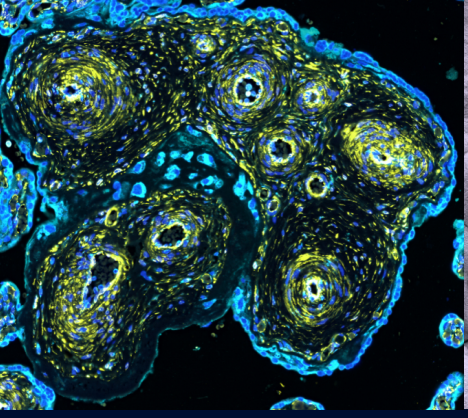
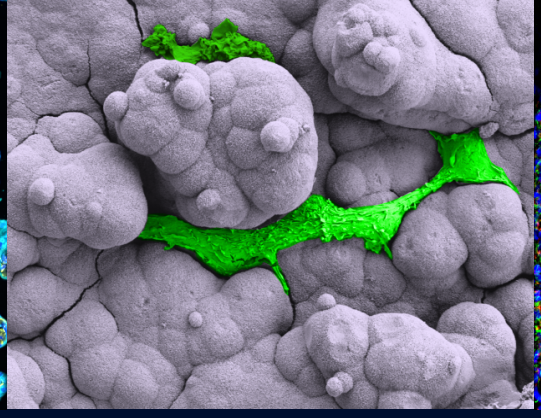
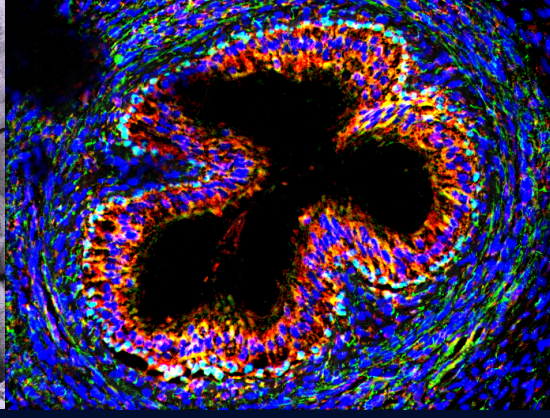




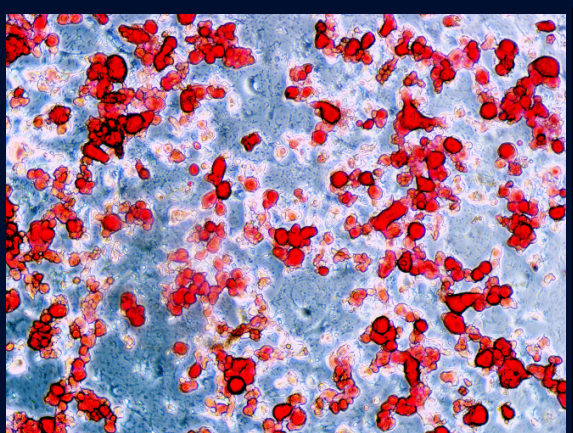
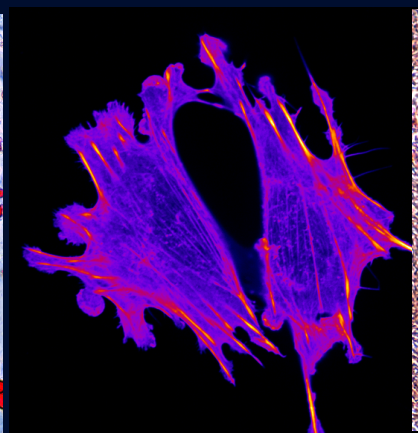
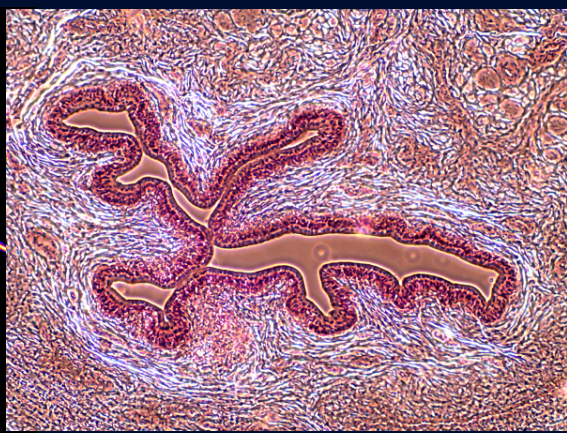
The

10th

Postdoc Research Day

UCONN HEALTH

The Jackson Laboratory
Leading the search for tomorrow's cures

We are delighted to celebrate the tenth anniversary of our annual UConn Health/Jackson Laboratory Postdoc Research Day with you. Today marks the tenth year where we have gathered together on a fall day to honor our postdocs and learn about the fantastic research happening here at UConn Health and the Jackson Laboratory for Genomic Medicine. Thank you for being a part of this special day. Our postdocs are proud to showcase the important work they are performing through a series of short Speak4Science talks and a full poster session. We also have the privilege of welcoming Dr. Bruce Spiegelman from the Dana Farber Cancer Institute and Harvard Medical School to give our keynote address. On the following pages, you will find a schedule as well as abstracts for all our presenters.

I would like to thank the Health Center Research Advisory Council and the Jackson Laboratory for Genomic Medicine for their support of this year's event as well as Jane Tran Sills for all her help in putting this together. I would also like to thank our PDRD Planning Committee:

Nisha Mahey (UConn Health)
Marianna Marino (UConn Health)
Katarina Milicevic (UConn Health)
Luke Trinity (Jackson Labs)
Ying Tang (UConn Health)

Be well,

Christopher D. Heinen, Ph.D.
Director of Postdoctoral Affairs
UConn Health

Cover image credits: (Top, from left to right) Ramalakshmi Ramasamy, Govindaraj Perumal, Nicholas Moskwa, (Bottom, from left to right) Marianna Marino, Milda Stanislauskas, Nicholas Moskwa

The 10th

UConn Health – Jackson Laboratory for Genomic Medicine

Postdoc Research Day

Thursday, September 17th, 2026

1:00	**Opening Remarks**	**Academic Rotunda**
1:10	**Speak4Science Part I** Short 4-minute talks from UCH and JAX postdocs	**Academic Rotunda**
2:05	**Coffee Break**	**Rotunda Lobby**
2:25	**Speak4Science Part II** Short 4-minute talks from UCH and JAX postdocs	**Academic Rotunda**
3:30	**Keynote Address:** Bruce Spiegelman, Ph.D. Stanley J. Korsmeyer Professor of Cell Biology and Medicine, Dana-Farber Cancer Institute and Harvard Medical School *The PGC1α/Irisin Axis: Connecting Muscle Metabolism with Brain Health and Resistance to Neurodegeneration*	**Academic Rotunda**
4:30	**Poster Session and Reception**	**Rotunda Lobby**
4:40	**Poster Presentations I (Odd numbers)**	**Rotunda Lobby**
5:20	**Poster Presentations II (Even numbers)**	**Rotunda Lobby**
6:00	**End**	

Our Speak4Science event will feature a series of 4-minute talks by our postdocs. Each speaker will use one slide to broadly introduce their area of research and why it excites them. To learn more about the details of their research, I encourage you to visit the poster sessions starting at 4:30 PM. The speaker roster and their corresponding poster numbers are listed below.

Name	Title	Affiliation	Poster
1. Alexander Arnuk	*DYRK1A and CD47 in AML immunotherapy*	JAX	1
2. Yamin Liu	*What can we learn from cows to fight cancer?*	UCH	2
3. Rajkishor Pandey	*CDH17-targated CAR-T cells for PDAC treatment*	UCH	3
4. Daniel Penarete-Accosta	*Patient susceptibility to colibactin*	JAX	4
5. Marianna Marino	*FGF2 and thermogenic fat activation*	UCH	5
6. Ramalakshmi Ramasamy	*When the placental clock runs too fast*	JAX	6
7. Iris Nakashima	*HAP2-mediated gamete fusion in plants*	UCH	7
8. Corie Owen	*Interfollicular communication in ovaries*	UCH	8
9. Dylan Krajewski	*BMEC EVs support leukocyte TEM*	UCH	9
10. Katarina Milicevic	*Probing early neurophysiological changes in AD*	UCH	10
11. John Stout	*Cortical control by corticothalamic neurons*	UCH	11
12. Ashok Dhinakaran	*Prolonged commensals train skin immunity*	JAX	12
13. Tasnif Rahman	*Multiscale models of tissue mechanics*	UCH	13
14. Nisha Mahey	*DNA repair in* S. aureus *persisters*	UCH	14
15. Anna Truong	*Non-canonical DNA repair in* Pseudomonas	UCH	15
16. Ranjuna Weerasekera	*Pathogenic* E.coli *and antibiotic tolerance*	UCH	16
17. Mubark Mebrat	*Elucidating PB3-ligand interactions*	UCH	17
18. Luke Trinity	*CMV reshapes the human immune system*	JAX	18
19. Aditya Kanwal	*Understanding TTNtv DCM to guide therapy*	JAX	19
20. Kiseki Washitani	*Altered lipid metabolism in CMD*	UCH	20
21. Pengyu Zong	*IL-31 signaling in ischemic stroke*	UCH	21
22. Manaswee Barua	*PPHOS for bone regeneration*	UCH	22
23. Govindaraj Perumal	*Morphology of male vs female aged macrophages*	UCH	23
24. Nicholas Moskwa	*Bladder confetti*	UCH	24
25. Pradeep Kumar	*DIY air purifiers reduce classroom PM*	UCH	25
26. Cristina Baquero	*Dissecting TME-driven GBM recurrence*	JAX	26

Keynote Presentation

Bruce Spiegelman, Ph.D.

