UCONN HEALTH
UNIVERSITY OF CONNECTICUT

The Jackson Laboratory

- ABSTRACT BOOK -

THE SIXTH ANNUAL POSTDOC RESEARCH DAY

Welcome to the sixth annual UConn Health/Jackson Laboratory Postdoc Research Day. We are delighted to be back in person this year to share and celebrate the excellent work our postdocs do here at UCH and JAX. As is our tradition, we will hear from a sampling of our postdocs giving their 4-minute Speak4Science talks. That will be followed by an in-person poster session where you will have a chance to hear more details about their research. 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 for their support of this year's event as well as Stephanie Holden for her help in putting this event together. Finally, I would like to thank the enthusiastic team of postdocs from our PDRD Planning Committee:

Feiyang Chen (UConn Health)
Lourah Kelly (UConn Health)
Amnah Siddiqa (The Jackson Laboratory)
Roberto Vazquez-Munoz (UConn Health)

Thank you for joining us in this celebration of our postdoctoral fellows and the great work they do to drive the research mission at our two institutions. I am very happy to see our community back together again.

Be well,

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

The Sixth Annual

Postdoc Research Day

Wednesday, September 21st, 2022

1:00	**Opening Remarks**	**Academic Rotunda**
1:20	**Speak4Science Part I**	**Academic Rotunda**
2:05	**Coffee Break**	**Under the Rotunda Entrance**
2:25	**Speak4Science Part II**	**Academic Rotunda**
3:15	**Poster Session**	**Rotunda Lobby**
4:45	**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, in person, from 3:15-4:45PM. The speaker roster and their corresponding poster numbers are listed below.

Name	Title	Affiliation	Poster
Moriah Turcotte	*Perinuclear ß receptors in hypertrophy*	UCH	N/A
Nancy Jaiswal	*Interaction between DNA polymerase ι and Rad23*	UCH	3
Georgia Doing	*Gene expression to study* S. epidermidis *strains*	JAX	4
Chengliang Wang	*Host-pathogen tug-of-war via pyroptosis*	UCH	5
Katrin Unterhauser	Klebsiella spp. *in NEC pathogenesis*	UCH	6
Roberto Vazquez-Munoz	*Dietary XOS alter mouse oral microbiota*	UCH	7
Lourah Kelly	*A novel avatar-guided mHealth platform*	UCH	9
Agnieszka Lukomska	*Role of microRNAs in axon regeneration*	UCH	10
Amit Gupta	*Role of cis-elements in translation*	UCH	11
Kwondo Kim	*Evolutionary insights from dog pangenome*	JAX	12
Shilpita Karmakar	*Epigenome dynamics in aged clonal hematopoiesis*	JAX	13
Marwa Elamin	*Sodium channel dysfunction in Dup15q syndrome*	UCH	14
Yamin Liu	*Controlled delivery RUX promotes aged bone healing*	UCH	15
Yuan Gui	*CNN2 regulates metabolism in CKD*	UCH	16
Manoshi Gayen	*Therapeutic potential of CX3CL1 derived peptides*	UCH	17
Amnah Siddiqa	*LC-MS metabolomics data processing using asari*	JAX	18
Shyam Kishor Sah	*Functional analysis of novel keloid-associated variants*	UCH	19
Cesar Zoni	*Heart transplant and donor cause of death*	UCH	20

Abstracts

1. Moriah Turcotte

Perinuclear β receptors in hypertrophy

Moriah Turcotte[1], Hrishikesh Thakur[2], Michael Kapiloff[2], Kimberly Dodge-Kafka[1]

[1]Department of Cell Biology, UCONN Health; [2]Department of Opthamology and Medicine, Stanford University, Stanford, CA

