Auditorium

Mike Piacentino, Faculty

Johns Hopkins University/Cell Biology

Renee Pepper, Post-doctoral fellow

Johns Hopkins University/Cell Biology

Valerie Thompson, Medical student, former DDP scholar

Johns Hopkins University

Milan Dower, PhD student, former SARE/CSM-CIP scholar

Johns Hopkins University/Biomedical Engineering

Ryan Huizar, MD-PhD student

Johns Hopkins University/Biochemistry, Cellular and Molecular Biology

Chin Patel, PhD student, former DDP scholar

Johns Hopkins University/Neurobiology and Neurosciences

James McCann, Technical Advisor, former JHU post-doctoral fellow

Mintz Law Firm

1:30-2:15PM	Scientific Session II Moderator: Jennifer Viveiros Kate M Henesey, University of Delaware, PhD student *A Structural and Mechanistic Exploration of Wnt Ligand Maturation* Giana I Vitale, NIH, Post-baccalaureate fellow *Oxygen Gradients Direct the Spatial Organization of Epithelial-to-Mesenchymal Transition in an in vitro Breast Cancer Tumor Microenvironment* Chaitali Khan, NIH, Post-doctoral fellow *Drosophila brain metastasis model uncovers injury-like response in the host triggered by tumor cells* Renee Pepper, JHU, Post-doctoral fellow *Skoupocytosis: a role for homeostatic microglia in synaptic proteostasis*	Mountcastle Auditorium
2:15-2:30PM	Break	-
2:30-3:30PM	**Keynote Address:** Chantelle Evans, Duke University Investigating the temporal dynamics of mitochondrial turnover in neurons	Mountcastle Auditorium
3:30-4:30PM	Poster Session II	Stile room
4:30-6:00PM	Social Hours	Greenhouse Cafe

Poster Session I

10:30-11:30 AM

Poster No.	Name	Poster Title
1	Stefania Mehedincu	American University
2	Rebecca Moffat	National Institute of Health
3	Kedhar Narayan	American University
4	Jelani O. Jarrett	Johns Hopkins University
5	Rohan Hosuru	National Institute of Health
6	Margery Chen	Johns Hopkins University
7	Giana I. Vitale	National Institute of Health
8	Peri Wivell	George Washington University
9	Jarin Taslem Mourosi	Catholic University
10	Yogeshwari Singh	University of Delaware
11	Grace Hillmer	American University
12	Neha Tripathi	Johns Hopkins
13	Willow Rock	Johns Hopkins University
14	Veronica Locker	American University
15	Hannah Haller-Hidalgo	Johns Hopkins University
16	Eleana Parajon	Johns Hopkins University
17	Rajan Jayasankar	Johns Hopkins University
18	Whitney S Sambhariya	Johns Hopkins University
19	Kate Henesey	University of Delaware
20	Tiffany Andohkow	National Institute of Health
21	Tanya Nesterova	Johns Hopkins University
22	Shinwon Ha	Johns Hopkins University
23	Sarah S. Kleb	George Washington University
24	Taqdees Gohar	Johns Hopkins University
25	Sounak Sahu	National Institute of Health

Poster Session II

3:30-4:30 PM

Poster No.	Name	Poster Title
1	Emma Morin	Johns Hopkins University
2	Stefania Mehedincu	American University
3	Xinyi (Cindy) Chen	Johns Hopkins University
4	Shravan Balasubramaniam	Johns Hopkins University
5	Natalia Maiorana	Johns Hopkins University
6	Tamara McErlain	National Cancer Institute
7	Ambarisha (Amrish) Samantaray	Johns Hopkins University
8	Hasset Tibebe	American University
9	Justin Pellicciotti	Johns Hopkins University
10	Niladri Sinha	Johns Hopkins University
11	Janelle Bellot	Johns Hopkins University
12	Sheetal Kooduvalli	Johns Hopkins University
13	Haoyuan Jing	Johns Hopkins University
14	Efren Barragan	El Paso Community College
15	Brooke Waechtler	Johns Hopkins University
16	Rachel Yang	National Institute of Health
17	Mark Allan Co Jacob	Johns Hopkins University
18	Sierra Williams-McLeod	Johns Hopkins University
19	Renee Pepper	Johns Hopkins University
20	Sebastian Lira	Johns Hopkins University
21	America Pinela	El Paso Community College
22	Brandon Onochie	University of Maryland, Baltimore County
23	Yanrui Guo	Johns Hopkins University
24	Janelle Bellot	Johns Hopkins University
25	Margarita Tsapatsis	Johns Hopkins University

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All Abstracts

KEYNOTE ABSTRACT

Investigating the temporal dynamics of mitochondrial turnover in neurons

Presenting Author: Chantell Evans, PhD
Duke University, Department of Cell Biology

Mitophagy, the selective removal of damaged mitochondria, is critical for neuronal maintenance and is implicated in neurodegenerative diseases, including Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). In this pathway, mitochondrial damage triggers phospho-ubiquitination of outer mitochondrial membrane proteins, which recruit autophagy receptors like optineurin (OPTN). Damaged mitochondria are sequestered in autophagosomes and eliminated from the cell via lysosome fusion and acidification. However, little is known about the temporal dynamics of autophagosome formation and subsequent lysosomal degradation in neurons. Previously, we found that damaged mitochondria persisted in the soma of primary hippocampal neurons twenty-four hours after treatment, but mitochondria were quickly degraded within an hour of treatment in non-neuronal cells. These findings indicate mitochondrial turnover could take days to complete in primary neurons, potentially contributing to neuronal vulnerability and neurodegenerative disease pathogenesis. Here, we investigate the temporal dynamics of OPTN-mediated neuronal mitophagy and identify the molecular mechanisms contributing to delayed mitochondrial turnover in neurons.

The role of Bone Morphogenetic Protein 2 (BMP2) in Bone Marrow Adipogenesis

Presenting Author: Kelechi Chukwuocha
Kelechi Chukwuocha, Anja Nohe
University of Delaware

Bone Morphogenetic Protein 2 (BMP-2), a potent growth factor in the TGF-Î² family, crucially maintains bone homeostasis by signaling through BMP receptor type IA (BMPRIa). While BMP-2 traditionally stimulates osteogenesis, it has been shown to potentially induce adipogenesis, posing a challenge in understanding the molecular switches governing these processes. High BMP-2 concentrations lead to BMPRIa cleavage, but the downstream effects and the factors determining osteogenesis or adipogenesis activation remain elusive.

Mesenchymal stem cells' differentiation into adipocytes relies on Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ) activation. However, the intricate interaction between BMP-2/BMPRIa and PPAR-γ in bone marrow adipogenesis is poorly understood. Our hypothesis posits that BMPRIa cleavage upon BMP-2 stimulation drives adipogenesis in bone marrow stromal cells.

Primary bone marrow stromal cells from C57BL/6 mice were cultured, stimulated with varying BMP-2 concentrations, and analyzed. Antibodies against BMPRIa and PPAR-γ, along with western blotting, uncovered BMPRIa cleavage. Immunostaining revealed the nuclear translocation of the cleaved segment, colocalizing with PPAR-γ. Caspase1 was identified as responsible for BMPRIa cleavage; inhibiting caspase1 not only downregulated PPAR-γ expression but also prevented BMPRIa cleavage and translocation.

This study reveals that caspase1-mediated BMPRIa cleavage drives adipogenesis in primary bone marrow stromal cells through nuclear interactions. Insights into these molecular mechanisms enhance our understanding of adipogenesis within the bone marrow.

Deciphering the molecular intricacies of BMP-2-mediated adipogenesis and regulating adipocyte numbers/functions may offer therapeutic avenues for addressing conditions such as low bone mineral density and osteoporosis.

Enzymatic Activity and Structure of E. coli's RNA Cap Decapping Protein: NudC

Presenting Author: Yogeshwari Singh
Yogeshwari Singh, Henry Anderson, Lingting Li, Imalka Mudiyanselage, Jared Schrader, Yu Zhang, Karl Schmitz, Jeremy G. Bird
University of Delaware

RNA stability relies on cap identity. Eukaryotes use methyl guanosine (m7G) cap, while prokaryotes use metabolite caps like NAD(H). These caps, acting as non-canonical initiating nucleotides (NCINs), are incorporated during transcription initiation. NAD(H) is the most studied NCIN cap, impacting RNA stability in both prokaryotes and eukaryotes. The Nudix hydrolase family enzymes remove 5â€²² caps in both domains. E. coli possesses NudC, a decapping enzyme removing NAD(H) and other NCIN caps, leading to RNA degradation by RNaseE enzyme. With NudC showing vital evolutionary conservation, I hypothesize that NudC serves as a universal decapping enzyme in E. coli, exhibiting varied specificities for distinct cap moieties. In vitro transcription (IVT) assays, which I helped develop in Bird Lab, were conducted, generating RNAs with diverse NCIN caps. Radiolabeled nucleotides labeled the RNA, followed by NudC incubation are run on TBE-Urea gel which separates RNA based on length. Radiolabeled RNA was visualized and analyzed using a Saphire Phosphorous screen reader. Structural studies on E. coli NudC were carried out through X-ray Crystallography to comprehend the molecular mechanism of NudC-dependent bacterial RNA-decapping reaction. NudC displays differing activities for specific capped RNA, exhibiting superior decapping activity for NADH-capped RNAs compared to NAD+ capped RNAs, and for longer RNA over short-length RNA. Structural data shows that the NudC active site can accommodate more than just the cap moiety. The addition of NCIN caps by RNAP on specific RNAs and the existence of enzymes responsible for removing these caps suggests that NCIN-capping is a highly regulated process that affects the transcriptome of the cell under different growth phases or conditions. This research and its results can help us understand RNA regulation and gene expression, which are fundamental processes used by all branches of life.

A Structural and Mechanistic Exploration of Wnt Ligand Maturation

Presenting Author: Kate M. Henesey
Kate M. Henesey, Senel S. Tektas, David L. Raden, Scott Tibbetts, Andrew W. Harmon, and Erica M. Selva
University of Delaware

Wnt signaling is fundamental for the development of all animals and is responsible for the homeostasis of renewable cell populations in adults. During development, reduced or absent Wnt signaling results in a range of developmental defects and aberrant signaling in adults is a primary driver of cancers. While the numerous Wnt ligands and target receptors in signal-receiving cells have been extensively studied, the maturation of virtually all Wnt ligands within signal-sending cells is relegated to highly conserved yet understudied components, Porcupine (Por) and Wntless (Wls). Initial characterization of their function within the model Drosophila melanogaster has informed much of our understanding of Wnt ligand maturation. Further characterization of the mechanisms by which these players interact to produce mature Wnt ligand is essential to better define how Wnt signaling is regulated. We discovered that Wls forms homodimers dependent upon intermolecular disulfide bridge formation, and Wls dimers interact with Wnt (2:1, Wls-Wnt). Remarkably, this provides a universal mechanism for Wls dimerization and an explanation for the uncharacterized Wnt hand-off between Por and Wls. My project aims to characterize disulfide bonded Wls dimer interactions and to characterize Wls dimer importance in Wnt posttranslational modification, secretion, and signaling. We have generated various tagged forms of Drosophila Por and Wls wild-type and conserved cysteine mutants that were used in co-immunoprecipitation and functional experiments to interrogate this. We show that a conserved cysteine amino acid pair within Wls Dimerization Domain 1 (DD1) is required for Wls dimer interaction with Wnt. Furthermore, conserved cysteines in DD1 are required for proper Wnt glycosylation, secretion, and canonical signaling. This work establishes Wls dimer function within a cell to produce functional Wnt ligand, impacting our comprehension of Wnt cellular dynamics, processing, and secretion mechanisms."