Bruce M. Spiegelman is the Stanley J. Korsmeyer Professor at Harvard Medical School and Dana-Farber Cancer Institute. Spiegelman received a B.S. with highest honors from the College of William and Mary, his PhD in biochemistry from Princeton University, and completed postdoctoral work at MIT. He then joined Harvard Medical School and Dana-Farber Cancer Institute. His research focuses on fat cell biology, diabetes and muscular diseases. Major honors include the Heinrich Wieland Prize in Lipid Research; the Bristol-Myers Squibb Award for Distinguished Achievement in Metabolic Research; the Rolf Luft award in Endocrionology; the Eliot P. Joslin Medal; the Transatlantic Medal (British Endocrine Society); the Naomi Berrie Award for Outstanding Achievement in Diabetes Research (Columbia University); the Frederick Banting Medal of the American Diabetes Association (their highest award); the Manpei Suzuki Prize for Diabetes Research, Japan, the Inbev Baillet-Latour Health Prize in 2015 and the Helmholtz Prize for Diabetes Research, Munich, Germany. Spiegelman has been elected to the National Academy of Sciences, American Academy of Arts and Sciences, and to the National Academy of Medicine. He has served as Chair of the Section on Metabolism and Medical Physiology of the National Academy of Sciences.

Abstracts

1. Alexander Arnuk

DYRK1A controls CD47 endocytic degradation and cancer immune surveillance

Won J Kim[1], Sadik Karma,[2,10*] Jeetayu Biswas[1*], Alexander Arnuk[2*], Cuijuan Han[2], Cynthia Castro[1], Carlo M Sevilla[7], Nina Fox[1], Jacob H Winick[1], Alexander M Lewis[1], Takeshi Fujino[1], Lizbeth M Navarrete[1], Charlotte Wishnack[1], Adriana Cuibus[1], Michael S Lee[1], Susan DeWolf[1], Kayla R Koenig[2], Abimbola E Lawal[2], Robert F Stanley[1], Sakthi H Rajendran[2,10], Joshua T Weinreb[1], Zachary Zaroogian[1], Hui Liu[3], Zhuoning Li,[3], Ileana C Miranda[4], Enes Dasdemir[9], Bofei Wang[8], Anurag Sharma[5], Hilda A Pasolli[5], Brian Eastman[6], Gopi K Mittapalli[6], Chi-Ching Mak[6], Matthew C Jarvis[6], Mara Monetti[3], Hussein A Abbas[8], Carine Bossard[6], Juan C Osorio[7], Omar Abdel-Wahab[1], Eric Wang[2,11,12]*

[1] Memorial Sloan Kettering Cancer Center, Molecular Pharmacology, New York City, New York;
[2] The Jackson Laboratory for Genomic Medicine, Genetics
[3] Memorial Sloan Kettering Cancer Center, Proteomics Innovation Laboratory, New York City, New York;
[4] Memorial Sloan Kettering Cancer Center, Laboratory of Comparative Pathology, New York City, New York
[5] Rockefeller University, Electron Microscopy Resource Center, New York City, New York
[6] Biosplice Therapeutics, Inc., Research, San Diego, California
[7] Memorial Sloan Kettering Cancer Center, Immuno-Oncology, New York City, New York
[8] University of Texas MD Anderson Cancer Center, Leukemia, Houston, Texas
[9] University of Houston, Biology and Biochemistry, Houston, Texas
[10] UConn Health, Genetics and Genomics
[11] UConn Health, Genetics and Genomics
[12] UConn Health, Institute for Systems Genetics

*These authors contributed equally

CD47 is a macrophage checkpoint broadly expressed on normal tissues and aberrantly upregulated in cancers including acute myeloid leukemia (AML). Antibody-based therapeutic blockade of CD47 has been limited by on-target toxicities in healthy cells, underscoring the need for lineage-specific strategies to selectively modulate CD47. Here, we performed a CD47 surface antigen-based CRISPR screen and identified the kinase DYRk1A as an AML-specific positive regulator of CD47 surface expression. CD47 regulation by DRYK1A depends on its kinase activity. Phosphoproteomics revealed multiple endocytic proteins as DYRk1A substrates, including the E3 ligase CBL, which dictate cell surface CD47 endocytosis and surface abundance. We developed the first ultra-selective DYRK1A/B inhibitor, SM13797 which selectively reduced CD47 expression on AML cells and had significant macrophage-dependent antitumor activity while sparing normal tissues. These data systematically define regulators of surface CD47 abundance on AML and propose a tumor-selective therapeutic approach of targeting CD47 through DYRK1A inhibition.

2. Yamin Liu

Evolution of collagen homeostasis in stromal fibroblasts underlie acquired resistance to cancer malignancy among placental mammals

Yamin Liu[1,2], Yasir Suhail[2,3], Wenqiang Du[1], Xihua Qiu[1,2], Khadija Wali[1], Anarghya Murthy[1,2], Junaid Afzal[4], Kshitiz[1,3,]

[1]Department of Biomedical Engineering, UConn Health
[2]Department of Biomedical Engineering, University of Connecticut, Storrs, CT
[3]Center for Cell Analysis and Modeling, UConn Health
[4]Department of Medicine, University of California San Francisco, San Francisco, CA

Among mammals, placental invasion is correlated with vulnerability to malignancy. Animals with invasive placentation (e.g. humans and rodents) are more vulnerable to malignancy of carcinomas, while those will no placental invasion (e.g. bovine) also can contain cancer metastasis locally. We have advanced an evolutionary framework to explain this correlation, termed Evolved Levels of Invasibility (ELI) which posits that it is the stromal evolution into a highly resistive state which has limited placental invasion, and secondarily, carcinoma dissemination. However, this framework is only correlative, and experimentally validated *in vitro*. Evolutionary hypotheses are extremely challenging to demonstrate, and confirm in the natural ecosystem. However, we have developed mouse models of “stromal avatar”, which can allow us to test the ELI framework, as well as mechanistically demonstrate the causal genetic correlates underlying the vastly differential rates of invasion across mammals. *In vivo*, bioluminescent A375 cancer cells co-mixed with hFbs or bFbs were implanted subcutaneously into SCID-beige/NBSGW mice and the progressive tumor development were observed and quantified using BLI. We found the hFbs group mice tumor size was smaller than bFbs group mice, but developed distal metastatic locii, as well as exhibited lower survivability. We further explored the genetic underpinnings underlying these differences by differential transcriptomics of hFbs, bFbs with or without the skin cancer cells, finding vast differences in gene expression encoding collagen subtypes, as well as matrix metalloproteinases. These included Collagen I and Collagen III being higher in cows, while Collagen VII, MMP3, MMP14 being higher in humans. Using gene perturbation combined with a quantitative nanotextured stromal invasion assay, as well as *in vivo* dissemination assays, we demonstrate the causality of these genes as critical regulators of stromal resistance in bFbs, while promoting vulnerability to invasion in hFbs. Our data support the idea that the evolution of stromal derived collagen rich ECM niche regulate the cancer metastases, which could provide important insights to develop rational antimetastatic therapeutics.

3. Rajkishor Pandey

Development of CDH17-targeted CAR-T cells for pancreatic adenocarcinoma treatment

Rajkishor Pandey[1], Kevin Staveley-O’Carroll[1], Eric T Kimchi[1], Guangfu Li[1,2]

[1]Department of Surgery, UConn Health
[2]Department of Immunology, UConn Health

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of carcinoma in the human pancreas (more than 95 % cases). Pancreatic cancer diagnosis in the early stage is challenging due to its asymptomatic where the chance of curing is greatest. In normal cells, cell adhesion

molecule cadherins help to maintain cell structure and functions appropriately. Candherin-17 (CDH-17) is one of the cadherin family surface proteins proficiently expressed in pancreatic tumor tissue and helps with tumor progression in the pancreas and other organs. Therefore, CDH-17 might be used as an immunotherapeutic target antigen against PDAC. At present study, the significant overexpression of CDH-17 was detected in different human & mouse tumor cell lines & human PDAC patients than normal peritumor tissue. Therefore, CDH-17 targeted nanobody based chimeric antigen receptors (CAR) have been designed in the lentivirus (LV) backbone. The designed and synthesized CDH17-CAR was transducted into jurkat T cells and human primary Pan-T cells with the addition of polybrene (8 µg/mL). Around 90 % of CAR expressions on the surface of T cells were detected. Furthermore, CAR-T has shown significant binding specificity with human and mouse PDAC cell lines on CDH-17-expresstion basis. Apart from binding specificity, human primary CAR-T cells and jurkat CAR-T cells showed significant cytotoxicity to different human PDAC cell lines based on CDH17 expression with the release of proinflammatory cytokines. Additionally, CAR-T cells suppressed the growth of PDAC cells in xenograft NSG mice in vivo study. Further, need to validate CAR-T cells as well as primary human PBMC derived CAR-T antitumor effects in the patient derived organoid model (PDOM).