Cardiac hypertrophy development is controlled by networks of signaling pathways, integrated by scaffold proteins that localize signaling enzymes and facilitate cross-talk between these pathways, leading to upregulation of hypertrophic transcription factors. Although the traditional dogma of adrenergic receptor signaling posits receptor activation begins at the plasma membrane, recent evidence has emerged showing receptor localization and activation at the nuclear envelope. In particular, the presence and activity of β1 and β3-adrenergic receptors are detected at the nuclear envelope. In addition, activity of G-protein subunits and downstream targets associated with GPCR signaling has been found at the nuclear envelope. Over the last several decades, our lab has studied a protein scaffold at the nuclear envelope, muscle A-Kinase Anchoring Protein (mAKAPβ), required for the induction of cardiac hypertrophy. mAKAPβ tethers various downstream targets of β-adrenergic receptors, and it is this localization that provides the framework for stress-related gene transcription in cardiomyocytes. Utilizing a FRET-based biosensor localized to mAKAP, we provide evidence that nuclear β receptors contribute to a nuclear cAMP microdomain that is dependent on expression of mAKAPβ. Furthermore, our immunofluorescent staining supports the hypothesis that nuclear β-receptors are sufficient to induce cardiomyocyte hypertrophy in adult and neonatal rat cardiomyocytes. Finally, we show potential therapeutic value of this research by demonstrating the ability to buffer this cAMP microdomain without impacting cytosolic cAMP levels.

2.

NOT PRESENTING

3. Nancy Jaiswal

Interaction between DNA Polymerase ι and Rad23 provides a link between DNA Damage Tolerance and DNA Repair pathways

Nancy Jaiswal[1], Nicholas W. Ashton[2], Roger Woodgate[2], Irina Bezsonova[1]

[1]Department of Molecular Biology and Biophysics, UConn Health, Farmington, CT; [2]Laboratory of Genomic Integrity, National Institute of Child Health and Human Development, National Institutes of Health, Medical Center Drive, Bethesda

DNA damage is caused by variable environmental agents such as UV radiations resulting in bulky DNA adduct which is eliminated through Nucleotide excision repair (NER) pathway. Recognition of the altered gene is the first step in this repair process, which involves a recognition complex formed by xeroderma pigmentosum complementation group C (XPC) protein and one of the Rad23 proteins (either Rad23A or Rad23B)[1,2]. The later steps of NER reaction includes preferential binding of XPC-Rad23 complex to the UV-damaged DNA followed by successive recruitment of other NER components to the lesion[3]. Here, we report that Rad23A and Rad23B can directly interact with DNA polymerase iota (Pol ι), a member of the DNA damage tolerance pathway. Pol ι is a low-fidelity translesion synthesis DNA polymerase, an emergency mutagenic polymerase, which is capable of DNA replication across DNA lesions[4]. Using solution NMR spectroscopy, we show that Ubiquitin Associated Domains (UBA) of Rad 23A and Rad23B effectively interact with DNA-binding domain of Pol ι with micromolar affinity. Chemical shift perturbation assays reveal the precise Pol ι binding interface on the surface of UBA1 and UBA2 domains of Rad23A. Interestingly, the resulting NMR mapping suggests that the Pol ι interface overlaps with known ubiquitin-binding interface of UBAs. Indeed, further binding competition experiments show that ubiquitin can effectively disrupt Pol ι/Rad23A complex by competing with Pol ι for Rad23A UBA1 and UBA2 binding. Other translesion synthesis DNA polymerases including Pol η, and Pol κ and Rev1 share a DNA-binding domain homologous to Pol ι[4], suggesting that they all may interact with Rad23 proteins in a similar manner. These results suggest that ubiquitin signaling plays an important role in switching between NER and DNA damage tolerance pathways.

4. Georgia Doing

Transcriptomics compendia for the study of novel *S. epidermidis* strains

Georgia Doing[1], Julia Oh[1]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT

Of the trillions of microbial cells associated with a human body, about half are identifiable at the genus level, a quarter are culturable, and only a handful have been isolated for extensive laboratory study. However, both species and strain-level genetic diversity are increasingly appreciated as important for microbial community function and clinical outcomes. While sequencing technologies readily unveil genetic diversity, the overwhelming number of species, strains, and genes in the microbiome with unknown function limits our ability to interpret available data. Extensively identifying roles for genes is important because modulators of microbial interactions and community dynamics will allow us to harness rich genetic microbial diversity to identify novel therapeutic targets and unidentified modulators of virulence. The abundance of public transcriptomics data allows for correlation analyses to identify patterns of co-expression, which can be used to infer function as it is associated with co-regulation in response to environmental cues. We are devising methods for the re-analysis of public datasets from closely related species of *S. epidermidis* and its well-studied pathogenic cousin, *S. aureus,* to hypothesize roles for yet uncharacterized accessory genes in *S. epidermidis* strains.