Mechanoresponsive Adaptability in Pancreatic Cancer Cells and Tissue

Presenting Author: Eleana Parajon
Eleana Parajon, Douglas N. Robinson
Johns Hopkins University

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer characterized by early systemic dissemination and poor patient prognosis. Our lab identified a PDAC program of upregulation of key highly mechanoresponsive cytoskeletal proteins that accumulate in response to mechanical stress. Cancer cells are highly adaptive and must continuously adjust to their ever-changing environment, including nutrient and oxygen availability, stiffness of the local matrix, and the neighboring cells. The mechanoresponsive proteins compose an adaptability program that allows cells to respond to alterations in many of these parameters. In humans, this mechanoresponsive machinery encompasses nonmuscle myosin II proteins (NMIIA, NMIIB, and NMIIC), alpha-actinins (ACTN4, but not ACTN1), and filamin (FLNB with FLNA to a much smaller extent). However, it is still unclear how the mechanoresponsive cytoskeletal machinery drives PDAC dissemination and metastasis. NMIIA, NMIIC, ACTN4, and FLNB are expressed at low levels in normal pancreatic ductal epithelia but become highly expressed in PDAC. We are utilizing a three-dimensional, reconstituted tissue model with engineered cells where we have altered the adaptability program using the CRISPR-Cas9 system. We are also investigating how these engineered cells influence neighboring cell behaviors and tissue mechanics. This study has revealed that knocking out NMIIC and ACTN4 leads to significant changes in cortical tension, cell roundness, metabolic activity, and cell motility of metastasis-derived pancreatic cancer cells. This effort will decipher how alterations in these proteins impact cell growth, shape, motility, and invasiveness and will guide future strategies for manipulating these proteins towards a therapeutic end.

Micelles using SAILs and HDESs

Presenting Author: America Pinela
America Pinela and Silvester Guillen
El Paso Community College

A surfactant is an amphiphilic molecule exhibiting both hydrophobic and hydrophilic properties. When combined with ionic liquids, this molecule forms surface-active ionic liquids (SAILs). Hydrophobic deep eutectic solvents consist of a hydrogen bond donor and a hydrogen bond acceptor. These solvents have special properties that make them useful for studying the formation and behavior of nanostructures, including micelles, under specific conditions. Two hydrophobic deep eutectic solvents and three surface-active ionic liquids were tested to determine if micelles or reverse micelles could be created using only water. Five solvents and three water concentrations were combined using sonication at varied temperatures for varying durations. X-ray scattering techniques, including wide-angle and small angle, were used to identify the presence of these structures.

Studying cotranslational folding using arrest peptide assay in live human cells.

Presenting Author: Hannah Haller-Hidalgo
Hannah Haller-Hidalgo, Xiuqi Chen, Kamena Kostova, Christian Kaiser
Johns Hopkins University

Folding into native structures is a prerequisite for the biological functions of most proteins. In the cell, proteins begin to fold cotranslationally, while the ribosome is synthesizing the nascent polypeptide. Cotranslational folding is particularly important for preventing misfolding of large multidomain proteins into potentially cytotoxic species. As the nascent protein emerges from the ribosome during synthesis, it interacts with the ribosome and other cellular components that guide productive folding. This complex network of interactions is not captured in in vitro experiments with purified components. To detect cotranslational folding in living cells, we established an in vivo reporter assay in cultured human cells, using a translation-stalling arrest peptide (AP). In proof-of-concept experiments, the bacterial protein elongation factor G (EF-G), which our group has investigated previously, was used to characterize the AP reporter assay in HEK293T cells. Ongoing studies with EF-G serve to map the cotranslational folding environment around mammalian ribosomes. Future experiments with the human EF-G homolog, eEF2, will help to define conservation of folding pathways among closely related proteins. The novel AP reporter assay described here will provide much-needed experimental information about cotranslational folding in eukaryotes, which is challenging to obtain with other methods. The assay is scalable and thus has the potential for high-throughput measurements, helping to bridge the gap between in vitro protein folding studies and folding in the natural environment of eukaryotic cells.

Assessment of heterogeneity within the Drosophila germline stem cell niche

Presenting Author: Jennifer Viveiros
Jennifer Viveiros, Ambarisha Samantaray, and Erika Matunis
Johns Hopkins University

Adult stem cells reside in dynamic, supportive microenvironments termed niches, which are generated by specialized niche cells. Niches are often complex and composed of functionally cooperative subpopulations of cells rather than uniform populations. Gaining insight into niche composition is fundamental to our understanding of tissue homeostasis. Using the Drosophila melanogaster testis stem cell niche as a model, we can further our understanding of stem cell niches by investigating their cellular composition and gene expression programs. This niche contains three cell populations: post-mitotic somatic hub cells (or niche cells), which are surrounded by two types of stem cells, germline stem cells (GSCs) that differentiate into sperm, and cyst stem cells (CySCs) that give rise to somatic support cells. Previous in situ hybridization (ISH) and immunostaining experiments have suggested that hub cells may not uniformly express the same transcriptional program, raising the possibility that they are a heterogeneous population of cells. Intriguingly, hub cells descend from somatic gonadal precursors (SGPs) that arise from three distinct parasegments (PS) in embryogenesis, suggesting that developmental origin could underlie previously observed heterogeneity. Here, we examine hub cell heterogeneity with respect to origin, position, and function within the hub. Using lineage tracing tools, we find that adult hub cells arising from PS 11 do not differ in position or endogenous signaling compared to their counterparts. We also observe that not all hub cells require expression of proteins integral to anchoring the hub to the basement membrane but loss of these proteins in the entire hub alone displaces the cluster of cells. This is supported by our results suggesting most hub cells contact the basement membrane while also making contacts with GSCs. Future work will examine the role of PS 11 cells in the adult hub and investigate differences in gene expression amongst hub cells.

The role of ubiquitin and multivesicular body formation in the stem cell niche of the Drosophila melanogaster testis

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Post-translational protein modifications such as ubiquitylation control protein interactions, stability, function, and localization, all of which are essential for maintaining optimal conditions in the cell. The Drosophila melanogaster testis stem cell niche serves as an ideal model to understand the role of ubiquitylation in stem cell maintenance. The niche supports two different population of cells- germline stem cells (GSCs) which later differentiate into sperm and cyst stem cells (CySCs) which support the germline. Here we find that RNAi-mediated knockdown of the endosomal sorting complexes required for transport (ESCRTs) in CySCs results in a dramatically enlarged niche due to an increased number of cells, a striking phenotype, as niche cells are normally quiescent. ESCRTs recognize ubiquitylated receptors in endosomes and control downstream formation of multivesicular bodies (MVBs) necessary to silence receptor signaling, leading us to a model where ESCRTs mediate signaling from CySCs to their niche. Consistent with disruption of endocytic trafficking and MVB formation we detected ubiquitin and early endosome accumulation in CySCs following ESCRT knockdown. We hypothesize that ubiquitylated and internalized receptors accumulate in endocytic vesicles and continue to signal to the niche, driving hub cell proliferation. Disruption in ESCRT functioning is seen in many diseases such as cancer and we hope to understand the etiology through pathways that lead to abnormal cellular communication.

Comprehensive Functional HIV Cure Strategy: The Role Of HDAC And PARP Inhibitors In The Clearance Of Latent Infections

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HIV remains a global pandemic, primarily due to latent reservoirs that are unresponsive to existing antiretroviral treatments. The ""Kick-and-Kill"" strategy for HIV cure involves using Latency Reversing Agents (LRAs) to reactivate dormant HIV-infected cells (""kick"") and subsequently leveraging the immune system to eliminate these reactivated cells (""kill""). Although the HDAC inhibitor (HDACi), the first FDA-approved drug for cutaneous T cell leukemia and one of the most extensively studied LRAs, did lead to a reduction in reservoir sizes in clinical trials, the effect was modest and short-lived, highlighting the limitations of HDACi. Thus, improving the latency reversal effect of LRAs is essential. Tankyrase, a member of the PARP enzyme family, influences Hippo and Wnt/Î²-catenin signaling pathways, with four FDA-approved PARP inhibitors (PARPis) targeting Tankyrase used in ovarian cancer treatment. Our approach involved assessing various inhibitors, targeting Tankyrase, Hippo, or Wnt/Î²-catenin pathways, alone or alongside vorinostat, to stimulate HIV expression in J-Lat cells which are the HIV latently infected model cell lines. Our results revealed that none of the inhibitors alone reactivated HIV expression. However, combining vorinostat with either Hippo inhibitors or Tankyrase, inclusive of FDA-approved PARPis, synergistically enhanced latency reversal by about threefold without impacting cell viability.

In conclusion, our study indicates that Tankyrase and Hippo signaling pathways substantially augment latency reversal when used in conjunction with vorinostat. This synergy underscores the potential of a dual therapy involving HDACi and PARPi. Considering the FDA approval of several HDACis and PARPis, this strategy offers a promising pathway for accelerating HIV treatment advancements.

The two-tether system spatiotemporally regulates bacterial cell division in E. coli

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During cell division, bacterial cells must spatiotemporally regulate synthesis and degradation of the septal cell wall (sPG) to avoid osmotic lysis. The physical basis behind the regulatory mechanisms that allow bacteria to divide is unknown, although it can open doors to novel antibiotic treatments that target the bacterial septal wall. Bacterial septal wall remodeling is performed by a milieu of 12 essential proteins in the midcell which are members of a macromolecular complex known as the divisome. Through theoretical modelling performed in synergy with single molecule biophysics experiments, we have recently discovered a plausible mechanism for the late stages of bacterial cell division in which assembled FtsZ filaments in the midcell act as a scaffold. Once all divisome proteins have assembled in the midcell, the Brownian ratchet mechanism is responsible for the corralling of sPG synthase FtsWIQLB and constriction regulator FtsN together to regulate the synthesis of a spatially smooth layer of sPG in E. coli. However, little is known about the early stages of bacterial cell division, in which FtsN and FtsWIQLB are present in lower concentration. In addition to this key difference, the FtsZ protoring has not yet fully condensed in the early stage. The FtsZ protoring is tethered to the inner membrane by bitopic membrane protein ZipA and membrane-associated protein FtsA. We propose a mechanism for the competing roles of ZipA and FtsA in early-stage bacterial cell division in E. coli, in which inactive FtsA negatively regulates and active FtsA positively regulates FtsZ protoring condensation; meanwhile, ZipA positively regulates FtsZ protoring assembly.

Outspread determines organ dimensions during salivary gland tubulogenesis

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Epithelial tubular organs are essential for viability in all higher multicellular organisms. Salivary gland (SG) development in the Drosophila embryo provides an excellent model system to study the processes by which a two-dimensional (2D) epithelial sheet morphs into a three-dimensional (3D) epithelial tube. This process is driven by morphogenetic signals acting on non-muscle myosin II (MyoII) to induce changes in cell shape and arrangement. We have identified the novel gene outspread (osp) as crucial for shaping the 3D structure of the SG; its cellular role and relevant molecular interactions, however, are not yet fully understood. We hypothesize that osp modulates MyoII activity to coordinate cellular changes during SG morphogenesis. To test this hypothesis, we employed a combination of genetic, molecular, and imaging techniques to dissect Osp molecular and cellular functions during the early stages of SG morphogenesis. We find that perturbations in osp expression change SG tube dimensions in much the same way as when we manipulate MyoII activity. In invaginating SG cells, Osp protein co-localizes with MyoII and a known myosin phosphatase on the apical medial domain of cells as they undergo apical constriction. We have discovered that one of the two PH domains of Osp is crucial for apical localization and Osp molecular function. Additionally, we identified a direct interaction between Osp and the membrane lipid PI (4,5)P2, suggesting that PI(4,5)P2 directs the apical localization of Osp. Interestingly, we found that Osp mediates condensate formation when expressed in cultured cells through its intrinsically disordered regions (IDRs); we are now testing the physiological significance of these Osp condensates. By uncovering the intricate interplay between Osp and key morphogenetic factors, including phosphoinositides and the Rho GTPase-MyoII pathway, we expect our studies will shed light on the molecular and cellular mechanisms shaping SG geometry.