4. Daniel Penarete-Acosta

Patient-specific susceptibility to colibactin-induced DNA damage in colorectal cancer

Daniel A. Penarete-Acosta[1], Arthur Szacik[1], Stephen Y. Li[2], Sarah Rizvi[2], Daniel W. Rosenberg[3], Sasan Jalili[1,2]

[1]Jackson Laboratory for Genomic Medicine
[2]Department of Immunology, UConn Health
[3]Center for Molecular Oncology, UConn Health

Colibactin is a genotoxin produced by *pks+ Escherichia coli* that has been associated with colorectal cancer development in humans. While key mechanisms of colibactin-mediated DNA damage have been increasingly defined, the host factors that mediate epithelial susceptibility to colibactin remain poorly understood. A significant challenge in addressing this question is the limited ability of conventional cell culture models to reproduce the physiological host-microbiota interface and support direct exposure of patient-derived colorectal epithelium to live colibactin-producing bacteria. To overcome this, we developed an advanced microfluidic chip platform that mimics key features of the colorectal microenvironment. The chip establishes a hypoxic gradient using oxygen scavenging and controlled gas diffusion across a porous membrane, while maintaining epithelial viability and differentiation during long-term coculture with bacteria. Using patient-derived colorectal epithelial cultures, we identified significant inter-individual heterogeneity in response to *pks+ E. coli,* with marked DNA damage accumulation upon exposure to colibactin in 70% of patients. Through a multi-omic approach, we identify novel genomic, transcriptomic and proteomic targets, suggesting that the biological state of the host epithelium is an important determinant of its response to microbial genotoxic stress. We anticipate this host-microbiota interaction model to significantly improve our capacity to develop novel therapeutic targets against microbiota-driven colorectal cancer.

5. Marianna Marino

Metabolic characterization of adipocyte specific FGF2 deletion under chow and high fat diet conditions in mice

Marianna Marino, PhD[1], Luigi Marino, PhD[1], Khadija B. Danazumi[1], Marja M. Hurley, MD[1], and Francesco S. Celi, MD, MHSc[1]

[1]Division of Endocrinology and Metabolism, School of Medicine, UConn Health

Background: Fibroblast Growth Factor 2 (FGF2) is a member of the FGF family with pleiotropic roles in the development and function of multiple organ systems. In the adipose tissue, FGF2 is synthesized and secreted by adipocytes. Notably, elevated FGF2 levels have been linked to obesity and to the pathogenesis of metabolic syndrome. However, the mechanisms underlying this correlation have not yet been clarified, and literature has so far reported conflicting results on FGF2's role in adipogenesis and thermogenic regulation.
Objective: To determine whether FGF2 plays a modulatory role in adipose tissue function and energy metabolism, we are examining the metabolic response under basal conditions and diet-induced metabolic stress of mice with adipose tissue-specific FGF2 knockout (aKO) generated by targeting *adipoq* expressing cells.
Methods: We are studying wild-type and FGF2 aKO mice of both sexes, at 3 and 6 months of age, fed with a regular chow diet or a long term 45% high fat diet. Comprehensive metabolic assessments, including body weight monitoring, glucose and insulin tolerance tests, indirect calorimetry, and body composition analysis is being conducted.
Preliminary data: Preliminary data show that, under regular chow diet conditions, no baseline metabolic differences occurred between wild type and aKO mice of either sex. Ongoing studies are now evaluating metabolic outcomes in mice exposed to a high-fat diet, which may reveal adaptive metabolic responses regulated by FGF2.
Conclusions: By elucidating the adipocyte specific role of FGF2, this work will allow us to understand its involvement in thermogenic and metabolic modulation, thereby advancing the current knowledge on FGF2 signaling in adipose tissue, with potential relevance for obesity and metabolic diseases.

6. Ramalakshmi Ramasamy

Spatial Mapping of Placental Senotypes in Preeclampsia: Trophoblast Senescence and the Molecular Control of Fusion

Ramalakshmi Ramasamy[1], Giray Naim Eryilmaz[1], Riley Sheldon[1], Cole Lorig[1], Sonja Suvakov[3], George Kuchel[2], Duygu Ucar[1], Elizabeth Enninga[3], Vesna Garovic[3], Paul Robson[1]

[1]The Jackson Laboratory for Genomic Medicine
[2]Center on Aging, UConn Health
[3]Mayo Clinic, MN

Cellular senescence has emerged as a fundamental mechanism in placental development, where transient, programmed senescence in syncytiotrophoblasts (STBs) and extravillous trophoblasts (EVTs) supports normal trophoblast turnover, invasion, and vascular remodeling. These developmental senescence programs exemplify antagonistic pleiotropy, in which pathways beneficial early in gestation may later contribute to disease when dysregulated. In preeclampsia,

these normal senotypes appear aggravated: we observe an excess accumulation of senescent, fused STB manifesting as increased syncytial knots with heightened senescence-associated secretory phenotype (SASP) alongside a marked rise in multinucleated EVTs. Here we apply Xenium spatial transcriptomics to preeclamptic placental tissue for the first time, together with H&E-based histomorphometric analysis to spatially resolve elevated senescence and SASP markers in preeclamptic versus control placentas, including *GDF15*, *CCL5*, and *SERPINE1* enriched within syncytial knots, and *CDKN1A* (p21) and *IGFBP3* elevated in multinucleated EVTs. Because both syncytial knot formation and, potentially, multinucleated EVT formation arise through trophoblast fusion, we further investigated the molecular regulation of fusion competence using matched placental and fetal membrane tissue. We find that villous cytotrophoblasts, which express *TFEB*, readily fuse into syncytiotrophoblast, whereas membrane cytotrophoblasts, which lack *TFEB* expression, do not fuse, identifying TFEB as a candidate master regulator distinguishing fusion-competent from non-fusing trophoblast. Using iPSC-derived syncytiotrophoblast models, we further demonstrate that *TFEB* loss impairs fusion and downregulates syncytialization genes including *ERVFRD-1* and *CYP19A1*. Together, our findings position preeclampsia as a disorder of aggravated placental senescence, and identify trophoblast fusion competence as a shared mechanism linking distinct senotypes across trophoblast lineages.

7. Iris Nakashima

Correlated light and serial section electron microscopy of *Arabidopsis* ovules during fertilization

Iris F. Nakashima[1], Nathaniel Ponvert[2], Mark Terasaki[1], Laurinda A. Jaffe[1], Mark A. Johnson[2], Rachael P. Norris[1]

[1]Department of Cell Biology, UConn Health
[2]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI

Flowering plants have two separate female gametes, the egg and the central cell, that must each fuse with one of two sperm cells delivered by a pollen tube. It is unclear how the two fertilization events are coordinated such that both sperm do not fertilize the same cell. Ultrastructural visualization of gamete membranes during this time could provide valuable information, but this is technically challenging to accomplish. Here we describe methods for obtaining *Arabidopsis thaliana* ovules at varying timepoints before, during, and after fertilization to analyze serial sections by electron microscopy. We first live-image preparations of pollinated pistils by confocal microscopy to visualize the time of pollen tube bursting. At precise time points after the pollen tube burst, we fix pistil preparations in glutaraldehyde, high-pressure freeze the samples, freeze substitute, then embed the plant tissue in resin. Blocks containing the pollinated ovules are serial sectioned with an automated tape collecting ultramicrotome (ATUM) to collect all sections through the interacting gametes. Using a scanning electron microscope with a backscatter detector, we have imaged ovules containing released sperm in the short window of time when fertilization occurs. In an ovule fixed seven minutes after sperm release, sperm were located in a cleft between the egg cell and central cell. One sperm cell was directly adjacent to both the egg and central cells, while the other sperm cell was adjacent only to the central cell. In an ovule fixed nine minutes after sperm release, cell-cell fusion had occurred, as indicated by a sperm nucleus inside the central cell in close proximity to the central cell nucleus. Using this approach, we will examine

more ovules in this timeframe, and we will compare them to ovules pollinated with sperm that lack the fusion protein HAP2.

8. Corie Owen

Luteinizing hormone-induced interfollicular communication in the mouse ovary

Owen C.M.[1], Yee S.P.[1,2], Jaffe L.A.[1]

[1]Department of Cell Biology, UConn Health
[2]Center for Mouse Genome Modification, UConn Health

Luteinizing hormone (LH) triggers the resumption of oocyte meiosis and ovulation in preovulatory ovarian follicles. These events have generally been viewed as autonomous responses occurring independently within each follicle. Here, however, we show that mouse preovulatory follicles can communicate with one another through an LH-induced paracrine signaling network. Isolated preovulatory follicles lacking LH receptors (Lhr-KO) resumed oocyte meiosis when co-cultured with LH-stimulated wildtype follicles, despite being unable to respond directly to LH. Oocytes within Lhr-KO follicles also resumed meiosis when exposed to conditioned medium from LH-treated wildtype follicles, demonstrating that diffusible factors mediate this interfollicular communication. Neutralizing antibodies against the epidermal growth factor receptor ligands epiregulin and amphiregulin inhibited the LH-induced interfollicular communication, identifying these LH-induced factors as key signaling molecules. Although epiregulin and amphiregulin are known to transmit LH signals within individual follicles, our findings indicate that they can also coordinate responses among neighboring follicles. Together, these results demonstrate that LH regulates a communication network between preovulatory follicles rather than acting solely at the level of individual follicles. Our findings raise questions about whether LH-induced paracrine factors regulate other stages of folliculogenesis in the ovary.