5. Chengliang Wang

Structural basis for specific recognition of Gasdermin family by *Shigella* IpaH7.8

Chengliang Wang[1], Sonia Shivcharan[1], Vijay Rathinam[1], and Jianbin Ruan[1, 2]

[1]Department of Immunology, University of Connecticut Health School of Medicine, Farmington, CT, USA; [2]Department of Molecular Biology and Biophysics, University of Connecticut Health School of Medicine, Farmington, CT, USA

Gasdermins (GSDMs) play integral roles in the immune response by executing pyroptosis that eliminates the replicative niche of intracellular pathogens, and by directly restricting the bacterial growth and spread. *Shigella*, a human-adapted enteropathogen, evade this host defense mechanism by triggering a ubiquitination-mediated proteasomal degradation of GSDMB and GSDMD using its virulence effector IpaH7.8. Here, we report the cryo-electron microscopy structure of *Shigella flexneri* IpaH7.8 in complex with human GSDMB. Our structure identifies a motif of three negatively charged residues in the loop near the N terminus in GSDMB as the structural determinant that is specifically recognized by IpaH7.8. Human, but not mouse, GSDMD contains the conserved residues, explaining the species specificity of IpaH7.8. Additionally, we find that IpaH7.8-binding and IpaH7.8-mediated ubiquitination directly inhibit the GSDMB pore-forming activity independent of ubiquitin-mediated proteasomal degradation. This work sheds light on the mechanisms of *Shigella* IpaH7.8 recognizing GSDMs, and reveals a multi-pronged inhibition of GSDMB by *Shigella* IpaH7.8.

6. Katrin Unterhauser

The role of *Klebsiella* spp. in pathogenesis of necrotizing enterocolitis

Katrin Unterhauser[1], Spencer Coleman[1], Karim Rezaul[1], M. J. Caimano[1,4,5], E. Jackson [6], D. Gratalo[6], M. D. Driscoll[6], Adam P. Matson[1,2,3]

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Necrotizing enterocolitis (NEC) is the most common intestinal inflammatory disease of premature infants with a mortality rate between 20-30%. NEC does not occur in utero or in germ-free animals, and antibiotics - disrupting the gut microbiota - increase the risk of disease. These findings indicate that intestinal bacteria play an important role in NEC pathogenesis. *Klebsiella* spp., Gram-negative and β-lactam resistant bacteria, have been associated with the disease in several studies. However, the precise role of these opportunistic pathogens in causing NEC remains unclear. Sequencing of preterm stool specimens showed *Klebsiella* spp. are colonizing the vast majority of preterm infants in the neonatal intensive care unit. More important, there is a higher abundance of *Klebsiella* spp. in NEC subjects versus non-NEC subjects. The genus Klebsiella comprise a wide diversity of species, including the *Klebsiella pneumoniae* species complex (KpSC) and *Klebsiella oxytoca* species complex (KoSC). 16S-23S amplicon sequencing is able to discriminate between species and based on this analysis our lab isolated the following strains from NEC patients: *K. oxytoca*, *K. grimontii*, *K. pasteurii*, *K. michiganensis* belonging to the KoSC and *K. pneumoniae* (KpSC). Intriguingly, the fecal microbiota of NEC patients was dominated by either KoSC or KpSC suggesting a possible competition between those in the gut. Furthermore, several of the KoSC isolates produce the cytotoxins Tilimycin and Tilivalline. Toxin production was confirmed via mass spectrometry, cytotoxicity assays and detection of toxin-related genes and proteins. Those toxins cause apoptosis in enterocytes and they are responsible for the intestinal pathology in antibiotic-associated hemorrhagic colitis. Our results clearly show the association of *Klebsiella* spp. with NEC. To determine how antibiotics, diet, microbiota- as well as host-derived metabolites influence toxin production and KpSC/KoSC competition will help to understand pathogenesis of this intestinal disease.