Loss of Tpl2 activation increases EGFR/Her2 signaling in transduced keratinocytes

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Cutaneous squamous cell carcinoma (cSCC) is the second most common form of skin cancer, affecting 1.8 million people per year. Dysregulation in mitogen-activated protein kinase (MAPK) signaling is frequently found in cSCC. Our laboratory has previously shown that tumor progression locus 2 (Tpl2) is tumor suppressive for cSCC. Mice lacking the Tpl2 gene (Tpl2 -/-) have an increased activation of receptor tyrosine kinases (RTKs) in the MAPK pathway, contributing to increased skin tumor incidence and progression to cSCC. We hypothesized that Tpl2-/- mice who developed cSCC have abnormal EGFR, Her2, and Her3 signaling in comparison to wild-type mice (Tpl2+/+). To test this hypothesis a two-step chemical carcinogenesis protocol was conducted. Sixty male and female wild-type and knockout mice were initiated once with DMBA and promoted with TPA twice with cSCC and then administering one of the two drugs (gefitinib and lapatinib) that inhibit receptors in the MAPK pathway. Tumors were removed from the mice and immunohistochemistry was performed to quantify the expression levels of EGFR, Her2, and Her3 on normal skin, benign tumors, and malignant tumors. Our findings indicate that Tpl2 -/- mice display higher numbers of papillomas and cSCCs in the two-step carcinogenesis study. Tpl2 -/- keratinocytes showed an increased signaling in EGFR and p-EGFR when Her2 was inhibited by lapatinib. Her2 and Her3 signaling was elevated in both genotypes when EGFR was inhibited by gefitinib. Collectively, the effects of gefitinib and lapatinib may have implications for effective skin cancer drugs.

PARIS (ZNF746) Mediates Dopamine Neuron Loss via DNA Methylation-Mediated Suppression of LMX1B in Parkinson's Disease

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Parkinson's Disease (PD) is characterized by motor dysfunction from the deterioration of dopamine neuron (DN), which are integral to movement, reward, and motivation. DNs are particularly susceptible to damage in PD and adversely affect the disease trajectory. Age-associated changes in the DNA methylome also regulate DNâ€™s transcription, particularly within the substantia nigra region, which is severely compromised in PD. However, the mechanisms driving DN degeneration in PD remain elusive. Therefore, this study aimed to investigate the biomolecular mechanism of DN death in PD. In our study, we identified the interaction between PARIS and the complex of DNA methyltransferases, which led to a complex that possibly explains the DNA methylation processes within the PD model. We then found an augmentation in the hyper-methylation of specific DNA sequences using several next generation sequencing screenings. Subsequently, we identified Lmx1b, a marker of DN and one that maintains the stability of DN, as one of our candidates from screening. We also sought to determine whether Lmx1b reduction results from hyper DNA methylation caused by PARIS in vivo. We developed Adeno-associated viruses-dCas9-demethylase with sgRNA system and administered it to mice that overexpressed PARIS. Through this, we confirmed that PARIS reduced Lmx1b expression by reversing DNA methylation through the dCas9 system. Furthermore, we applied these tests to human DN derived from embryonic stem cells. We observed an increased level of PARIS along with a decrease in LMX1B levels. And we identified reduced LMX1B expression in TH-positive cells from the midbrain of PD patients. Our findings reveal the substantial role of PARIS in DN attrition in PD through the methylation-mediated repression of Lmx1b. Our study findings could illuminate DN methylation patterns specific to PD, especially those associated with Lmx1b, facilitating the development of future pharmacological strategies targeting human DN.

Investigating the Origin of Prostate Macrophages through Genetic Lineage Tracing

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The prostate immune microenvironment is highly heterogeneous, containing distinct populations of cells performing different roles in prostate organogenesis and the promotion or resolution of different prostatic diseases. Under disease conditions, different subsets of macrophages can either promote or resolve inflammation, causing tissue damage or aiding in tissue repair. Previously, it was believed that tissue-resident macrophages were maintained and repopulated by blood-circulating monocytes derived from progenitors in adult bone marrow (BM). Recent studies have revealed that several tissue-resident macrophage populations arise from embryonic precursors in the yolk sac (YS) or fetal liver (FL) prior to birth and continue to maintain themselves throughout adulthood. We hypothesized that distinct populations of macrophages in the mouse prostate arise from embryonic origins and contribute to the heterogeneous cellular landscape throughout prostate development and disease progression. To identify yolk-sac derived macrophages in the mouse prostate, we genetically labeled embryonic macrophages using a yellow fluorescent protein (YFP) reporter in Cx3cr1CreERT2/+; R26REY/+ mice. Prostate tissues containing lineage-labeled cells were then collected during different stages of organ development to assess their presence and localization in the prostate. Through immunohistochemical and immunofluorescence staining for YFP, macrophage marker F4/80 and proliferation marker Ki-67, we show that the mouse prostate contains distinct populations of macrophages which originate from embryonic precursors. Further, these cells proliferate locally within the prostate and persist in the tissue after puberty. This data indicates a tissue-specific role of embryonic macrophages in regulating prostate organogenesis. Applying this lineage-tracing method to models of prostatic diseases will further expand on the involvement of embryonically derived macrophages in their pathogenesis.

Spatiotemporal modulation of growth factors directs the generation of multilineage mouse embryonic stem cell-derived mammary organoids

Presenting Author: Sounak Sahu

Sounak Sahu, Sarthak Sahoo, Teresa Sullivan, T. Norene O' Sullivan, Sevilay Turan, Mary E. Albaugh, Sandra Burkett, Bao Tran, David Salomon, Serguei Kozlov, Karl R. Koehler, Mohit K. Jolly, Shyam K. Sharan

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Stem cells have a remarkable capacity to not only self-renew and differentiate into multiple lineages but also to self-assemble and self-organize into complex and functional tissues and organs. Using directed differentiation, here we report an optimized state-of-the-art in vitro condition for the generation of multilineage ESC-derived mammary organoids (MEMOs), that mimic the tissue organization of a functional mammary gland. We have customized an organoid culture system by self-organizing mouse ESCs that differentiate to form complex skin and associated skin appendages. We use stepwise modulation of BMP, TGFÎ², and FGF signaling pathways to co-induce surface ectoderm and mammary mesenchymal cells within an embryoid body. We show that sequential activation of BMP4, Parathyroid hormone, and subsequent inhibition of hedgehog signaling in the spherical cell aggregate can block the formation of hair follicles in the dermal mesenchyme and promote mammary lineages, concomitant with embryonic mammary commitment. We recapitulate the dermal-epidermal interaction in these organoids and the development of mammary lineages characterized by hormone-sensing luminal and myoepithelial cells. The lactogenic hormonal stimulation led to the secretion of milk proteins into the organoid lumen further confirming the functional capacity of mammary organoids. Using single-cell-RNA sequencing, we identified gene expression profiles that demonstrate the presence of mammary-specific epithelial cells, fibroblasts, and adipocytes. MEMOs undergo ductal morphogenesis in Matrigel and can reconstitute the MG in vivo. Further, we demonstrate that the loss of function in placode regulators LEF1 and TBX3 in mESCs results in impaired skin and MEMO generation. In summary, our MEMO model is a robust tool for studying the development of ectodermal appendages, and it provides a foundation for regenerative medicine and disease modeling.

Evaluating HIV-1 Infectivity and Virion Maturation Across Varies Producer Cells with a Novel FRET-Based Detection and Quantification Assay

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HIV virion maturation is a vital life cycle phase involving the cleavage of Gag Polyprotein into functional units. Developments in HIV-1 treatment have emphasized a need for a more comprehensive understanding of the virion maturation process. The small virion particles size requires electron microscopy to assess maturation, a labor-intensive method subject to human bias. This has led to a variation in reported maturation efficiency, ranging from 80-99% across laboratories. We previously developed a FRET-based virion visualization system that enables rapid, reliable, and unbiased differentiation between mature and immature viruses using fluorescent microscopy. Though T-cells are predominantly recognized as the primary producers of HIV-1, much existing research on maturation has been conducted using kidney cell lines due to the optimization for laboratory manageability. This approach has led to a gap in understanding virion production by T-cells. This study extends previous research by further investigating virion maturation in T-cell line Jurkat cells and comparing these findings with the well-documented maturation process in 293T cells The maturation rate was characterized using HIV-1 Env pseudotyped, single-rounded viruses labeled with the HIV-1 Gag-iFRETÎ"Env for fluorescent microscopy. The relative infectivity was measured by TZM-bl assay. The 293T-derived virions showed a maturity rate of 81.79%, which is consistent with findings from previous studies. Jurkat cells demonstrated a reduced maturation rate of 68.67%. The relative infectivity of viruses produced from Jurkat cells was 65.3%, with the infectivity of viruses derived from 293T cells set as 100, indicating that the infectivity of viruses derived from Jurkat cells was correlated with virion maturation efficiency in comparison to viruses derived from 293T cells. This observation shows the dynamic nature of virus-host interactions and their implications for virion production and infectivity.

Determining the role of ApoD in avian neural crest cell migration

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Natalia Maiorana, Stephanie Peralta, and Michael L. Piacentino

Neural crest cells (NCC) are multipotent progenitors, giving rise to a diverse array of cell lineages within the developing embryo. Early in embryonic development, NCCs migrate throughout the growing embryo in response to chemokine signaling. However, evidence suggests that leading and following NCCs differ in their migratory behaviors, prompting us to ask how these different behaviors are coordinated. To this end, we found that signaling by the bone morphogenic protein (BMP) pathway peaks to initiate NCC migration from the midbrain in avian embryos. Using differential RNA sequencing to identify BMP targets during migration, we found the glycoprotein Apolipoprotein D (ApoD) to be downregulated by BMP signaling. ApoD is a member of the lipocalin family of lipid transporters and is responsible for the transport of arachidonic acid during oxidative stress and inflammation in the adult nervous system. Using hybridization chain reaction-based fluorescent in situ hybridization (HCR-FISH) in wild type embryos, we found APOD expression restricted to a subset of follower cells during early phases of midbrain NCC migration but were surprised to find it absent from the leading cells. From this observation, we hypothesized that ApoD contributes to collective NCC migration by regulating follower cell migration. To test this, we manipulated APOD expression by performing ex ovo electroporation in gastrulating chicken embryos. We then examined migration patterns using immunohistochemistry at the 8- and 9-somite stages. Using a splice-blocking morpholino to knock down APOD expression, we found significantly reduced NCC migratory area, with the follower cells selectively delayed in their migration. These results identify ApoD as a novel regulator of early NCC migration and implicate arachidonic acid-dependent signaling as a critical mediator of collective cell migration during early vertebrate development.

Negative feedback regulation of UV-induced ribotoxic stress response controls programmed cell death

Presenting Author:Niladri Sinha

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While exposure to ultraviolet (UV) radiation has long been known to damage DNA to elicit the DNA damage response (DDR), it also damages RNA triggering transcriptome-wide ribosomal collisions to elicit a ribotoxic stress response (RSR). However, the relative contributions, timing, and regulation of these pathways in determining cell fate is unclear.

In this study, we used time-resolved phosphoproteomics, chemical-genetic, single-cell imaging, and biochemical approaches to uncover a chronological atlas of signaling events activated in cells responding to UV damage. We determined that global ribosomal collisions activate the RSR kinases GCN2 and ZAK within minutes of UV exposure, which remodel the early signaling landscape of cells responding to UV stress. We discovered that UV-induced apoptosis is almost entirely mediated by the RSR kinase ZAK and not through the DDR. Further, we identified two negative-feedback modules that regulate ZAK-mediated apoptosis in response to UV stress: (1) GCN2 activation triggers a negative-feedback loop that attenuates ZAK-mediated RSR-signaling by preventing the accumulation of collided ribosomes, and (2) ZAK's kinase activity leads to autophosphorylation of a conserved phosphodegron that enables ZAK to regulate its stability through ubiquitin-mediated proteasomal degradation by the CUL1-RBX1-SKP1 (CRL1)-Î²-TrCP E3 ubiquitin ligase complex. These events tune ZAK's activity to the level of ribosomal collisions to establish regimes of homeostasis, tolerance, and death and reveal its key role as the cellular sentinel for nucleic acid damage.