9. Dylan Krajewski

Polarized release of brain microvascular endothelial cell-derived extracellular vesicles supports leukocyte transendothelial migration in vitro

Dylan Krajewski.[1], Joel S. Pachter[1]

[1]Department of Immunology, UConn Health

Abundant evidence indicates extracellular vesicles (EVs) are mediators of intercellular communication. Previous reports from this group further showed that EVs from brain microvascular endothelial cells (BMEC) transferred the tight junction protein (TJ) claudin-5 (CLN-5) to leukocytes *in vitro* and during experimental autoimmune encephalomyelitis, leading us to hypothesize that such interaction might facilitate transendothelial migration (TEM) by a “zipper mechanism” whereby CLN-5 molecules on leukocyte-bound EVs temporarily replace those at interendothelial junctions. Such a mechanism is likely to be under strict spatiotemporal control. A corollary to this is that EVs are released from BMEC in a polar manner, such that only those released from the apical surface interact with leukocytes, while those from the basolateral surface bind perivascular and/or parenchymal targets. To assess polar EV release from BMEC and subsequent BMEC EV:leukocyte interactions during TEM using transwell assays, flow cytometry, nanoparticle tracking, super-resolution and live-cell time-lapse imaging were used. It was shown

that EV release was highly polarized, as EVs accumulated predominantly in the chamber facing their membrane of origin. Supportive of polarized exosome release, apical and basolateral membrane material appeared segregated into discrete late endosomal populations. Consistent with a role in TEM, apical-derived BMEC EVs preferentially associated with adherent leukocytes, with TEM suppressed when EV release was inhibited. Polarized EV release was maintained under physiological flow, wherein CLN-5^{+} EVs exhibited near exclusive release at the apical surface, possibly reflecting their predisposition toward interacting with circulating immune cells.

10. Katarina Milicevic

Probing Early Alzheimer's Disease Physiology with Dual Glutamate-Voltage Imaging in the Same Brain Slice

Katarina D. Milicevic[1,2], Nicholas J. Matejak[1], Naimah E. Carter[1], Amal M. Abdulkadir[1], and Srdjan D. Antic[1,2]

[1]UConn Health
[2]University of Connecticut, Institute for the Brain and Cognitive Science (IBACS), Storrs, CT

Two competing hypotheses in Alzheimer's disease (AD) propose that early circuit dysfunction arises from impaired synaptic transmission or disrupted glutamate uptake. Here, we tested both mechanisms by measuring synaptically evoked membrane-potential responses using voltage imaging and glutamate transients using glutamate imaging in acute brain slices from a transgenic mouse model of AD. Cortical responses were quantified across progressively increasing stimulus intensities. In both modalities, response amplitude increased monotonically and approximately linearly with stimulus intensity, with no evidence of nonlinear scaling. Kinetic analyses further revealed no significant genotype-dependent differences in response rise or decay time across imaging modalities or ages, indicating that the temporal dynamics of evoked cortical responses remain largely preserved. Pharmacological disinhibition with 4-aminopyridine (4-AP) produced a greater increase in glutamate signal amplitude at P50 compared to P100, suggesting age-dependent differences in cortical excitability rather than AD-specific effects. Complementary UPLC-MS proteomic analyses identified AD-associated protein alterations across three time points (P50, P100, and P200). At P50, inflammatory markers were already upregulated, while several neuronal proteins (e.g., tetraspanin-3, synaptotagmin) were downregulated. By P200, proteins associated with immune function (complement C4-B, tumor necrosis factor-α), lysosomal pathways (progranulin), and amyloid/lipid metabolism (clusterin, apolipoprotein E) were upregulated, whereas proteins involved in dopamine transport, intracellular calcium regulation (neurogranin), and synaptic transmission (synaptophysin) were reduced. Notably, inflammatory markers (CD180 and ISG15) were elevated at both P50 and P200, indicating sustained immune activation. Collectively, these findings demonstrate that early prodromal AD is characterized by increased cortical response amplitudes without accompanying changes in response kinetics or input-output transformations. Increased sensitivity to disinhibition at P50 further suggests elevated network excitability at earlier stages. Proteomic alterations in immune-related pathways may represent early molecular signatures of disease progression, preceding later synaptic dysfunction.

11. John Stout

Signal Control by Layer 6 Corticothalamic Neurons

John Stout[1], Katarina Milicevic[1], Krystina Jorgensen[1], Srdjan Antic[1], and Timothy Spellman[1]

[1]Department of Neuroscience, UConn Health

Communication between the prefrontal cortex and thalamus supports executive function and is disrupted across neurodevelopmental disorders. A major pathway linking the prefrontal cortex with the thalamus originates from layer 6 corticothalamic (L6CT) neurons. Although defined by their projections to the thalamus, we hypothesized that prefrontal L6CT neurons also form intracortical connections with local inhibitory interneurons to regulate higher-order cortical signaling. We combined two-photon calcium imaging, optogenetics, behavioral assays, cell-type-specific slice electrophysiology, and chemogenetics to define the intracortical contributions of L6CT neurons. We found that L6CT neurons naturally encode information about recent choice errors and, when optogenetically stimulated, suppress competing representations among neurons encoding correct choices. In a smaller population of cortical neurons, L6CT stimulation produced gain modulation of higher-order representations, altering response amplitude while preserving task preference. Chemogenetic suppression of the thalamus revealed that L6CT neurons primarily regulate deep-layer cortical neurons through local intracortical connections. Consistent with these findings, slice electrophysiology demonstrated monosynaptic excitation of deep-layer inhibitory interneurons by L6CT neurons, providing a circuit mechanism for lateral suppression of prefrontal pyramidal neurons. Together, these experiments reveal an intracortical L6CT circuit for regulating prefrontal representations during executive control, with potential relevance to neurodevelopmental disorders.

12. Ashok Dhinakaran

Cutaneous commensals shape protective barrier immunity in skin

Ashok Kumar Dhinakaran[1], Daniel Peñarete-Acosta[1], Sarah Rizvi[1,2], Arthur Szacik[1], Benjamin Johnson[3], Sasan Jalili[1,2,4]

[1]The Jackson Laboratory for Genomic Medicine
[2]Department of Immunology, UConn Health
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Skin is a major barrier tissue where commensal microbes and immune cells interact to maintain homeostasis and protect against pathogen invasion. Commensal bacteria such as *Staphylococcus epidermidis* can enhance cutaneous immunity and improve resistance to subsequent infection, yet the cellular mechanisms underlying this protective state remain incompletely understood. In particular, the cellular mediators of commensal-induced protection, the kinetics of this response, and how prolonged commensal exposure transitions from immune activation to durable barrier adaptation are not fully understood. To address this, we used murine skin colonization models combined with minimally invasive microneedle-based sampling and confirmatory biopsies to longitudinally profile local immune, epithelial, and microbial responses following short- and prolonged *S. epidermidis* exposure. Protective responses were evaluated

after challenge with *Staphylococcus aureus*. Skin immune populations were characterized by multiparameter flow cytometry, with emphasis on CD4 T cells and $CD3^+CD4^-CD8^-$ double-negative T cells, including discrimination of γδ T cells and other innate-like lymphocyte populations. Functional polarization was assessed using IL-17A- and IFNγ-associated readouts together with local inflammatory and antimicrobial profiling. Transcriptomic analyses were used to define temporal changes in inflammatory, immune, and epithelial barrier programs. Our findings support a time-dependent model of commensal conditioning in which early *S. epidermidis* exposure is associated with IL-17-, NF-κB/TNF-, chemokine-, and antimicrobial programs, whereas prolonged exposure shifts toward epidermal differentiation, keratinization, junctional, and barrier-repair programs. This transition is accompanied by remodeling of CD4 and double-negative T cell populations, including γδ T cells, and enhanced resistance to subsequent *S. aureus* colonization. Together, these findings suggest that prolonged commensal exposure establishes a protective barrier-defense state through coordinated immune conditioning and epithelial reprogramming rather than simple direct bacterial antagonism. This work provides a translational framework for understanding how commensals program protective skin immunity and for identifying immune and microbial signatures associated with enhanced barrier defense.

13. Tasnif Rahman

Vivarium-Tyssue: A Platform for Compositional Multi-scale Cell Vertex Models

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Background Vertex models are a widely used class of mathematical models used to represent the geometry and dynamics of tissues – wherein cells are represented by the polygons or polyhedrons and allows for rapid hypothesis testing and phenomenological validation against *in vitro* and *in vivo* data. However, available simulation tools make it difficult to incorporate subcellular and tissue scale interactions that regulate these putative cellular forces without rewriting or editing the tool itself. Thus, we have created a tool based on the Python-based vertex model software Tyssue using our Vivarium integrative modeling platform that allows external models to dynamically change model parameters and trigger events such as cell division, death and differentiations in a modular fashion.