7. Roberto Vazquez-Munoz

Influence of oral pre- and pro-biotics on murine oral bacterial microbiota composition.

Roberto Vazquez-Munoz[1], Angela Thompson[1], Takanori Sobue[1], and Anna Dongari-Bagtzoglou[1]

[1] Dept. of General Dentistry, University of Connecticut Health Center

Oropharyngeal candidiasis is a common infection in immunocompromised hosts. Work from our group and others has shown that *C. albicans* virulence can be modified by lactic-acid bacteria which are members of the oral microbiome in health. The oral microbiota composition may be influenced by probiotics (live bacteria) and/or prebiotics which are nondigestible food supplements that favor probiotic growth. We recently reported that lactobacilli-enriched oral microbiota are associated with reduced *C. albicans* virulence in an immunocompromised mouse oral infection model. We isolated, sequenced and characterized an *L. johnsonni* strain (strain MT4) from the oral cavity of these mice. Strain MT4 displayed significant anticandidal properties in biofilm models *in vitro*. As the cultivable *Lactobacillus* population in the murine GI tract is dominated by *L. johnsonii*, we hypothesized that MT4 may dominate the autochthonous murine oral microbiota and may be responsible for attenuating *Candida* virulence *in vivo*. We conducted a survey of the cultivable lactobacillus and discovered that 64% of female C57BL/6 mice were colonized by MT4, and that MT4 represented >90% of the cultivable oral lactobacilli. Dietary Xylo-OligoSaccharides (XOS) have been shown to increase *Lactobacillus* species in the murine gut microbiota, although their impact on the oral microbiota is unknown. We thus hypothesized that oral administration of XOS given with or without the probiotic strain MT4 may further increase the oral lactobacillus burdens in mice. Mice received a daily XOS-enriched diet, with or without MT4 supplementation after the XOS diet was stopped. Control groups received a standard diet. Contrary to our expectations, the oral Lactobacillus biomass in the XOS dietary group were significantly reduced. In addition, MT4 supplementation increased the lactobacilli numbers to levels significantly higher than those in the control groups. Our current results suggest that, 1) the effect of prebiotics may differ in different parts of the GI tract; 2) while XOS reduced the oral lactobacillus biomass, probiotic supplementation --*i.e.*, with MT4- can restore the oral microbiota. Our future work will focus on the role of XOS-induced changes and the effect of MT4 strain supplementation on *C. albicans* virulence *in vivo*. This work expands the understanding of the effect of prebiotics and probiotics on the oral microbiome.

8. Sonia Shivcharan

Human epithelial cell sensing of intracellular bacterial invasion.

Sonia Shivcharan[1], Skylar Wright[1] and Vijay Rathinam[1]

[1]Department of Immunology, UConn Health

Epithelial cells at the mucosal sites are part of the first line of defense against microbial invasions. Epithelial cells have specialized mechanisms to sense intracellular invasion of pathogens. A non-canonical form of inflammasome with caspase-4 senses cytosolic invasion by gram-negative bacteria such as *Salmonella Typhimurium* and *Burkholderia Thailandensis* in human epithelial cells by monitoring the cytosol for lipopolysaccharide, a major cell wall component of gram negative bacterial pathogens. Caspase-4 sensing of bacterial LPS triggers its protease activity, which in turn leads to the proteolytic activation of a membrane pore-forming protein called gasdermin D (GSDMD). Cleaved GSDMD migrates to the plasma membrane and forms pores, leading to a lytic form of cell death called pyroptosis. Recent studies have reported that in human cells interferon inducible guanylate binding proteins facilitate caspase-4 recruitment to the cytosolic bacteria and bring about GSDMD-mediated pyroptosis and IL-18 release. A key event concurrent with bacterial escape is phagosomal rupture/destabilization which results in exposing otherwise hidden host endosomal glycans. Recognition of these glycans intracellularly leads to accumulation of galectins 3, 8 and 9 on the damaged membrane. In this study, we are examining the role of galectins and guanylate binding proteins in the sensing of bacterial invasions by the caspase-4-noncanonical inflammasome. We will discuss our findings on how these different cytosolic proteins co-ordinate cell-intrinsic host defense against intracellular bacterial pathogens and the consequent immune responses.