Investigating the Relationship between the Pancreatic Tumor Microenvironment and Cancer Health Disparities

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The incidence and mortality rates for pancreatic cancer are highest among African Americans. Several factors including lower socioeconomic status, lack of accessible quality health care, biology, and genetics are all contributors to these poor health outcomes. However, the biology of pancreatic cancer pathogenesis in Black patients has largely been ignored. Furthermore, increasing evidence from published studies on other cancer types highlight immunobiological differences between tumors from Black and White patients. Therefore, we hypothesize that pancreatic cancer health disparities observed between Black and White patients are being driven by immunobiological differences, that if better understood, could reduce this disparity. In this study, multiplexing immunohistochemistry was used to assess the immune and stromal cells in pancreatic cancer tissues from Black and White patients. Tissue microarrays constructed from archival pancreatic cancer tissues from patients (n = 90) matched on sex, age, stage, and time of surgery were used for this study. Our study found the CD8+ cytotoxic T cells and Tryptase+ mast cells were significantly decreased in cancer compared to benign tissue. CD4+ helper T cells and FAP+ fibroblasts in Black patients, specifically in Black women, were significantly increased in cancer compared to benign tissue. Additionally, FoxP3+ regulatory T cells were significantly increased in cancer compared to benign tissue, regardless of race. CD163+ M2 macrophages were significantly increased in cancer compared to benign tissue in Black patients. CD163+ M2 macrophages and FAP+ fibroblasts showed a positive correlation in tumor from both Black and White patients albeit not significant for White patients. Elevated FoxP3, FAP, and CD163 expression seen in tumor is consistent with published studies in predominantly white populations. However, future studies will be aimed at determining the significance of increased CD4 expression observed in Black women.

G-Protein-Coupled Receptor 84 Regulates Acute Inflammation in Normal and Diabetic Skin Wounds

Presenting Author: Paula Cooper

Paula O. Cooper, Sarah S. Kleb, Satish K. Noonepalle, Najuma S. Babirye, Veronica M. Amuso, Rohan R. Varshney, Michael C. Rudolph, Tanvir K. Dhaliwal, Darlene V. Nguyen, Miguel F. Mazumder, Brett A. Shook

George Washington University

Skin wound healing relies on a coordinated immune response composed of multiple overlapping phases. The innate immune response is influenced by countless factors concentrated at the site of injury. Among the regulatory factors for myeloid and lymphoid cell function, are lipids. Herein, we investigate a medium-chain fatty acid (MCFA) receptor, G-protein-coupled receptor 84 (GPR84), for its potential to propagate lipid-induced pro-inflammatory signaling during skin wound healing. We found Gpr84 expression to be upregulated by a variety of cell types shortly after inury, when pro-inflammatory signaling is most necessary. Through in vivo administration of a pharmacological antagonist and the MCFA decanoic acid (DA), GPR84 signaling was identified as a key component of myeloid cell inflammation in normal murine skin wounds. Treatment with the GPR84 antagonist early during wound-induced inflammation subsequently decreased wound closure and tissue repair, revealing lasting effects of GPR84 modulation during wound inflammation. Diabetic skin wounds heal slowly due in part to impaired early myeloid cell inflammation. Thus, we probed murine diabetic wounds for the components necessary for GPR84 signaling. Gene expression analysis showed a robust increase in Gpr84 in murine diabetic (db/db) wounds early after injury; however, immunostaining indicated impaired injury-induced dermal adipocyte lipolysis. Concordantly, lipidomic analysis revealed a significant reduction in MCFAs. Local injection of DA to diabetic mouse (db/db) wounds rescued myeloid cell numbers and improved subsequent wound closure and revascularization. Thus, GPR84 presents a readily targetable lipid signaling pathway for manipulating injury-induced tissue inflammation with beneficial effects on acute diabetic wound healing.

The Role of G Protein-Coupled Receptor 84 in Skin Wound Healing

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Inflammation is an essential stage during the tissue wound healing process, and its magnitude affects subsequent healing phases. Cell-cell communication plays a major role in the initial response of wound healing, where tissue-resident cells release proinflammatory signals to fight infection and recruit immune cells. G protein-coupled receptor 84 (GPR84), a medium chain fatty acid (MCFA) receptor, has been shown to contribute to myeloid cell function during tissue inflammation. Its expression increases in many cell types during inflammation, and it plays a role in myeloid cell chemotaxis, phagocytosis, and cytokine secretion. However, its role in skin wound healing is understudied. Through tissue immunostaining and quantitative RT-PCR, we show that multiple cell types increase the expression of GPR84 after injury. In particular, we find that keratinocytes and adipocytes express GPR84. We hypothesized that GPR84 signaling in these tissue-resident cells will contribute to their pro-inflammatory function during the inflammation phase of tissue repair, thus influencing overall wound healing. Here, we use genetic mouse models to selectively delete GPR84 in keratinocytes and adipocytes and investigate delays in wound healing. Using flow cytometry and tissue immunostaining, we assess changes in tissue inflammation and subsequent tissue repair by measuring re-epithelialization, and revascularization. As prolonged inflammation contributes to chronic, non-healing wounds, GPR84 reveals itself as a therapeutic target with clinical agonists and antagonists capable of modulating inflammation to improve wound healing outcomes.

Multiple Myeloma protects adipocytes from apoptosis by altering survival pathways

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Multiple Myeloma (MM), characterized by malignant B-cells in the bone marrow, is the second most common blood cancer in the United States. Although overall survival has increased three-fold in the last several decades, MM remains an incurable disease with a median life span of 5-8 years post-diagnosis. Obesity is a risk factor for MM, although the mechanisms by which obesity contributes to MM pathogenesis have not been fully elucidated. We have previously shown that adipocytes from obese individuals activate drug resistance mechanisms in MM cells. The purpose of this study was to further evaluate the bidirectional relationship between MM cells and adipocytes, to identify whether MM cells increase adipocyte survival pathways. Human adipose-derived stem cells (ASCs) were isolated from normal (BMI = 20-25 kg/m2), overweight (25-30 kg/m2), or obese (30-35 kg/m2) individuals undergoing abdominal liposuction. ASCs were differentiated into adipocytes before being co-cultured with two multiple myeloma cell lines (RPMI 8226 or U266B1). The cells were also treated with multiple myeloma drugs including bortezomib or a triple (bortezomib, dexamethasone, and lenalidomide) drug cocktail. Our findings indicate that multiple myeloma contributes to adipocyte viability by increasing the presence of survival factors BCL-2 and BCL-xL. Additionally, co-cultured adipocytes showed extended lifespan in comparison to non-co-cultured adipocytes. In conclusion, our study provides more insight into the bidirectional MM/adipocyte relationship that contributes to disease progression.

Mmsdh as a bridge between cortical dynamics and metabolic activity

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The cytoskeleton, a vital cellular framework comprised of structural and contractile proteins, plays a crucial role in maintaining cell morphology, motility, and division. In Dictyostelium discoideum, cytoskeletal proteins assemble into macromolecular structures known as contractility kits (CKs), which sense and accumulate at sites of mechanical stress, reinforcing the cortical cytoskeleton. Among these proteins, myosin II, cortexillin I, IQGAP1, and IQGAP2 form essential components of CKs, with myosin II monomers polymerizing into bipolar thick filaments (BTFs) to generate contractile force. Previously, our lab studied the phenotypic consequences of a phosphomimetic myosin II variant that is incapable of assembling into BTFs, leading to defects in cell growth and division. Surprisingly, in our efforts to identify genes capable of suppressing these defects, we discovered an unexpected candidate: mmsdh. Traditionally recognized as an enzyme within valine catabolism, mmsdh emerged as an interactor of CK components through a proteomic analysis of CK components. While mmsdh is conventionally metabolic, its involvement in the cortical cytoskeleton presents a novel and unexplored avenue of regulatory activity. We hypothesize that mmsdh mediates metabolic activity and cortical dynamics crosstalk through the CK system. To investigate this hypothesis, we created overexpressed and knocked down mmsdh cell lines. We will assess cytoskeletal parameters such as myosin II BTF assembly, cortical tension, and mechanoresponsiveness. In all, we plan to conduct metabolic studies including ATP determination, Seahorse Assays, and metabolomics to elucidate the cytoskeletal effects of mmsdh's interactors and validate the potential for crosstalk. By unraveling the mechanism of crosstalk mediated by mmsdh, our study aims to provide insights into a novel pathway of cytoskeletal-metabolic regulation that has not been explored previously.

Developing cryptic ACTL6B as novel TDP-43 loss of function biomarker

Presenting Author: Margery Chen
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Introduction

The nuclear clearance and cytoplasmic aggregation of splicing repressor TAR DNA/RNA-binding protein-43 (TDP-43) occur in amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) and approximately 50% of Alzheimer's disease (AD), driving neuron loss. However, it is not clear how early such loss of function occurs in human disease as there is no method of detecting TDP-43 dysregulation in living individuals. Since the loss of TDP-43 leads to cryptic exon inclusion, we propose that cryptic exon-encoded peptides may be detected in patient biofluids to indicate TDP-43 pathology.

Methods

We developed and characterized antibodies against cryptic actin-like protein 6B (ACTL6B) through protein blot analysis using TDP-43-knockdown (KD) SH-SY5Y neuroblastoma cell lysates. To test the antibodyâ€™s sensitivity and specificity for cryptic ACTL6B in human brains, we used immunofluorescence staining on control and FTLD-TDP brain tissues. We developed a Meso Scale Discovery (MSD) ELISA using the novel antibody.

Results

While the wild-type (WT) ACTL6B antibody detected an expected band in both WT and KD lysates, the cryptic ACTL6B antibody detected a band in only KD SH-SY5Y. Using this antibody, we detected ACTL6B signal in FTLD, but not control, brain tissues. The cryptic ACTL6B staining colocalizes with neurons that display TDP-43 nuclear depletion and/or cytoplasmic aggregation. Using transfected SH-SY5Y cells to overexpress either cryptic or WT ACTL6B, the MDS ELISA was sensitive and specific for cryptic ACTL6B.

Conclusions

Our findings provide evidence that our cryptic ACTL6B antibody is sensitive and specific for the ACTL6B cryptic peptide in both SH-SY5Y cells and human brain tissues. This antibody could be used to determine TDP-43 nuclear clearance/loss of function upon pathological staining, as our cryptic ACTL6B antibody stains some non-immunoreactive neurons for cytoplasmic phosphorylated TDP-43 but shows TDP-43 nuclear depletion.

Dynamic Intracellular Localization of Vasohibins Regulates Microtubule Detyrosination

Presenting Author: Sebastian Lira
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Microtubule (MT) detyrosination, which is catalyzed by Vasohibins 1 and 2 (VASH1/2), is a highly conserved post-translational modification involved in cell polarity and MT stability. Despite their impact on MT dynamics, the signaling pathways that control VASH1/2 function are largely unexplored. VASH1/2 preferentially acts on polymerized MTs, which are predominantly cytosolic in mammalian cells. Based on this, we hypothesized that cytoplasmic localization of VASH1/2 is critical for MT detyrosination. Here, we report that using live cell microscopy and immunofluorescent assays, we determined that VASH1/2 were primarily cytosolic in HeLa cells and that the nuclear-localized catalytic domain of VASH2 was unable to detyrosinate MTs. Using predictive algorithms, we identified a putative Nuclear Export Signal (NES) and a putative phospho-inhibited Nuclear Localization Signal (NLS) in VASH2. We used site-directed mutagenesis and pharmacological inhibition of Exportin-1 to validate the NES. By screening multiple mutations around the putative NLS, we identified S314 to be a key residue, whose phosphorylation inhibits the NLS since VASH2(S314A) mutant showed strong nuclear localization. By using a kinase inhibitor screening assay, we further identified CDK4 and CDK6 as potential candidates that inhibit VASH2 NLS. Based on these observations, we propose a model of intracellular spatial regulation of VASH2 function through competing nuclear export and import, acting downstream of kinases and phosphatases. In parallel, we have identified a highly similar mode of regulation for VASH1, suggesting a conserved mechanism. Finally, we have also discovered a conserved NES in the Tubulin Tyrosine Ligase (TTL) enzyme, which mediates MT tyrosination, and we are currently examining its role in TTL function. To summarize, we have demonstrated a role of nuclear transport in modulating MT detyrosination through dynamic subcellular localization of VASH1/2 downstream of kinases and phosphatase

Pericyte derived lipids alter disseminating tumor cell fate decisions

Presenting Author: Tamara McErlain
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National Cancer Institute

Metastasis accounts for 90% of cancer related mortality and is aided by the formation of a pre-metastatic niche. Perivascular cells, including pericytes, initiate development of a pre-metastatic microenvironment upon activation by circulating tumor derived factors, becoming synthetic, migratory, and proliferative. The microenvironmental changes associated with perivascular cell activation increases metastasis. Extravital imaging demonstrated that disseminated tumor cells (DTC) interact with pericytes on extravasation into the lung. We hypothesized that direct pericyte-DTC interaction in the early metastatic microenvironment could confer a survival advantage to DTC. Co-culture experiments were used to assess DTC fate after pericyte contact. Primary lung pericytes transfer lipids to metastatic breast tumor cells (4T1), but not to non-metastatic cells (67NR). Gene expression data from metastatic 4T1 cells after direct co-culture with pericytes demonstrated activation of pathways related to syncytium formation. In normal physiology, pericytes act in a syncytium to regulate blood flow by responding to mechanochannel activation induced by changing blood pressure. We hypothesized that direct contact with tumor cells activates mechanosensitive pericytes to initiate lipid transfer. Lipid transfer was dependent on direct contact and was not observed with transwell assays. Mechanosensitive calcium channel inhibitors significantly reduced the transfer of lipids to 4T1 cells. Intracardiac injection of 4T1 cells isolated from co-culture demonstrated increased lung colonization indicated by enrichment in the number of small lesions compared to the monoculture group, and lower proliferation indicated by reduced ki67 status compared to the monoculture group. These data suggest that tumor cells are reprogrammed by direct pericyte contact in the early metastatic lung to aid persistence and colonization.