Methods

We used Vivarium 2.0 interface to wrap Tyssue's Euler solver as a Vivarium process which runs the vertex model internally. This process can then interact with other processes through reading and writing from shared data-stores: (i) it outputs the tissue state at each simulation interval, which can then be read by external models that model cellular processes like stochastic cortical tension and cell divisions; (ii) these external processes run their models and output serialized function calls that to a "behaviors" store instructing the vertex model what to change; (iii) the vertex model then reads and executes these functions at the beginning of the next simulation step. This allows any number of external models to dynamically change the vertex model without the need to be run in the vertex-model Euler loop.

Results

Our tool runs multi-scale vertex model simulations of various biological phenomena. The simulations can be composed using a json specification file improving shareability, uses parallel processing for each model to maintain performance, and is modularly composable.

14. Nisha Mahey

Post-fluoroquinolone treatment molecular events and nutrient availability modulate *Staphylococcus aureus* antibiotic persistence

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Staphylococcus aureus is a bacterial pathogen associated with about one million deaths per year globally. *S. aureus* can infect diverse host sites, including skin and the airway, many of which are nutrient-limited. At these infection sites, competition with immune cells can further deprive *S. aureus* of metabolites, forcing the bacteria to enter a state of reduced metabolic activity. While lower metabolic activity may help contain the growth of the pathogen, it can also enhance *S. aureus*'s survival during antibiotic treatment. Here, we focus on *S. aureus*'s response to fluoroquinolones (FQs), which inhibit topoisomerases needed for nucleic acid synthesis and can lead to double-stranded DNA break (DSB) formation. We investigated how the stationary phase *S. aureus* survives FQ treatment. We demonstrate that even in the stationary phase, when nucleic acid synthesis levels are minimal, loss of DNA break repair enzymes reduces *S. aureus*'s survival. Using genetic and imaging approaches, we found that both survivors and cells that die induce DNA damage responses after FQ treatment terminates, and DNA repair enzymes are needed mainly during this recovery period. We found that starving *S. aureus* after treatment significantly increases FQ persistence, even in cells lacking the ability to repair DSBs. Our data suggest that *S. aureus* in nutrient-limited infection sites can delay the resumption of nucleic acid synthesis after treatment, allotting more time for resolving trapped DNA-topoisomerase complexes. This study demonstrates that both the nutritional environment and the molecular events during post-FQ recovery are critical determinants of *S. aureus* survival. Collectively, these findings point to processes that could be targeted to sensitize *S. aureus* to FQs and ultimately improve treatment outcomes.

15. Anna Truong

Non-canonical double-stranded DNA break repair mechanisms in *Pseudomonas aeruginosa*

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Infections caused by the gram-negative pathogen *Pseudomonas aeruginosa* are commonly treated with fluoroquinolone (FQ) antibiotics, which target the bacterial topoisomerase enzymes DNA gyrase and DNA topoisomerase IV. Inhibition of these essential enzymes results in the accumulation of deleterious double-stranded DNA breaks (DSBs), ultimately causing genome instability and death. However, rare *P. aeruginosa* persister cells that do not exhibit classical resistance genotypes can survive FQ treatment and serve as a reservoir for relapsing infections. Hence, we aim to elucidate DSB repair mechanisms that enable *P. aeruginosa* persistence against FQs. We demonstrate that two canonical DSB repair pathways, RecA-mediated

homologous recombination and Ku-/LigD-mediated non-homologous end-joining (NHEJ), are dispensable for *P. aeruginosa* DNA repair following treatment with the FQ levofloxacin, implicating the involvement of alternative pathways. To interrogate further, we employ a genomic DSB reporter system where two inverse I-SceI endonuclease restriction sites are incorporated into the *P. aeruginosa* chromosome. Expression of I-SceI generates chromosomal DSBs with non-complementary 3' overhangs, which are repaired in surviving cells. Sequence analysis of the repaired junctions reveals that *P. aeruginosa* can engage in non-canonical DSB repair activities, including LigD-independent NHEJ and Ku-/LigD-independent microhomology-mediated repair (MHMR) of three to eight base pairs. Both NHEJ and MHMR pathways involve bidirectional resection of one to hundreds of nucleotides up and downstream of the I-SceI cleavage sites, and these repair outcomes are ablated upon genetic deletion of *recA*. Moreover, we are uncovering how *P. aeruginosa* can repair DSBs consisting of 3', 5', and blunt ends by identifying the essential enzyme players of non-canonical NHEJ and MHMR using transcriptomic approaches. Altogether, our findings provide mechanistic details into elusive DSB repair pathways in *P. aeruginosa*, informing how globally threatening pathogens overcome genotoxic stress from clinically important antibiotics.

16. Ranjuna Weerasekera

The role of RpoS in metabolic reprogramming and antibiotic tolerance of uropathogenic *Escherichia coli*

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Recurrent urinary tract infections (UTIs) are among the most common bacterial infections, with approximately 80% caused by uropathogenic *Escherichia coli* (UPEC). Antibiotic treatment failure is a major contributor of recurrent UTIs. Besides acquiring antibiotic resistance, UPEC can become tolerant to therapeutics because they have entered a phenotypic state that allows them to survive antibiotics. Understanding the mechanisms that drive antibiotic tolerance is essential for advancing effective therapeutic strategies. In our current study, we treated a panel of non-resistant UPEC strains with the fluoroquinolone (FQ), levofloxacin. We found a strain that exhibited FQ tolerance in a nutrient-dependent manner. When we compared the genomes of the susceptible and tolerant UPEC strains, we identified a point mutation in the *rpoS* gene, which deactivates the major general stress response regulator. Using genetic mutants, we show that loss of RpoS led to increased FQ tolerance in strains that were originally killed by the FQs when the mutants were cultured in peptone-containing minimal media and in artificial urine. To deduce the molecular basis of FQ tolerance, we performed NMR-spectroscopy-based metabolomics, LC-MS/MS-based proteomics, genetics, and biochemical assays to investigate how urine-associated metabolites modulate FQ sensitivity. We found that strains with functional RpoS sustains RNA transcription when they are grown in media with peptone, which sensitizes them to FQs. Our work suggests that RpoS contributes to phenotypic variation in UPEC, allowing distinct bacterial subpopulations to adapt to and survive under adverse conditions within the urinary tract.

17. Mubark Mebrat

Structural and Biochemical Characterization of *Klebsiella pneumoniae* PBP3

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Peptidoglycan is an essential component of the bacterial cell wall and is cross-linked by penicillin-binding proteins (PBPs), which are the primary targets of β-lactam antibiotics. In *Klebsiella pneumoniae (k. spp.)*, PBP3 is an essential component of the divisome that catalyzes peptidoglycan cross-linking during septum formation and is an important β-lactam target. Despite the clinical importance of PBP3, the molecular basis governing its interactions with β-lactams and contribution to antibiotic resistance remains poorly understood. Functional studies revealed that penicillin G–acylated PBP3 undergoes unusually rapid acyl-enzyme hydrolysis compared with other characterized PBPs. To investigate the molecular basis of PBP3 substrate interactions, we established and optimized a platform to structurally and biophysically characterize the PBP3 soluble domain. Recombinant expression and purification were optimized to yield up to 60 mg/L of purified protein, enabling isotopic labeling and biomolecular NMR studies of this challenging 55 kDa target. Crystallization and structure-determination conditions were further optimized, enabling determination of the first X-ray crystal structure of *k. spp* PBP3 at 2.8 Å resolution. The structure revealed extensive structural flexibility, particularly within the N-terminal region. Together, these results establish a structural and biophysical platform for investigating PBP3 substrate interactions and its interactions with β-lactam antibiotics.

18. Luke Trinity

CMV-specific clonal expansion and immune remodeling revealed by human PBMC profiling

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Cytomegalovirus (CMV) is a ubiquitous herpesvirus that establishes lifelong latency and drives durable remodeling of the immune system. Because CMV infection preferentially expands cytotoxic and innate-like lymphocyte states, it provides a powerful human model for studying immune memory beyond conventional antigen-specific T cell responses. We systematically characterized CMV-associated immune remodeling across six human cohorts, including two newly generated datasets, using single-cell RNA sequencing, T cell receptor (TCR) sequencing, and flow cytometry. To identify CMV-associated clonal responses, we integrated single-cell TCR data with a large database of known CMV-reactive clones and predictive modeling (CMVerify). We also examined longitudinal immune changes in an individual who seroconverted from CMV-negative to CMV-positive.

CMV-positive adults showed the expected expansion of CD4+ and CD8+ TEMRA cells, adaptive NK cells, and gamma delta T cells, along with increased frequencies of GZMK+ CD8+ T cells and

atypical B cells and a reduction in CD56dim NK cells. In the seroconverter, CMV primary infection was accompanied by rapid shifts in circulating immune cell composition, including expansion of Vd1 gamma delta T cells and adaptive NK cells. Single-cell TCR analyses identified novel CMV-specific clonal expansions that were reproducible across two independent cohorts. Within the CD8+ lineage, CMV-specific clones were enriched in GZMK+ CD8+ and CD8+ TEMRA cells, while within the CD4+ lineage, Th1 cells and CD4+ TEMRA cells showed clonal expansion. This integrative study defines CMV-driven remodeling of the human immune system as a coordinated expansion of both adaptive and innate-like memory compartments. In addition to conventional TEMRA responses, CMV-associated expansion of Vd1 gamma delta T cells and adaptive NK cells highlights the breadth of the latent CMV response and its profound impact on circulating immune cells.