9. Lourah Kelly

Development of an avatar-guided mobile health intervention for emerging adults with alcohol misuse and suicidal thoughts

Lourah Kelly[1], Kristyn Zajac[1], Caroline Easton[2], Anthony Spirito[3], & Howard Tennen[4]

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Emerging adults have the highest rates of alcohol use disorders, suicidal ideation, and suicide attempts compared to any other age group. These problems are highly comorbid, with alcohol intoxication serving as a proximal risk factor for suicidality. However, utilization of outpatient treatment services for alcohol or mental health is extremely low in this population, even following emergency department (ED) visits for either problem. Emerging adults are likely to be responsive to mHealth interventions, and mHealth is well-suited to the ED. mHealth can address an urgent public health need by bridging the gap between discharge and outpatient care and by supporting emerging adults who do not access outpatient care. This study seeks to design an evidence-informed interactive, avatar-guided mHealth intervention to provide ongoing education, skills practice, mood and behavior monitoring, and personalized feedback to reduce alcohol misuse and suicidality. This mHealth platform will be developed based on a review of commercial suicidality and alcohol mHealth apps, in-person integrated interventions, and an existing avatar-based mHealth platform. The study aims to: 1) seek feedback on the proposed mHealth intervention avatar, content, and features from a national youth advisory board, consumers (e.g., emerging adults with alcohol misuse and suicidality; *n*=25), clinical experts in emerging adult alcohol and suicidality treatment (*n*=10) and ED staff (*n*=10) creating an alpha version of the intervention. Results of the content analysis of commercial suicidality apps and preliminary drafts of the avatar-guided mHealth intervention features and content will be presented. Data collection with experts and emerging adults is ongoing. Themes from qualitative and quantitative feedback from experts and emerging adults will inform the programming of the intervention, which will be tested in a usability and pilot feasibility trial. This intervention has the potential to reduce drinking and suicidal thoughts among emerging adults using a developmentally sensitive and evidence-informed intervention.

10. Agnieszka Lukomska

Developmentally regulated microRNAs play a role in retinal ganglion cell survival and axon regeneration after optic nerve injury

Agnieszka Lukomska[1], Jian Xing[1], Matthew Frost[1], William Theune[1], Ashiti Damania[1] and Ephraim F. Trakhtenberg[1]

[1]Department of Neuroscience, University of Connecticut School of Medicine

Retinal ganglion cells (RGCs) are central nervous system (CNS) projection neurons that do not spontaneously regenerate axons disrupted by optic neuropathies, such as those resulting from optic nerve trauma, ischemia, and glaucoma. RGC capacity to grow long axons declines sharply after birth, and several factors that are developmentally regulated in RGCs were discovered to contribute to the regenerative failure. No clinical treatments exist to date that could help patients with axonal injuries. Thus, the failure of RGC and other CNS axons to regenerate after injury remains a major unmet problem. Here, we investigated the roles of developmentally regulated microRNAs (miRNAs) in RGC survival and axon regeneration after optic nerve injury, using a well-established murine *in vivo* model of optic nerve crush (ONC). We used bioinformatic analysis of small-RNA-seq data, which we generated for the developing RGCs at various ages, in order to identify miRNAs that are developmentally regulated in maturing RGCs. Then, we pre-treated the RGCs with intraocularly injected AAV2 vectors, which expressed either miRNA mimics or anti-miRNA shRNAs. Next, we performed ONC and evaluated the effects on RGC survival and axon regeneration. We found several novel miRNA targets, which either promoted or inhibited RGC survival and axon regeneration. Thus, the identified developmentally regulated miRNAs play a role in RGC survival and axon growth, and present

potential therapeutic targets for treating optic neuropathies and glaucoma, as well as axonal injuries in other white matter tracts of the CNS.