Investigating epigenetic inheritance during asymmetric cell divisions in C. elegans

Presenting Author: Yanrui Guo
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Asymmetric histone inheritance was first discovered in the asymmetric cell division (ACD) in Drosophila melanogaster male germline stem cells (GSCs). The disruption of this asymmetry results in both stem cell loss and progenitor germ cell tumors. However, whether asymmetric histone inheritance is a general developmental mechanism crucial for cell fate decisions, and whether it interacts with established extrinsic developmental pathways are questions that remain unanswered. In this study we approach these unknowns through investigation of histone densities in established asymmetric cell divisions in C. elegans. Throughout the C. elegans cell lineage, ACDs result in two daughter cells with different developmental fate potentials. Through CRISPR-Cas9-mediated gene editing, live cell imaging, and immunostaining, we measured histone H4, H3, and H3 histone variants. We find that specific lineages display asymmetric histone inheritance. Interestingly, these divisions are also coordinated by conserved cell-signaling pathways including both the Wnt signaling and tyrosine-kinase signaling pathways. To further investigate the connection between this intrinsic and extrinsic regulation of histone inheritance, we will dysregulate extrinsic signaling through genetic alleles and RNAi, and measure the resulting effects on histone asymmetry. Together, these studies will provide novel insights into the crosstalk between cell signaling pathways and epigenetic inheritance during development.

Drosophila brain metastasis model uncovers injury-like response in the host triggered by tumor cells

Presenting Author: Chaitali Khan
Chaitali Khan and Nasser M Rusan
National Institute of Health

The tumor microenvironment, constituted by resident and recruited cells, is critical for metastasis to secondary organ sites. Several studies have investigated the role of recruited immune and stromal cells in metastasis; however, the complexity of the current organ models has limited our understanding of tissue-resident cells and the heterogeneity among cell types known to support tumor metastasis. Simpler model organisms such as Drosophila provide a tractable system to investigate the function of tissue-resident cells in the context of whole organ and organism. However, to date, very few Drosophila metastasis models exist. To overcome this, we developed a reliable model for studying metastatic tumors at the secondary organ sites by modifying the classical allograft transplantation assay. Our method demonstrates robust metastasis in adult Drosophila organs, including the brain and ovaries. We used this model to investigate one of the least understood cancer phenomena: how a tumor invades the brain surface and breaches the blood-brain barrier (BBB). Our system allows unprecedented analysis of cellular dynamics of brain tumor invasion at different metastatic stages. Fly brains contain a structure equivalent to the mammalian BBB, comprising two sets of surface glial cells, the perineurial glia (PNG) and the sub-perineurial glia (SPG). Further, extracellular matrix (ECM) proteins form the outermost layer of the brain -the basement membrane (BM). Our analysis of the early stages of invasion revealed that tumors remodeled the BM via a collective mode of tumor migration and invasion. We discovered that BBB cells were remodeled yet remained associated with the migrating invasive front. In addition, the tumor-BBB interface at the invasive front contained disorganized ECM components, suggesting that matrix secretion might be essential for brain metastasis. Furthermore, the invasion sites recruited tumor-associated macrophages (TAMs) and exhibited upregulation of Jun-Kinase i

Spatial Localization of Cancer Stem Cell Phenotypes, CD8+ Cytotoxic T cells, Vasculature, and Hypoxia in 4T1 Tumors: A Mouse Model of Triple-Negative Breast Cancer

Presenting Author: Rebecca Moffat
Rebecca L. Moffat, Elise L. Femino, Ana L. Gonzalez, Leandro L. Coutinho , Lisa Ridnour, Stephen J. Lockett, and David A. Wink
National Institute of Health

Triple-negative breast cancer (TNBC) is an extremely aggressive form of cancer with high likelihood of recurrence following treatment. Moreover, treatment options are limited, because of a lack of molecularly-targeted therapies. Populations of cancer stem cells (CSCs) within tumors lead to this likelihood of recurrence and pose crucial complications for treatment of aggressive forms of cancer, such as triple-negative breast cancer, as these cells allow for renewal, differentiation, drug resistance, and tumorigenicity. A look inside the tumor environments in which these cells reside provides understanding of their function. Regions of hypoxia and inflammation have been known to induce stress responses within tumor environments, it is hypothesized that these stress responses induce cancer stem cells and drive them to migrate up the oxygen gradient towards vasculature, ultimately promoting metastasis. Cell surface markers such as CD44+, CD44v6+, CD49f+, and CD24- can be used to identify cells that maintain cancer stem cell properties within tissues. The spatial localization and distance of cancer stem cells in relation to regions of hypoxia and vasculature informatively reveals aspects of their function. In the 4T1 tumors, a syngeneic mouse model of human triple-negative breast cancer, CSCs were found to have decreased populations in tumors where the active NOS2 protein had been knocked out in the mouse (NOS2KO), compared to wild-type mice. Further study is needed to understand the spatial relationship between hypoxia and CSCs. This will be done using cultured live cells in the Restricted Exchange Environment Chamber (REECs), which imposes a defined gradient of hypoxia and nutrients across the cell culture. Cancer stem cells, likely the origin of cancer metastasis, may pose a vital target in cancer treatment as they maintain resistance to conventional chemotherapy and treatments of radiation.

Targeting lipid metabolism in breast cancer metastasis

Presenting Author: Rohan S Panaparambil
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Metastasis is the leading cause of cancer mortality, particularly in breast cancer, where 5-year survival rates drop from over 90% for localized disease to 30% for metastatic disease. Despite this, the molecular processes and dependencies underpinning metastasis remain poorly understood. Lipids are a major class of biomolecules which play a major role in enabling aggressive proliferation by way of supplying membrane biomass, a source of energy, and activation of pro-growth signaling pathways. We hypothesized that breast cancer metastases must activate lipid metabolism through the sterol regulatory element-binding protein (SREBP) pathway, which turns on the expression of genes necessary for lipid synthesis and uptake upon activation. To test this hypothesis, we isolated tumor clusters from primary mouse mammary tumors and embedded them in a 3D culture system that models metastatic colony formation, and studied the effect of pharmacological SREBP inhibitors on colony formation ex vivo.

We found that SREBP inhibition led to reduced colony formation and induced apoptosis in clusters isolated from two GEMM models of breast cancer, and reduced mRNA expression of a panel of genes involved in fatty acid, cholesterol, and lipoprotein metabolism. Furthermore, we found that cholesterol was sufficient to rescue growth of 2D cultures treated with SREBP inhibitors. By using cyclodextrins to modulate plasma membrane cholesterol, we found that PM cholesterol depletion led to reduced colony size and sensitivity to SREBP inhibition, while PM cholesterol enrichment led to increased colony size and resistance to SREBP inhibition. Taken in sum, these results suggest that cholesterol is a limiting metabolite in breast cancer metastasis, and the SREBP pathway promotes metastatic establishment, growth, and survival by activation of genes necessary to supply the cell with cholesterol.

Skoupocytosis: a role for homeostatic microglia in synaptic proteostasis

Presenting Author: Chenxu Guo

Johns Hopkins University

Every hour homeostatic microglia make direct contact with every synapse in the mammalian brain. While it is hypothesized microglia are evaluating the internal state of synapses, the purpose and consequence of microglia-synapse interactions remain fundamentally unknown. Recently we observed microglia intricately removing parts of synapses that contain proteolytic organelles. We hypothesized microglia were contributing to synaptic proteostasis and named this process skoupocytosis after the Greek word for garbage, skoupidia. We first observed this phenomenon in the electron microscopy 3D reconstruction from the Allen Brain Institute of the visual cortex of a P36 mouse. Using correlative electron microscopy, we identified these organelles colocalize with the ESCRT0 protein, Hrs, marking amphisomes-like structures. To capture the dynamics of this process we used live cell fluorescence imaging of a neuron-microglia co-culture model and observed neuron specific fluorescently tagged Hrs is taken up from synapses by microglia. While this occurs under basal conditions, inducing neuronal hyperexcitability increased Hrs uptake. Skoupocytosis was also observed in the intact nervous system using live in vivo two-photon imaging. Using adeno-associated virus, we expressed fluorescently tagged Hrs specifically in neurons of Cx3CR1-eGFP, GFP labelled microglia, mice. Interestingly, the number of microglia containing Hrs increased throughout life from P16, P45 P120 and P240. These data suggest proteolytic organelles produced by homeostatic activities are taken up in vivo. As the proteolytic burden increases throughout life, and during synaptic activity, we propose this previously unknown role of homeostatic microglia, skoupocytosis, contributes to the imperative balance of synaptic proteostasis in the central nervous system.

Skoupocytosis: a role for homeostatic microglia in synaptic proteostasis

Presenting Author:Renee Pepper
Renee Pepper, Jacqueline Griswold, Eunbin Park, Frances Middleton-Davis, Shigeki Watanabe
Johns Hopkins University

Every hour homeostatic microglia make direct contact with every synapse in the mammalian brain. While it is hypothesized microglia are evaluating the internal state of synapses, the purpose and consequence of microglia-synapse interactions remain fundamentally unknown. Recently we observed microglia intricately removing parts of synapses that contain proteolytic organelles. We hypothesized microglia were contributing to synaptic proteostasis and named this process skoupocytosis after the Greek word for garbage, skoupidia. We first observed this phenomenon in the electron microscopy 3D reconstruction from the Allen Brain Institute of the visual cortex of a P36 mouse. Using correlative electron microscopy, we identified these organelles colocalize with the ESCRT0 protein, Hrs, marking amphisomes-like structures. To capture the dynamics of this process we used live cell fluorescence imaging of a neuron-microglia co-culture model and observed neuron specific fluorescently tagged Hrs is taken up from synapses by microglia. While this occurs under basal conditions, inducing neuronal hyperexcitability increased Hrs uptake. Skoupocytosis was also observed in the intact nervous system using live in vivo two-photon imaging. Using adeno-associated virus, we expressed fluorescently tagged Hrs specifically in neurons of Cx3CR1-eGFP, GFP labelled microglia, mice. Interestingly, the number of microglia containing Hrs increased throughout life from P16, P45 P120 and P240. These data suggest proteolytic organelles produced by homeostatic activities are taken up in vivo. As the proteolytic burden increases throughout life, and during synaptic activity, we propose this previously unknown role of homeostatic microglia, skoupocytosis, contributes to the imperative balance of synaptic proteostasis in the central nervous system.

Unveiling Novel Therapeutic Target and Insight into Nuclear Expulsion

Presenting Author: Rachel Yang
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National Institute of Health

Apoptosis, a programmed form of cell death, has been an essential cellular process for maintaining homeostasis. Recently, our group discovered that apoptotic cancer cells with high levels of Padi4 undergo nuclear expulsion (NE) and release a DNA/protein structure known as nuclear expulsion products (NEPs) into the extracellular space, which is crucial for metastatic outgrowth. However, the regulatory machinery governing NE, beyond Padi4, remains largely unknown.

In this study, we aim to identify other regulators involved in the NE process and further characterize the molecular aspects of NE. In our research, we initially observed variations in nuclear expulsion patterns among individual tumor cells within the overall population, leading us to hypothesize that the diversity of nuclear expulsion patterns may stem from differential genomic profiles arising from heterogeneity.