19. Aditya Kanwal

Establishing a "poison-peptide" induced murine model of truncated Titin-associated dilated cardiomyopathy and exploring therapeutic avenues.

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Titin truncating variants (TTNtvs) are the most common pathogenic genetic variants identified in individuals with dilated cardiomyopathy (DCM). Previous work has shown that TTNtv expression is associated with reduced levels of full-length Titin (TTN) protein, along with impaired sarcomere structure and function in affected patients. Despite their high prevalence, the pathogenic mechanisms by which TTNtvs drive disease remain incompletely understood, with ongoing debate between haploinsufficiency and poison-peptide (or dominant-negative) models of action. Investigating the role of TTNtvs in DCM has been challenging, largely due to the lack of robust in-vivo models. Although murine systems provide a valuable experimental platform for genetic manipulation, most existing models fail to develop the severe and progressive DCM phenotype observed in patients, thereby limiting mechanistic insight and therapeutic exploration.

Here, by using allele specific CRISPR activation, we demonstrate that poison-peptide mechanism is sufficient to drive dilated cardiomyopathy in a murine model harboring a TTNtv, similar to human patients. Leveraging this first-of-a-kind model, we systematically interrogate the molecular, cellular and physiological consequences of TTN truncation and establish a relevant in-vivo platform for studying disease progression and evaluating therapeutic interventions. Lastly, we also test a non-allele specific activator to show improvement in cardiac function and survival in these DCM mice, paving way for a prospective therapeutic.

20. Kiseki Washitani

Lipid metabolic signatures reveals potential biomarkers in craniometaphyseal dysplasia

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Craniometaphyseal dysplasia (CMD) is a rare genetic bone disorder characterized by progressive thickening of craniofacial bones and flared metaphyses of long bones. Hyperostosis of craniofacial bones results in cranial nerve compression leading to visual impairment, hearing loss, or severe headache. CMD is inherited as an autosomal dominant (AD) trait with mutations in the progressive ankylosis (*ANKH*) gene and an autosomal recessive (AR) form carrying a mutation in the gap junction protein alpha 1 (*GJA1; Cx43*) gene. We have generated $Ank^{KI/KI}$ mice and $Cx43^{KI/KI}$ mice and reported that both models replicate many features of human CMD. Currently, treatment for CMD is largely limited to surgical intervention, and no biomarkers have been established. Clinically useful biomarkers can be used to facilitate early diagnosis and disease monitoring, enabling timely clinical intervention. Here, we aim to identify biomarkers for CMD using mass spectrometry (MS)-based metabolomics studies and lipidomic analyses. In this study, we examine human urine and both mouse models for CMD. We showed increased bone marrow adipose tissue in $Ank^{KI/KI}$ and $Cx43^{KI/KI}$ mice compared to their wild type littermates, suggesting altered lipid metabolism in both CMD AD and AR mice. We further showed that the size of lipid droplets in bone marrow-derived adipocyte cultures are significantly increased in CMD mutant mice by BODIPY staining. Interestingly, we noted that several classes of lipid metabolites are significantly less abundant in $Ank^{KI/KI}$ mouse serum by lipidomic analyses. Moreover, lipid metabolic pathway is commonly affected in CMD patients and mice. Taken together, our results suggest that lipid handling is divergently dysregulated between peripheral and bone marrow adipocytes in CMD mice and several lipid metabolites may serve as potentially biomarkers for CMD.

21. Pengyu Zong

Ultra-deep single-nucleus sequencing identifies IL-31 signaling as a promoter of ischemic brain injury

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Ischemic stroke is a devastating disease and a leading cause of death and long-term disability worldwide. Despite advances in recanalization therapies, effective pharmacological therapies to protect the brain from ischemic injury remain lacking. This therapeutic gap is largely due to our incomplete understanding of the molecular mechanisms and cell populations that drive ischemic brain injury. Identifying previously unrecognized injury-associated cell populations and signaling pathways may reveal new therapeutic targets. To identify novel therapeutic targets, we leveraged the latest next-generation RNA sequencing technology, 10x Genomics GEM-X v4, to perform

ultra-deep single-nucleus RNA sequencing of the ischemic brain in a mouse model of ischemic stroke induced by middle cerebral artery occlusion (MCAO).

We achieved more than 50,000 reads and detected over 5,000 genes per cell, enabling us to identify previously unrecognized ischemia-induced neuronal populations. These neuronal populations were characterized by high expression of cell death- and ischemia-associated markers, as well as particularly high expression of both IL-31 receptor subunits, IL-31RA and OSMR, suggesting a potential role for IL-31 signaling in ischemic brain injury. Consistent with this finding, in vitro treatment with IL-31 exacerbated ischemic neuronal injury. In vivo, intracerebral administration of IL-31 similarly worsened ischemic brain injury, whereas treatment with either an IL-31-neutralizing antibody or an IL-31 receptor-blocking antibody significantly attenuated injury. In summary, our study identifies IL-31 signaling as a previously unrecognized mediator of ischemic brain injury and demonstrates that targeting the IL-31/IL-31 receptor pathway may represent a promising therapeutic strategy for ischemic stroke.

22. Manaswee Barua

Bioactive Polyphosphazene-based Composites for Bone Regenerative Engineering

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The reconstruction of critical-size bone defects remains a formidable challenge in orthopaedic surgery. Current bone grafting strategies spanning autografts, allografts, and synthetic scaffolds, are frequently compromised by donor-site morbidity, infection risk, limited availability, immune rejection, and suboptimal cellular integration. Recently, biodegradable polyphosphazenes (PPHOS) have emerged as promising candidates for bone regenerative engineering due to their synthetic flexibility, tunable properties, metabolizable degradation products, and established biocompatibility. Furthermore, reinforcing PPHOS with bioceramic such as akermanite ($Ca_2Mg[Si_2O_7]$) can strategically enhance mechanical integrity while imparting/improving osteogenic, osteoinductive, pro-angiogenic, antibacterial, and piezoelectric properties. We hypothesize that a mechanically competent PPHOS-PLGA-akermanite composite, exhibiting *in situ* hierarchical interconnected pore formation, will undergo controlled degradation to release amino acids and bioactive ions (Ca^{2+}, Mg^{2+}, PO_4^{3}), thereby coupling scaffold resorption with collagen biosynthesis and osteogenesis and serve as a promising platform for functional bone regeneration. In this study, we synthesized novel lysine-functionalized bioactive PPHOS polymers via macromolecular substitution, designed to release amino acids upon degradation. These polymers were then used to fabricate a novel mechanically competent PPHOS-PLGA-akermanite composite. These composites are stable under ambient conditions and degrade via *in situ* pore formation while releasing bioactive inducerons designed to enhance collagen deposition, vascularization, and osteogenesis. These properties make this bioresorbable composite a

promising biologics-free candidate for investigating bone regeneration in load-bearing bone defect models. Together, these findings establish a versatile, biologics-free platform for bone regenerative engineering that is amenable to clinical translation.

23. Govindaraj Perumal

Scanning Electron Microscopy Study Reveals Sex-Based Differences in Morphology of Aged Murine Macrophages

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Aging macrophages contribute to impaired bone healing. The Kuhn lab is developing a therapeutic approach of localized delivery of senolytics to overcome the negative effects of aged macrophages. Our previous studies showed that macrophages cultured on a transitory barrier layer (TBL) of calcium phosphate delivering IL-4 were delayed in early phenotype switching to a reparative phenotype for 2 days. To determine if the sharply crystalline TBL affects the attachment and spreading of macrophages, we undertook a scanning electron microscopy (SEM) morphology study. We hypothesized the TBL would restrict macrophage spreading relative to non-treated tissue culture plastic (nTCP). TBL coatings were formed according to our previous publication. BMDMs were harvested from either male or female aged mice (18 months+) and cultured in MCSF (20 ng/ml), then seeded onto TBL or nTCP. One of three stimuli were applied: no stimuli, a pro-inflammatory stimuli (M1, 100 ng/ml IFN-γ overnight (18 hours), followed by the addition of 50 ng/ml LPS for 6 hours and imaged at 24 hrs (D1)), or a pro-reparative stimuli (abbreviated as M2, 20 ng/ml IL-4 added on Day 3 and imaged on D6). Spreading and elongation were reduced on the TBL relative to plastic for both male and female cells for all stimuli. The proinflammatory stimuli enhanced the circumference of male cells more than female cells. The pro-reparative stimuli did not alter the morphology of either male or female cells. Surprisingly, there were striking morphological differences between aged male and female macrophages on both materials. Female BMDMs were more elongated and spindle-shaped than the male BMDMs, independent of the stimuli applied. Bulk RNA-sequencing studies are now being conducted to relate morphological changes to phenotypical changes.