To investigate the hypothesis and identify novel machinery of NE, we generated a panel consisting of single clones across multiple tumor cell lines. Subsequent analysis like live cell imaging, qPCR and immunoblots allowed us to select clones with distinct NE patterns and unchanged padi4 levels. Through the use of multiple apoptosis inducers and viability assays, we confirmed that the different NE patterns are independent of the apoptosis process. Notably we observed that vimentin, a candidate component of NE regulatory machinery, influenced expulsion tendencies independently of Padi4. Further analysis, such as RNA sequencing, will be conducted to identify additional candidates.

Our aim in this study is to explore options targeting the key machinery components of NE, refine strategies to target cancer metastasis, and investigate relapse reduction directions through combination with existing anticancer therapy. In summary, our findings provide promising evidence of additional key modulators of NE, independent of the previously identified Padi4, which may have significant clinical implications.

Reconstitution of Dictyostelium discoideum Contractility Kits

Presenting Author: Brooke Waechtler
Brooke Waechtler and Douglas Robinson
Johns Hopkins University

The cell's ability to undergo shape change is a central feature in many cellular processes, including cytokinesis, motility, and migration. To make these shape changes, the cell harnesses the contractility machinery, which also is poised to sense and respond to mechanical cues from its environment. These proteins construct large-scale assemblies at the cell cortex to give the cell its shape. These proteins also preassemble in the cytoplasm, forming protein complexes that we have termed Contractility Kits (CKs). Cells build two types of CKs: the mechanoresponsive and the non-mechanoresponsive. The mechanoresponsive CKs accumulate at sites of mechanical stress. Non-mechanoresponsive CKs, antagonize the mechanoresponsive CKs. The preassembly of distinct contractility proteins into CKs likely allows for the delivery of important cytoskeletal proteins to the cortex quickly and synchronously in response to mechanical cues. Although we have a good understanding that these proteins do interact, there is currently little structural information about how these contractility kits are forming and what proteins are directly interacting. To determine the structure of these CKs, I am using a reductionist biochemical approach to see what CK proteins are directly interacting with each other. Specifically, I am purifying multiple proteins within the contractility system (fluorescently tagged myosin II, Cortexillin I, Discoidin 1, IQGAP1, and IQGAP2) and will determine the KD between these proteins. I will also reconstitute the cortex by creating Giant Unilamellar Vesicles (GUVs) and will test how the addition and subtraction of different contractility proteins affects the mechanical properties of the GUV and the localization of these proteins. By uncovering the molecular mechanisms of cellular mechanics, we will develop a better understanding of a wide range of biological processes and facilitate future biological engineering.

Determining the Mechanism of 4-HAP Drug Analogues on Myosin II Bipolar Thick Filament Assembly

Presenting Author: Rajan Jayasankar
Rajan Jayasankar, Brooke Waechtler, Douglas Robinson
Johns Hopkins University

A cell's ability to respond to mechanical stimuli, otherwise known as mechanoresponsiveness, has become an attractive target for therapeutics due to their consequential effects on growth and proliferation. Cancer cells have been shown to have distinct mechanical properties which can be targeted with small molecule drugs. Our lab discovered that the small molecule 4-hydxyacetophenone (4-HAP) modulates cell stiffness by increasing nonmuscle myosin IIC bipolar thick filament (NMIIC BTF) formation in human cells. However, its molecular mechanism of action remains unknown. Here, we aim to determine if the molecular analogues of 4-HAP also produce a change in the mechanical phenotype of cells while simultaneously determining potential binding sites for 4-HAP on NMIIC. Using TIRF and confocal microscopy, we are testing if 4-HAP analogues 2-hydroxyacetophenone and 3-hydoxyacetophenone increase the assembly of myosin II bipolar filaments in Dictyostelium discoideum, the model system where we first discovered the activity of 4-HAP. We are also using fractionation assays to further quantify the impact of drug dosing on myosin II bipolar thick filament formation. Concurrently, we are also purifying NMIIC to test if 4-HAP directly binds to NMIIC using a multitude of binding assays such as fractionational BTF assembly assays and thermal shift assays. Better understanding of 4-HAPâ€™s mechanism and its effects on BTF formation will further guide new therapeutic development for targeting NMIIC in cancer.

Optimization and Application of ActuAtor as a Molecular Tool to Explore the Physiological Roles of FUS Condensates in Cell Pathology of ALS

Presenting Author: Xinyi (Cindy) Chen
Xinyi (Cindy) Chen, Saki Takayanagi, Takanari Inoue
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The RNA-binding protein fused in sarcoma (FUS) typically localizes within the nucleus, where it regulates RNA expression and splicing. However, gain-of-function mutations in FUS protein sequences lead to the mislocalization of FUS to the cytoplasm, where it forms condensates with other cytosolic proteins and RNAs. The aberrant formation of FUS condensates is believed to contribute to the pathogenesis of ALS. We have previously designed the molecular tool actuator for inducibly deforming organelles and protein condensates through actin polymerization. Here, we further optimized ActuAtor for efficient FUS dispersion with minimum effect on actin machinery, and applied ActuAtor to investigate the physiological roles of FUS condensates in cell pathology. We first modified ActuAtor construct and co-expressed these derivatives in COS-7 cells with mutated FUS constructs to identify the optimal derivative. Our result indicates that ActA183 which lacks a motif for interacting with actin regulators exhibits both comparable efficiency to the full-length ActA and preferable cytosolic localization after adding nuclear export signal (NES). Thus, we identified the NES-ActA183 as the optimal ActuAtor derivative for the physiological studies of condensates. Previous research has demonstrated that FUS condensates colocalize with stress granules (SGs), which are proposed to be involved in protein synthesis and suppress mRNA translation when cells are under stress. The binding of FUS to SGs is thought to impede SGsâ€™ normal dissociation, thereby harming normal cell functions. By using the optimized ActuAtor to disperse the condensates, we observed the release of mRNA from the FUS-associated SGs. Further experiments on the translation activity will support our hypothesis that ActuAtor-mediated condensate dispersion can reverse translation suppression triggered by SG formation. The research may help determine the role of condensates in the pathogenesis of neurodegenerative diseases.

Membrane compression by synaptic vesicle exocytosis triggers ultrafast endocytosis

Presenting Author: Haoyuan Jing
Haoyuan Jing, Tyler H. Ogunmowo, Sumana Raychaudhuri, Grant F. Kusick, Shigeki Watanabe, Jian Liu
Johns Hopkins University

Compensatory endocytosis keeps the membrane surface area of secretory cells constant following exocytosis. At chemical synapses, clathrin independent ultrafast endocytosis maintains such homeostasis. This endocytic pathway is temporally and spatially coupled to exocytosis; it initiates within 50 ms at the region immediately next to the active zone where vesicles fuse. However, the coupling mechanism is unknown. Here, we demonstrate that filamentous actin is organized as a ring, surrounding the active zone at mouse hippocampal synapses. Assuming the membrane area conservation is due to this actin ring, our theoretical model suggests that flattening of fused vesicles exerts lateral compression in the plasma membrane, resulting in rapid formation of endocytic pits at the border between the active zone and the surrounding actin-enriched region. Consistent with model predictions, our data show that ultrafast endocytosis requires sufficient compression by exocytosis of multiple vesicles and does not initiate when actin organization is disrupted, either pharmacologically or by ablation of the actin-binding protein Epsin1. Our work suggests that membrane mechanics underlie the rapid coupling of exocytosis to endocytosis at synapses.

Correlation of repetitive behaviors in deer mice with striatal mRNA expression of endogenous opioids and mu, delta, kappa, and dopamine receptors

Presenting Author: Justin Pellicciotti
Augustine, Farhan; Doss, Shawn; Pellicciotti, Justin; Mahate, Sahar; Singer, Harvey S.
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Deer mice provide a valuable naturally occurring animal model for investigating pathophysiological mechanisms underlying repetitive behaviors. Prior investigations using this model have identified abnormalities in the cortico-basal ganglia circuitry, including alterations within the indirect pathway and levels of endogenous opioids in the frontal cortex. In this study, the behaviors of n=7 mice were quantified, and their brains were sectioned. Using analysis of in-situ mRNA hybridization (RNAscopeÂ®) in four striatal regions (dorsomedial, dorsolateral, ventromedial and ventrolateral), mRNA levels of dopamine receptors (DRD-1 [D1] and DRD-2 [D2]), endogenous opioids (prodynorphin [p-dyn] and proenkephalin [p-enk]), and opioid receptor expression (Oprm1 [mu], Oprd1 [delta], Oprk1 [kappa]) were quantified. We hypothesized that mRNA levels of these molecules would correlate (positively or negatively) with the number of repetitive movements performed by the mice. Statistical analyses were performed using Spearmanâ€™s rank correlation coefficients with a False Discovery Rate (FDR) procedure to address the small sample size. Results identified a positive correlation between total activity and DRD-2 mRNA in the dorsolateral striatum, indicating an imbalance in the dopamine system that may influence repetitive behaviors in deer mice. This result has several underlying pathophysiological implications, including the potential effects of dopaminergic alterations on several cortical-basal ganglia-thalamo-cortical pathways, and also takes a step forward toward future investigations to explore the roots of repetitive behaviors.

Molecular Dynamics Simulation of Viral Protein U (VPU) Association with the Cellular Membrane

Presenting Author: Kedhar Narayan
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American University

Viral protein U (Vpu) is a retroviral accessory protein important in the release of replicated virions from the plasma membrane of host cells. It acts by counteracting the antiviral host protein BST2, which anchors virions to the membrane, inhibiting their release. Vpu interacts with BST2 within the membrane, leading to its downregulation and removal from the membrane. Key to this interaction is the residues of alanine (A) at positions 22 or 25 and tryptophan (W) at positions 30 or 33 in Vpu of SIVgsn99CM71 strain, which was reported to antagonize human BST2. Each A and W form the AW motif, which is recognized for its significance in BST2 antagonism. There is no structural information available for Vpu associated with the membrane. The transmembrane protein structure prediction programs suggest that the A residues are likely located inside the membrane, while the W residues are positioned closer to the edge of the membrane. It remains unclear whether either or both W residues are exposed outside the inner membrane domain. We hypothesize that the positioning of W residues, whether inside or outside the inner membrane domain, is crucial for characterizing the neutralization effect of Vpu on BST2. We performed molecular dynamics simulations to explore Vpu association with the plasma membrane over 200 nanoseconds (5 frames per nanosecond; 1,000 frames total). Solvent-accessible surface area was then assessed, which quantifies whether each residue is accessible from the outside of the membrane. The results indicate that the A22, A25, and W30 residues consistently remain within the membrane across all frames. In contrast, W33 is found both inside and outside the membrane (partially exposed outside during 149 frames out of 1,000). This implies that the effectiveness of Vpu in neutralizing BST2 may be constrained by the membrane environment and conditions that affect the positioning of W33 residue within the inner membrane domain.

Oxygen Gradients Direct the Spatial Organization of Epithelial-to-Mesenchymal Transition in an in vitro Breast Cancer Tumor Microenvironment

Presenting Author: Giana I. Vitale
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Introduction:

Triple negative breast cancer (TNBC) is a metastatic and recurrent breast cancer subtype, with five-year survival rates as low as 13%. Expression of proinflammatory proteins NOS2 and COX2 and the hypoxic tumor microenvironment (TME) promotes epithelial-to-mesenchymal transition (EMT), initiating metastasis. In this study, epithelial (E) and mesenchymal (M) phenotypes are defined by E-cadherin (ECAD) and vimentin (VIM) expression, respectively. We hypothesize that hybrid (E/M) phenotypes, heavily implicated in metastatic potential, organize along cell-generated hypoxic gradients and are spatially correlated with COX2 and NOS2 expression.

Methods:

Restricted exchange environment chambers (REECs) mimic the cell-generated gradients of oxygen (O2) of solid tumors in 2D cell culture and facilitate fluorescence microscopy. These gradients form within a few hours of REEC application, resulting in a compact cell disk formed by a combination of cell death and migration. We cultured 4T1 cells in REECs for up to 7 days. At 0, 12, 24, 48, 96, and 168 h, fixed cells were imaged with multiplexed immunofluorescent microscopy. Cells positive for E or M phenotypes were defined as z- score normalized intensities above a threshold. Spatial phenotype analyses were conducted.