24. Nicholas Moskwa

Bladder confetti: Testing miniaturization and implantation

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Interstitial cystitis or Bladder Pain Syndrome (BPS) causes patients significant reduction in quality of life from the pain and emotional toll. Around 1% of the American population suffers from BPS.

Currently, BPS dominate treatment is oral painkillers, which have systemic risks. A topical treatment would reduce this systemic painkiller usage and lower overall drug dosages. However, the bladder is difficult for drug delivery because urine continually washes away medications. We are designing a novel form of drug delivery involving thousands of microscopic films laced with drug on one side and an artificial glycosaminoglycans (GAGs) on the other side that intercalates with the bladder's natural GAG layer. In our preliminary work, we hypothesize that film can be miniaturized for delivery and visualized within the bladder lumen after injection. Films were cast from processed silk fibroin. Several strategies were tested for film miniaturization and injectability. Films were injected into postmortem animal bladders. Histological staining and fluorescent microscopy assessed the film's tissue interaction. Various markers were validated for highlighting specific cell subpopulations using microscopy. We found that a bead mill could pulverize the film small enough for injection through a 23G needle. The film's composition determines the film's ability to resuspend. Raw silk films could not be resuspended in water. Silk films chemically bonded with synthetic GAGs could be suspended in water or 10% ethanol and were injectable. The injected films could be stained using DAPI, Sudan black and hematoxylin. Staining made the films identifiable inside the bladder. Staining markers for fibroblasts and the three epithelial subpopulations were validated. The films can be miniaturized and injected into the lumen of the bladder through a 23G needle. This novel form of drug delivery will be the first effort that effectively introduces topical treatments into bladder and potentially relieve BPS and other conditions.

25. Pradeep Kumar

Evaluation of Student-Built CR DIY Air Purifiers for Reduction of Indoor Particulate Matter in Elementary School Classrooms

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Children spend a substantial proportion of their day in classrooms, where exposure to infectious airborne particulate matter (PM) may adversely affect health and learning. Low-cost do-it-yourself (DIY) 4 filter Corsi Rosenthal (CR) Box air purification systems constructed from box fans and high-efficiency filters have emerged as a scalable approach for improving indoor air quality, but field-scale evaluations under real classroom conditions remain limited. Using a "low-touch" public health approach, researchers at UConn provided ready-made air purifiers and resources. College students and staff decorated the units, while Wi-Fi-enabled timers automated their daily operation. This study evaluated college student-built DIY air purifiers in nine occupied classrooms in a urban Connecticut elementary school during the 2023–2024 school year. The analysis was restricted to occupied school hours on non-holiday weekdays to characterize true student exposure. Real-time PM1, PM2.5, and PM10 concentrations were measured using QuantAQ MODULAIR-PM monitors. Indoor-to-outdoor (I/O) relationships were evaluated using regional ambient monitoring data, and a counterfactual model was used to estimate indoor concentrations expected in the absence of purification during a period of elevated outdoor pollution to assess the efficacy of the intervention. DIY air purifiers significantly reduced school-wide average PM1 and PM2.5 concentrations by 30% and 31%, respectively ($p < 0.001$), whereas PM10 reductions were smaller and more variable. During a particularly polluted (wildfire) period, observed indoor concentrations were substantially lower than counterfactual predictions, with reductions of 54% for PM1, 59% for

PM2.5, and 33% for PM10. I/O comparisons revealed that fine particles easily infiltrated the building from outdoor pollution, whereas larger coarse particles (PM10) were driven by everyday activity inside the room, such as walking and stirring up settled floor dust. These findings demonstrate that student-built CR DIY air purifiers can meaningfully reduce indoor concentrations under occupied classroom conditions and maintain safe indoor air quality even during extreme regional pollution events. This low-cost, scalable intervention may be particularly valuable for schools with aging infrastructure or limited resources.

26. Cristina Baquero

Dissecting microenvironment-driven mechanisms of glioblastoma recurrence

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Glioblastoma (GBM) is the most aggressive primary brain tumor in adults, and recurrence remains inevitable despite standard treatments. This highlights the urgent need to understand the mechanisms driving therapy resistance. Emerging evidence suggests that the tumor microenvironment undergoes substantial remodeling during recurrence, including changes in neuronal and glial cell composition, which may create supportive niches for tumor survival. At the same time, the microenvironment has been shown to shape transcriptional states linked to resistance in GBM cells, yet the specific factors remain unclear. To investigate these mechanisms, we employed 3D glioblastoma cerebral organoid (GLICO) models by integrating iPSC-derived cerebral organoids with fluorescently labeled patient-derived glioblastoma stem cells. These models recapitulate key features of human GBM, including cellular heterogeneity, spatial organization, and clinically relevant transcriptional programs. Following exposure to standard-of-care treatment, including temozolomide and radiotherapy, we performed single-cell RNA sequencing and proteomic profiling to characterize therapy-associated cellular states and molecular changes in both the tumor and microenvironment compartments. In parallel, multiplex immunofluorescence imaging was used to map treatment-associated alterations in tissue architecture and cellular organization. Analyses revealed treatment-associated transcriptional changes across both tumor and microenvironment compartments, including shifts toward NPC-like and inflammatory GBM cell states and transcriptional changes in neuronal populations within the tumor microenvironment. Together, these findings suggest that therapy-induced microenvironmental remodeling may contribute to GBM regrowth after treatment.

27. Lauren Crowley

Cognitive reserve does not protect against frailty in depressed older adults

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Background: Frailty, or reduced functional reserve, and depression in older adulthood have a bidirectional relationship. In addition to frailty, depression is associated with reduced cognition in

dementia-free older adults. However, one study suggests higher cognitive reserve (CR) may protect against developing mobility impairment in older adults. The relationship between cognition and frailty in depressed older adults is less understood, in particular whether higher CR protects against frailty.

Objective: This retrospective, exploratory study aimed to investigate the relationship between cognition, depression, and motor functioning in depressed older adults. We hypothesized CR has an attenuating effect on the relationship between frailty and depression.

Methods: Data were from 192 depressed participants in the Neurobiology of Late-Life Depression (NBOLD) study. The sample was elderly (M=70.9 years), well-educated (M=15.8 years), predominantly female (65.6%) and White (90.1%). Participants completed the Montgomery-Asberg Depression Rating Scale (MADRS) and were administered cognitive and motor tests at baseline and 1- and 2-year follow-up. Spearman correlational analyses were conducted between total MADRS score and motor variables as well as their change scores at each timepoint. Multiple linear regression models controlling for demographics evaluated whether CR variables (i.e., Shipley Vocabulary Test, years of education) had a moderating effect on the relationship between depression and motor functioning.

Results: Spearman correlations showed 2-year change in dual walking task speed is correlated with 2-year change in MADRS total score (r(s)=0.283, P=0.009). However, multiple linear regression did not show a moderating effect of either CR variables on that relationship after controlling for demographics.

Conclusion: CR does not moderate the relationship between depression and motor functioning as originally hypothesized. CR does not protect against frailty in depressed older adults in the way that it protects against cognitive decline. Targeted physical and functional therapies should be emphasized to prevent frailty in depressed older adults regardless of cognitive status.

28. Ali Zelan

National Inventory of Early Childhood Intervention Personnel Preparation Programs

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High-quality early childhood intervention services depend on a well-prepared workforce capable of supporting the developmental and educational needs of young children during the critical birth-to-five period. Personnel preparation programs play a central role in preparing professionals to deliver these services; however, limited national information exists regarding the availability and characteristics of these programs. This study aims to develop a national inventory of personnel preparation programs to examine the current landscape of early childhood intervention programs across the United States. The purpose of this study is to identify the number, distribution, and characteristics of personnel preparation programs that prepare professionals for careers in early childhood intervention. Data are being collected from institutions of higher education across all 50 states, the District of Columbia, and U.S. territories. Information includes program type, degree level, credential or licensure pathway, delivery format, and other key program characteristics. The National Inventory will provide a comprehensive overview of the current landscape of personnel

preparation programs and serve multiple stakeholders. It will offer prospective students a centralized resource for identifying preparation programs while providing researchers, institutions of higher education, credentialing agencies, and policymakers with empirical data to evaluate workforce preparation. The inventory will also establish a baseline for examining the alignment of existing programs with professional standards and recommended competencies for the early childhood intervention workforce. Findings from this study will contribute to the growing knowledge base on early childhood intervention personnel preparation by documenting program availability, credentialing pathways, and variation across states. The inventory will identify strengths and gaps in the current preparation system and provide evidence to inform policy, program development, and workforce planning. Additionally, it will establish a foundation for future research examining changes in personnel preparation over time and the relationship between program characteristics and workforce capacity to serve young children with disabilities, developmental delays, and their families.