Results:

Over 7 days in the REECs, the proportion of VIM+ cells increased, initially in hypoxic regions. After 48 h, the VIM+ population maximizes in normoxic regions. E/M cells increased in all regions over time. COX2 and NOS2 expression increased significantly in response to O2 gradients, with COX2 present in the normoxic region and NOS2 forming a ring-like pattern in the hypoxic region centered on the O2 source. These results show that COX2 and NOS2 contribute to the organization of E/M phenotypes in a hypoxic gradient.

Impact:

These results provide insight into how high coexpression of COX2 and NOS2 influences EMT, the metastatic cascade, and profoundly impacts patients' survival.

Interrogating the relationship between small molecule 4-HAP and contractile protein myosin II

Presenting Author: Emma Morin
Brooke Waechtler, Emma Morin, Douglas Robinson
Johns Hopkins University

Cell mechanics are controlled by the mechanobiome, composed in part by an array of proteins involved in the contractility network, including nonmuscle myosin II (NMII). These proteins assemble at the cell cortex and sense mechanical stimuli; however, a population of these contractile proteins reside in the cytoplasm, providing an available pool that helps respond to these mechanical stimuli. The relative fractions of the populations of cytoplasmic vs. cortical proteins are tuned in a cell type-specific manner, setting the level of mechanoresponsiveness appropriate for each cell type. We have identified a small molecule, 4-hydroxyacetophenone (4-HAP), as a modulator of this balance where 4-HAP causes specific paralogs of NMII to over-assemble and accumulate at the cortex. This over-assembly increases cell stiffness and cortical tension in cell types that express specific NMII paralogs. Treatment with 4-HAP also decreases human cancer metastasis in mouse models. In humans, 4-HAP affects NMIIB and NMIIC, the latter of which is of particular interest, as NMIIC is upregulated in specifically in pancreatic cancer. Although we know how the addition of 4-HAP affects NMIIC, the molecular mechanism by which 4-HAP drives NMIIC assembly remains unknown. We hypothesize that 4-HAP directly binds to NMIIC, promoting its assembly and accumulation at the cortex. We are purifying NMIIC (as well as its different domains) to perform binding assays to test if 4-HAP is directly binding to NMIIC, and if so, to what domain it binds. Understanding the binding mechanism of 4-HAP to NMIIC will provide greater knowledge as to how 4-HAP decreases cell movement, which could have a great impact in treating diseases that are characterized by altered cell mechanics, such as metastatic cancer.

Exploring Potential Biomarker for Response to Metronomic Chemotherapy in Gastrointestinal Cancer Patients

Presenting Author: Efren Barragan

Efren Barragan, Valeria Lopez, Ryley Stewart, Saeedeh Darvishi, Diana Prospero, Hector Padilla, Raul A Pineda, Giacomo Allegrini, Paola Orlandi, Maria Laura Manca, Guido Bocci, Giulio Francia

El Paso Community College

Metronomic chemotherapy can produce clinical and preclinical antitumor activity in a number of malignancies. We previously reported a study of a selection of 14 cytokine plasma levels in phase II clinical trial of metronomic UFT (a 5- fluorouracil prodrug; 100 mg/twice per day p.o.) and cyclophosphamide (CTX; 500 mg/m2 i.v. bolus on day 1 and then 50 mg/day p.o.) plus celecoxib (200 mg/twice a day p.o.) in 38 patients with advanced refractory gastrointestinal tumors (Valenzuela et al). Here we present the analysis of one additional cytokine that we had not previously evaluated; thus, we assessed plasma levels of ENA78 (CXCL5) in patients in this trial.

The data we obtained was divided into 2 subsets; patients with 28-day ENA78 (CXCL5) values lower than 971.05 pg/ml (group 1) and patients with greater or equal values (group 2). Group 1 subjects showed a median PFS of 3 months (interquartile range 3.5 months) while those in group 2 was 2 months (interquartile range 1 month; P=0.025). Cox regression analysis showed that patients with higher 28-day ENA78 levels at the optimal cut-off they were 3 times (95% CI: 1.2-9.7) more likely to have a PFS of less than 6 months compared to subthreshold patients.

Our hypothesis is that systematic analysis of different cytokine levels in patients undergoing metronomic chemotherapy will reveal a cytokine signature of patients that will show significant responses to the metronomic regimen, as well as those patients that are not likely to benefit from this approach. The data presented here suggests ENA78 (CXCL5) may be a useful marker to identify gastrointestinal cancer patients likely to benefit from metronomic chemotherapy.

PTEN knockout improves survival of transplanted human retinal ganglion cells

Presenting Author: Whitney Stuard Sambhariya
Whitney Stuard Sambhariya, Aru Nagalingam, Jian Du, Will Yutzy, Thomas V Johnson
Johns Hopkins University

Background:

Optic neuropathies, characterized by retinal ganglion cell (RGC) dysfunction and death, cause irreversible vision loss. RGC transplantation holds potential for restoring vision, but fewer than 1% of RGCs transplanted into the eyes of mice persist for more than a few weeks. Inhibition of phosphatase and TENs in homolog deleted on chromosome 10 (PTEN) increases RGC survival and axon regeneration after optic nerve injury. Here, we evaluated the effects of PTEN deletion on RGC survival and engraftment following intravitreal transplantation.

Methods:

Human embryonic stem cells (hESCs) expressing tdTomato from the endogenous BRN3B locus were subject to PTEN deletion using CRISPR. Purified RGCs differentiated from parental (WT) hESCs or PTEN knockout (KO) hESCs were transplanted in immunosuppressed (daily s.c. cyclosporine) Lama1n naive mf223 or C57BL/6J mice pretreated with intravitreal pronase (37,500 RGCs cells/eye). Transplantation of WT RGCs into one randomly selected eye and PTEN KO RGCs contralaterally was performed. At 2wk post-transplantation, retinas were processed for whole-mount confocal microscopy and immunolabeling was performed. Confocal z-stacks were processed in ImageJ and Arivis Pro.

Results:

PTEN KO was confirmed in hESCs and differentiated RGCs through DNA sequencing, RT-PCR, and Western blot. PTEN KO RGCs demonstrated significantly greater survival after transplantation compared to WT RGCs. PTEN KO RGCs grew longer and more complex neurites, with dendrites laminating within the IPL and axons coursing through the RNFL and into the optic nerve head.

Impact:

PTEN KO significantly improves survival of RGCs following transplantation and augments neurite outgrowth. Ongoing analyses will characterize the dendritic architecture of donor RGCs and evaluate synaptogenesis with the retinal neurocircuitry. Genetic engineering of donor RGCs represents a promising strategy to achieve RGC repopulation in optic neuropathies through cell transplantation."

SMYD3 INHIBITION USED TO IDENTIFY AND BLOCK MAIN METASTATIC PATHWAYS WITHIN PROSTATE CANCER

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Aberrant lysine methylation of histone and non-histone proteins mediated by deregulated lysine methyltransferases (KMT) and lysine demethylases (KDM) has been associated with numerous malignancies. SMYD3, a member of the SMYD family of lysine methyltransferases, is overexpressed in multiple cancers including prostate cancer. Increased expression levels of SMYD3 in prostate cancer cells promotes survival, migration, invasion, and metastasis, indicating that it plays a significant role in prostate carcinogenesis. However, the molecular and the biochemical mechanisms that regulate substrate identification and lysine methyltransferase activity of SMYD3 remain undefined. We hypothesize that SMYD3 has a preferred substrate through which it facilitates development and progression of malignancy. In prostate cancer cells, using subcellular fractionation and immunofluorescence we found SMYD3 to be primarily localized in the cytoplasm. Preliminary data indicates that subcellular localization of SMYD3 changes when nuclear export is blocked. Essentially, our goal is to study the contribution of different regions of the uniquely regulated proteins. Structurally, SMYD3 contains a conserved SET domain, which catalyzes methylation, a zinc finger MYND domain, a cysteine rich post-SET domain and a TPR-like region comprising C-terminal domain (CTD). Through immunofluorescent imaging, we aim to define the role of the different domains of SMYD3 in regulating its localization.

TDP-43 depletion promotes conversion of endogenous tau to drive death of neurons

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TAR DNA-binding protein 43 (TDP-43) is an RNA-binding protein that represses the splicing of nonconserved cryptic exons, however, when TDP-43 function is impacted cryptic exons are incorrectly spliced into mRNA, promoting nonsense-mediated decay. This is referred to as TDP-43 proteinopathy. Alzheimer's Disease (AD) has two characteristic factors when looking at the biology of the disease, neuritic plaques and neurofibrillary tangles. Although it was initially thought that abnormal accumulation of A in the brain triggers the aggregation of tau which leads to neurodegeneration, it has been shown that A accumulation is required but not sufficient. A second is needed to drive the conversion of wild type to hyperphosphorylated tau. Due to an initial finding which showed that loss of TDP-43 exacerbates neurodegeneration in the hippocampus of mice, we hypothesised TDP-43 proteinopathy to be the necessary second.

To test this theory, a mouse model was designed using a tamoxifen inducible Cre recombinase system to allow for TDP-43 to be deleted. Once TDP-43 was depleted, the right hippocampus was directly injected with tau using AAV. At 19 months of age, immunohistochemical analysis was done on these mice to identify hyperphosphorylated pathological tau tangles derived from endogenous mouse tau using two independent antisera, AT8 and pS422. At 19 months of age it was shown that loss of TDP-43 does indeed facilitates age-dependent pathological conversion of wild-type tau in the hippocampus, cortex, and particularly in the entorhinal cortex, where where tauopathy is thought to initiate in the brains of AD cases.

CRISPR Engineering of Bacteriophage T4 Nanoparticle as a Next-Generation Flavivirus Vaccine Design Platform

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Dengue and Zika belong to the Flavivirus genus, causing major public health concerns globally. The licensed live attenuated dengue vaccine is not effective, and there is no Zika vaccine available. Here, we present a novel approach utilizing the T4 bacteriophage as a vaccine platform engineered through CRISPR technology. The T4 bacteriophage platform is safe, can produce a robust immune response without adjuvant, doesn't require low-temperature storage, is inexpensive, and can be administered intranasally. The T4 bacteriophage has two non-essential proteins (Hoc and Soc), to which foreign antigens can be attached or fused with in order to display them on the phage head. For the dengue and Zika vaccines, a receptor-binding domain of the envelope protein, known as Domain III, will be incorporated with the phage Soc protein. DIII can fold independently and can induce serotype-specific antibodies. Initially, this incorporation provides approximately 50 copies per capsid, while T4 can display around 870 copies of the Soc protein. Therefore, to increase the copy number, we hypothesized testing different regulatory elements (promoters, RBS, codon usage) in the phage genome. Interestingly, replacing the native Soc promoter with a strong late promoter of the tail sheath structural gene (gp18), or deleting an 11 bp riboswitch-like element from upstream of the Soc gene, exhibited approximately 5 and 8-fold increases in the DIII domain copy number, respectively. Riboswitch-mediated down-regulation of a phage structural gene is intriguing and consistent with the hypothesis that such reductions would economize resources, maximize phage yields, and might also streamline assembly pathways. Engineered T4 nanoparticles hold promise for improving the T4 bacteriophage vaccine platform. In conclusion, our engineered T4 bacteriophage nanoparticle platform emerges as a next-generation solution, offering a new paradigm in vaccine development.

Comparative analysis of retinal organoids cultured across distinct protocols

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Stem cell-derived retinal organoids (ROs) facilitate investigations into retinal development, transplantation therapies, and disease modeling. While RO transplantation may hold the potential to restore vision in patients with retinal disease, its translational use is currently impeded due to heterogeneity among ROs. This heterogeneity that presumably results from the differences in culturing protocols complicates the interpretation of experimental outcomes. To investigate the influence of distinct culturing protocols on the composition of ROs, we used single-cell and single nuclei RNA seq published datasets of ROs (GSE142526, GSE183684, GSE201356, and EGAS00001004561) each generated using its unique culturing protocol. The organoids were dissociated into a single-cell suspension and run on the 10X Genomics Chromium single-cell platform. Raw data was processed using the 10X Genomics cellranger count and analyzed using the Seurat 5.0 R package. The combined dataset underwent normalization, variable feature selection, scaling and integration. Uniform Manifold Approximation and Projection (UMAP) dimension reduction was performed and clusters were computed. Cell types were then identified using a list of known marker genes.

We found that divergent culturing protocols yield unique gene expression profiles and heterogeneous cell type populations in ROs, with specific protocols favoring the prevalence of specific cell types. Additionally, the presence of astrocytes and brain and spinal cord-like cells in cultured ROs contrasts with their absence in established retinal development datasets. The precision of RO specification and cultivation needs to be further refined for the development of safe and effective clinical treatment involving the augmentation of ROs to restore visual function.

Mouse Model of Maternal Nicotine Vaping Drives Fetal Innate Immunity Dysregulation

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Maternal nicotine vaping during pregnancy can have detrimental effects on fetal growth and development. The innate immune system serves as the first line of defense against infections and plays a crucial role in protecting the developing fetus from potential pathogens. This system can also be negatively impacted in several ways, including reduced immune cell function, alterations in inflammatory response, epigenetic changes, increased susceptibility to infections, and elevated oxidative stress. The overall goal of this work is to therefore evaluate the effects of maternal nicotine vaping on the development of the innate immune system of the developing fetus. Here, we exposed pregnant female C57/BL6 mice to nicotine vapor via a whole-body exposure system starting at embryonic day 14 each day through gestation. An increase in the production of pro-inflammatory cytokines and chemokines was found in mouse pups exposed to nicotine vapor in-utero in several tissues, including the lung and ileum. Our finding suggest that in-utero nicotine vapor exposure may have discernible effects on the integrity of the neonatal gut barrier and cause immune dysregulation.

Epigenetic Inactivation of CTHRC1 enhances Immune Suppressive Microenvironment in Breast Cancer Metastasis

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Breast cancer metastasis remains a leading cause of cancer deaths among women. Migration of breast cancer cells (BCC) to distant organs and their survival through immune evasion marks its late stage, causing lower response to treatment. Due to its heterogeneity, BC presents different subgroups, with Triple-negative breast cancer (TNBC) reported as the most aggressive with the poorest prognosis. In our project, we employ 4T1 mouse cell lines, a mouse model of spontaneous TNBC metastasis, to study the role of Collagen Triple Helix Repeat Containing 1 (CTHRC1) in the microenvironment of the metastatic site. We discovered that CTHRC1, a glycoprotein secreted into the extracellular matrix by fibroblasts, is epigenetically inactivated during colonization at the distant site. Our in-vivo studies showed a significant decrease in lung metastasis in mice after orthotopic injection with CTHRC1 overexpression cell lines. Upon immune profiling of the lung using flow cytometry, we observed that the overexpression of CTHRC1 also decreased immune suppressive cells including MDSCs, PD1+, and LAG3+ T cells. This was proven to be T cell-dependent using nude mice and immunocompetent mice. From our observations, we hypothesize that CTHRC1 inhibits the metastatic progression of TNBC by decreasing the immune suppressive microenvironment of the distant site. Moreover, through mass spectroscopy and ELISA, we further report the mechanism of interaction of CTHRC1 with Galectin 3 to inhibit LAG3+ T cell activation. This study highlights the role of CTHRC1 in the progression of metastasis in TNBC by inhibiting LAG3+ T cell-mediated immune suppression. Its epigenetic inactivation might be a critical step for the metastatic outgrowth of BCC. Overall, this project serves to improve our understanding of breast cancer metastasis for better therapeutic interventions.

A protein-based tool to model physical states of danger signals in cells

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Cells encounter non-microbial materials whose physical state can pose a threat to the integrity of cell architecture. It is critical for cells to distinguish between diffuse and solid states of molecules. The ill effects of abnormal states are evidenced in neurodegenerative, cardiovascular, allergic, and autoinflammatory disease. In these diseases, immune cells activate a homologous pathway between solid particulates, seemingly independent of their chemical composition. Despite the physiological significance of such a surveillance system for molecular assembly states, it is unclear how cells identify danger signals at a molecular level based on the state of self-assembly. This is largely due to a lack of experimental approach with which we can alter the state of self-assembly without changing molecular composition. Here, we seek to develop a protein-based tool to model different assembly states across the same building block which can be deployed inside living cells. Using an existing series of recombinant proteins that crystallize in the cytosol, our strategy includes the addition of intrinsically disordered regions and/or oligomerization domains in order to form more liquid-like phases such as liquid droplets and hydrogels. While developing the tool, we characterized expression in non-immune cells. We, like others, observed minimal cytotoxicity of the protein crystals. In agreement with the results of a previous study, we found colocalization of autophagic machinery with protein crystals. Moreover, with this tool, we began to assess the inflammatory response of innate immune cells through co-culture with cells containing a given molecular assembly. Further development of this tool could facilitate deeper inspection of immune pathways such as those involving pattern recognition receptors. In turn, the study may contribute towards the effort to uncover therapeutic targets for chronic immune dysregulation in diseases associated with abnormal molecular self-assembly.

Fly-FUCCI in the Drosophila Testis Niche: Investigating the correlation between the cell cycle phase of GSCs and their survival

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Germline stem cells (GSCs) transmit genetic information to future generations; These cells are particularly resistant to damage and can survive toxic conditions. Knowing how DNA damage is repaired in this cell type is important for understanding how this function is preserved. Also, learning why a GSC with DNA damage survives or is eliminated from the tissue can expand our understanding of both therapies and stem cells. A common, lethal type of DNA damage is the double-strand break. The cell cycle phase dictates which DNA double-strand break repair pathway is available upon damage. Homologous recombination (HR) is used in S, G2, and Mitosis, while Non-homologous end joining (NHEJ) is used during G1. NHEJ is mutagenic, while HR is not. I have developed an assay to determine the responses of individual GSCs in the fly testis to DNA damage. This experiment uses Fly-FUCCI to identify a potential link between the cell cycle phase and GSCs that remain in the niche after exposure to ionizing radiation (IR). I hypothesize that the GSCs surviving IR will preferentially be in the G2 or S phase, where HR repair is accessible. Fly-FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator) is a genetic tool that allows the visualization of cell-cycle activity and differentiation between G1, S, and G2 phases with fixed imaging. To express the FUCCI tool specifically in the germline, I used the Gal4-UAS system. To analyze the cell cycle in the germline, I paired the germline-specific driver, nos-GAL4, with the Fly-FUCCI transgene. 1 day after exposure to irradiation, I expect most of the remaining GSCs to be either yellow or red (G2 or S) and no GSCs will be green alone. This result would suggest that the cell cycle does play a role in GSC survival.

Long-term Tracking of Hematopoietic Clonal Dynamics and Mutations in Nonhuman Primate Post-Autologous Lentiviral-barcoded Hematopoietic Stem and Progenitor Cell Transplantation

Presenting Author: Rohan Hosuru

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Hematopoietic stem and progenitor cells (HSPCs) autologous transplantation-based gene therapies are promising for treatment of genetic blood disorders. Investigating the long-term clonal dynamics of HSPC output and disease-related mutations following lentiviral-mediated gene therapy is important to gain insights regarding efficacy and safety. We established an autologous lentivirally-barcoded HSPC transplantation rhesus macaque (RM) model allowing quantitative interrogation of primate hematopoiesis, with relevance to human HSPC biology. Healthy RMs underwent total body irradiation, followed by transplantation of autologous HSPCs transduced with a lentivirus library containing high diversity genetic barcodes, uniquely labeling HSPCs and their progeny. We quantitatively characterized a broad range of clonal behaviors, including contribution levels, clonality, diversity, stability, and lineage biases in 5 barcoded RMs for up to 121 months post-transplantation.

We revealed stable multipotent long-term hematopoietic clonal output of T, B cells, monocytes, and granulocytes produced by a highly polyclonal repertoire of HSPCs in all 5 macaques for up to 121 months post-transplantation. Distinct clonal differences existed between early short-term (prior to 2 months post-transplantation) and long-term clones (persisting up to 121 months), excluding the emergence of some large T clones due to adaptive peripheral expansions. Clonal succession after stable hematopoietic reconstitution was minimal. Granulopoiesis, reflecting active HSPC production, remained stable over time in terms of clone number and clonal diversity across all animals. Our data collectively suggests steady-state hematopoiesis is driven by multipotent progenitor cells.

Tracking of a panel of clonal hematopoiesis-related acquired somatic gene mutations comparing pre-/early timepoint samples with the longest follow-up timepoint post-transplantation is ongoing.

Extracellular matrix stiffness regulates chromosome loss and genetic variation

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Genomic instability, the inability of a cell to pass on its genetic information accurately, is a hallmark of cancer. Aneuploidy, or an abnormal number of chromosomes, is observed in approximately 85% of solid tumors. During cancer progression, changes in the mechanical microenvironment can physically restrict cancer cells and induce errors in DNA replication or mitosis. Using chromosome reporter cell lines, we investigated whether a three-dimensional (3D) tumor microenvironment contributes to aneuploidy and found that increased matrix stiffness increases mitotic aberrations that may lead to chromosome missegregation. Our findings indicate that stiff microenvironments suppress mitosis, increase micronucleus formation, and enhance chromosome loss, thus highlighting a potential role for mechanical confinement in chromosome segregation. We also find that inhibiting myosin-II increases chromosome loss without affecting spheroid growth. The variance in chromosome loss across cancer spheroids increases per Luria-Delbruck's theory of heritable genetic change and is also consistent with the emergence of colonies with chromosome loss. Overall, our findings indicate that increased matrix stiffness increases heritable genomic instability and tumor heterogeneity -a deeper understanding of which could contribute to synergistic treatments for cancer.

Uncovering the role of canonical cell polarity proteins in the maintenance of the Drosophila testis niche

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Stem cell niches are local microenvironments that provide essential signaling cues to govern the fate of resident stem cells. Understanding the various ways through which niches maintain and regulate stem cells is still an active area of research. We use the well characterized testis stem cell niche of Drosophila melanogaster to better understand the nature of stem cell microenvironments. This niche consists of 10-16 quiescent somatic cells, referred to as niche cells, that maintain two stem cell populations, namely the germline stem cels (GSCs) and the cyst stem cells (CySCs). Previous work by our lab has shown that elevated EGFR signaling within niche cells results in loss of their quiescence. Additionally, cell polarity proteins have been shown to mediate EGFR signaling within cyst cells, which originate from the same precursors as the niche cells. In this work, we ask two questions: 1. what are the expression patterns of the canonical cell polarity proteins in niche cells, and 2. do polarity proteins contribute to niche cell quiescence in a cell-autonomous manner? Here, we characterize and quantify the abundance of cell polarity proteins (Dlg, Scrib, Lgl, and Crb) at different interfaces of niche cells. We find that while cell polarity proteins are found across niche cell membranes, they are slightly more concentrated at niche cell - stem cell interfaces (as compared to niche cell - niche cell interfaces). Additionally, we assessed the requirement of cell polarity proteins in maintaining niche quiescence and identity through expression of cell-specific RNA-interference and lineage tracing, respectively. Future work will aim to investigate the crosstalk between the EGFR pathway and cell polarity proteins in the context of maintaining niche cell quiescence.

Examining the role of glutamate receptors within the Drosophila stem cell niche

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Adult stem cells are maintained within environments of complex, supportive cells referred to as a niche. Niche cells produce essential signaling molecules that are necessary to maintain homeostasis within a tissue. The male Drosophila melanogaster germline stem cell niche is an effective model for studying such signaling due to its well-characterized structure. This niche is comprised of mature niche cells referred to as the hub, cyst stem cells, and germline stem cells, which will develop into mature sperm. Understanding the hub cells is of particular interest, as they are required to maintain stem cell populations. Previous single nuclei RNA sequencing work performed in collaboration with our lab has suggested that glutamate receptors are expressed in hub cells, a population of cells not thought to use such signaling pathways. Glutamate receptors are ionotropic receptors that bind glutamate to move calcium down its concentration gradient, a process essential to motor contractility in flies. Within flies, these receptors have four subunits "GluRIIA (or GluRIIB), GluRIIC, GluRIID, and GluRIIE. However, glutamate receptor activity has not been characterized within the testis. Through the use of enhancer trap lines, we have found that these subunits have varying levels of expression within hub cells. Future work will investigate the role of glutamate receptors in the testis and understand whether the niche is composed of excitable cells.