29. Sanaz Keshavarz Shahbaz

Patient-derived lung epithelial progenitor platforms for autologous lung cancer models

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Lung cancer remains the leading cause of cancer-related mortality worldwide, yet the epithelial-intrinsic mechanisms underlying early tumor-associated phenotypes and disease heterogeneity remain incompletely understood. Patient-derived epithelial progenitor models provide a physiologically relevant system to investigate airway epithelial biology while preserving patient-specific characteristics. We established a patient-derived lung epithelial progenitor biobank from 20 adjacent lung tumor tissues, including 12 lung adenocarcinoma (LUAD) and 8 lung squamous cell carcinoma (LUSC) samples; 11 male and 9 female patients. Following expansion and quality control, 15 NSCLC-derived samples were successfully maintained as progenitor cultures, including 9 LUAD and 6 LUSC samples, from 8 male and 7 female patients (ages 48–82 years). Progenitor cells were expanded as organoids and differentiated at air–liquid interface (ALI). ALI differentiation was performed across four experimental runs incorporating LUAD-, LUSC-, and normal-donor-derived cultures.

We evaluated epithelial differentiation, remodeling, immune-associated phenotypes, and antiviral responses following infection with influenza A/PR8 and A/Netherlands strains. All immunofluorescence (IF) staining was quantitatively analyzed using histocytometry, including epithelial, remodeling, immune-associated, and viral markers. Patient-derived ALI cultures

retained distinct epithelial characteristics following differentiation. LUAD-derived cultures demonstrated altered epithelial differentiation, including reduced secretory differentiation, increased Vimentin expression, and altered E-cadherin/N-cadherin patterns, consistent with enhanced epithelial plasticity. Baseline expression of immune-associated markers, including HLA-DR, HLA-DM, CD74, and MX1, varied among patient-derived cultures, highlighting inter-patient heterogeneity. Following influenza infection, LUAD-derived ALI cultures consistently exhibited lower viral NP and HA signals than LUSC-derived and normal-donor cultures, indicating subtype-specific differences in antiviral responses. Variation in baseline interferon-associated responses did not fully explain differences in viral susceptibility.

Overall, patient-derived lung epithelial progenitors preserve disease- and patient-specific epithelial programs through organoid expansion and ALI differentiation, providing a platform to investigate early epithelial reprogramming, epithelial plasticity, antiviral responses, epithelial–immune interactions, aging, and therapeutic resistance.

30. Caroline Haney

Single-cell transcriptional profiling reveals altered immune cell states in the peritoneal microenvironment of endometriosis

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Endometriosis is a common, chronic inflammatory disease affecting an estimated 1 in 10 individuals assigned female at birth worldwide, yet the immune environment inside the abdominal cavity, also known as the peritoneal cavity, where the disease develops remains poorly understood. We studied peritoneal fluid (PF), a unique compartment in direct contact with abdominal organs where endometriosis lesions establish and grow. The PF creates an immune-rich milieu impacted by endometriosis chronic inflammation that can sustain and drive lesion growth, shaping the local immune and inflammatory environment in which endometriosis progresses. Using single-cell RNA-sequencing, we compared the cell composition of PF from patients with and without (Ctrl) endometriosis collected at time of surgery by the UCH OB/Gyn MIGS team. Our data revealed widespread changes in immune cell behavior, including shifts in T-cell activation, dendritic cell proportions, and natural killer (NK) cell function in endometriosis vs Ctrl PF. These changes suggest that immune cells within the peritoneal cavity are being reshaped by endometriosis-driven persistent inflammation. We also found that epithelial and fibroblast cells from both endometriosis and Ctrl patient's eutopic endometrium could grow when placed in PF supernatant, suggesting that the PF itself provides a hospitable environment that could support the growth of endometriosis cells outside the uterus. Together, these findings describe a coordinated pattern of immune changes tied to chronic inflammation in endometriosis peritoneal cavity. This suggests that endometriosis involves interactions between local tissue and broader immune signaling. Together, these findings reveal a coordinated pattern of immune dysregulation driven by chronic inflammation in the endometriosis peritoneal cavity, linking local tissue changes to broader immune signaling. By characterizing this immune landscape at single-cell resolution, our work supports a model of endometriosis as a systemic condition.

31. Xuan Ye

Genome-wide profiling of RNA 2'-*O*-methylation in neurons and identification of putative orphan snoRNA targets

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We have compared genome-wide patterns of RNA 2'-*O*-methylation (Nm) between two isogenic pairs of neurons. Each pair includes one line harboring a small deletion of orphan box C/D snoRNAs (SNORD116s) from the paternal chromosome 15q11-q13 region. One isogenic pair also differs in expression of SNORD113/114 snoRNAs from chromosome 14q32.2. Wild-type and modified cells were differentiated into cortical neurons, and genome-wide patterns of Nm identified. Nm profiles of rRNA between stem cells and neurons were essentially the same and unaffected by orphan snoRNA expression, except that 18S Um354 is unmethylated in stem cells and largely methylated in neurons. We further identified thousands of Nm sites in mRNAs, lncRNAs and small RNAs. Most of these are shared between all 4 induced neuronal cell lines and many are also observed in HEK293T cells. Surprisingly, most mRNA Nm sites do not exhibit canonical complementarity to snoRNAs, but a number exhibit strong complementarity to U3 snoRNA, not previously shown to direct Nm. Evidence from cross-linking and sequencing of hybrids (CLASH) suggests that U3 is proximally associated with a subset of 2'-*O*-methylation events. Finally, we identify a number of apparent canonical targets of SNORD113, SNORD114 and SNORD116 snoRNAs. These data present a comprehensive characterization of the Nm landscape in neurons and, for the first time, allow the assignment of Nm sites targeted by specific orphan snoRNAs associated with neurodevelopmental and other disorders.

32. Victor Fraile-Agreda

Novel experimental platforms to examine antibody- and macrophage-mediated clearance mechanisms for *Treponema pallidum*, the syphilis spirochete

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Syphilis, caused by the sexually transmitted spirochete *Treponema pallidum*, remains a major global health challenge. Development of a safe and effective vaccine is a major priority for syphilis research. It is widely believed that antibodies against surface-exposed determinants of *T. pallidum*

are essential for protective immunity, although their protective mechanisms are poorly understood. Efforts to study protective immunity against *T. pallidum* have been hindered by the unusual molecular architecture of the organism's outer membrane, the historic inability to propagate *T. pallidum in vitro,* and the lack of robust experimental systems to model host-pathogen interactions *in vitro.*

Previous work from our laboratory has demonstrated that heat-inactivated immune rabbit sera and antisera directed against selected extracellular loops of *T. pallidum* outer membrane proteins are bactericidal *in vitro*. Using GFP-expressing Nichols and SS14 reference strains of *T. pallidum*, our studies herein demonstrate antibody-mediated impairment of bacterial viability at early stages of replication, with progressively greater effects at later time points, ultimately resulting in bacterial cell death. To better approximate the *in vivo* environment, complement-based assays showed that active complement inhibited the bactericidal effect of immune rabbit sera, whereas heat-inactivated complement did not, suggesting that complement may modulate antibody-mediated activity against *T. pallidum*.

Opsonophagocytosis studies of *T. pallidum* have historically relied upon rabbit peritoneal macrophages; rabbit bone marrow-derived macrophages (BMDMs) potentially provide a more controlled, differentiation-defined system for investigating macrophage – *T. pallidum* interactions. Along these lines, we have successfully isolated and differentiated from rabbits BMDMs that display characteristic macrophage morphology, expression of canonical macrophage-associated markers, and functional phagocytic activity using *E. coli*-coated beads, while *T. pallidum* opsonophagocytosis assays remain ongoing.

Together, these studies establish novel experimental platforms to delineate functional antibodies and macrophage-mediated immune responses against *T. pallidum*, with potential relevance for future syphilis vaccine development.

33. Niti Jani

Spatial profiling of tumor reveals distinct immune microenvironments in early vs late onset colorectal cancer

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Incidence of colorectal cancer (CRC) has increased in individuals <50 years, described as early-onset CRC (EOCRC). Although the precise etiology is unclear, EOCRC is associated with external exposures that may alter the tumor immune microenvironment (TIME). We hypothesized that EOCRC develops a distinct immune landscape compared with late-onset CRC (LOCRC). Using imaging mass cytometry (IMC) we compared the composition and spatial organization of the TIME at single-cell resolution. 142,992 cells from 18 regions of interest (ROIs) from 7 EOCRC and 10 LOCRC patients were analyzed using Steinbock computational pipeline. EOCRC tumors showed significantly higher (2-fold, p=0.047) infiltration of regulatory T cells (Tregs; $CD3^+CD4^+FOXP3^+$) than LOCRC after normalization to cell numbers. Spatial analysis revealed distinct patterns of

immune cell organization, including increased Treg avoidance of $GZMB^+$ neutrophil-like (CD15+) cells and increased interaction between CTLs (CD8a) and T-helper cells (CD3+, CD4+) in EOCRC. Patch-based analysis demonstrated higher Treg enrichment in EOCRC (1.6-fold, $p=0.032$). In contrast, EOCRC tumors exhibited lower CTLs (0.8-fold, $p<0.0001$) and T-helper cells (0.6-fold, $p=0.00054$) within stromal regions, with a trend toward increased intraepithelial lymphocytes (IELs; $CD8a^+$). Higher Treg abundance (1.6-fold, $p<0.0001$) in EOCRC was confirmed by immunohistochemistry in four independent EOCRC and LOCRC tumors. These findings suggest subtle changes in spatial distribution of immune cells in TIME that may contribute to EOCRC progression.

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