11. Amit Gupta

NaP-TRAP: A novel approach to study the *cis*-elements in translational regulation

Amit Gupta[1], *Jean-Denis Beaudoin*[1]

[1]*Department of Genetics and Genome Sciences, UConn Health*

Precise regulation of translation is critical to maintaining normal homeostasis and cellular fate. Translational is majorly regulated by the coordinated action of *trans*-acting factors and *cis*-regulatory elements. While there are multiple approaches available to investigate the role of RNA binding factors in translation, understanding the role of *cis*-regulatory elements in mRNA translation remains particularly challenging due to the lack of robust techniques to measure their activity in a high-throughput manner. To deepen our understanding of the *cis*-regulatory code entrenched in UTRs in translational regulation, we have developed a novel approach named: Nascent peptide mediated Translating Ribosome Affinity Purification (NaP-TRAP). NaP-TRAP is highly efficient in measuring the regulatory activity of UTR elements on translation. The robustness of NaP-TRAP has been validated in multiple model systems, including zebrafish embryos, HEK293 cells, and stem cells. Furthermore, to accurately identify and understand the sequence-specific functionality of *cis*-elements, we have designed libraries with >10,000 variable 5' UTR regions and >17,000 variable 3' UTR regions for use in massively parallel reporter assays (MPRAs). We have coupled these libraries with NaP-TRAP-Seq and we aim to test these libraries in different biological conditions. Using this approach, we will unravel the governing role of UTRs in translational regulation which may direct us toward predictable tuning of translational regulation via UTRs engineering.

12. Kwondo Kim

The chromosome-scale pangenome highlights structural variations linked to the evolution of dog

Kwondo Kim[1], *Feyza Yilmaz*[1], *Pille Hallast*[1], *Charles Lee*[1]

[1]*The Jackson Laboratory for Genomic Medicine, Farmington, CT*

Dogs were the first domesticated species and have been closely linked to human dispersal and culture over the last 10,000 years after their domestication. Today, approximately 450 dog breeds are globally recognized, each with unique traits and morphologies. Although considerable progress has been made in understanding the genetic basis of dog domestication and its genetic diversity, the role of structural variation behind them is poorly understood. In this study, we assembled eight chromosome-scale genomes from six dog breeds (Basset Hound, Beagle, Cocker Spaniel, German Shepherd, Maltese, and Shih Tzu) with reference genome quality and constructed a structural variation map including ~87Mb sequences not found in the canFam3.1 reference genome. Based on the map, we genotyped population-scale short-read sequencing samples and discovered structural variations that are potentially responsible for dog domestication and breed formation. Our findings give insight into the role of structural variations in the evolution of dogs and demonstrate the advantage of pangenome representation in studying the diversity of dogs.

13. Shilpita Karmakar

Deciphering the impact of aging and somatic mutations on clonal hematopoiesis via single cell transcriptome

Shilpita Karmakar[1], Xiaowen Chen[1], Yang Liu[1], Jennifer Trowbridge[2#], Hideyuki Oguro[1,3#], Sheng Li[1,2,4,5]

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Aging is a process of overall systemic deterioration of the whole body and the biggest risk factor to cancer. Aged hematopoietic stem cells (HSCs) express a protooncogenic phenotype that suppresses the HSC differentiation. Age related clonal hematopoiesis (CH) involves abnormal expansion of HSCs that are known to have somatic mutations in genes encoding DNA demethylase *Tet2* and DNA methyl transferase (*Dnmt3a*) that regulates the HSC epigenome and are one of the major drivers of CH which is recurrently detected in AML and MDS. Its loss in hematopoietic stem and progenitor cells (HSPCs) lead to stem cell self-renewal and confer a competitive advantage to these cells. With the growing elderly population in US and increasing economic burden for AML treatment, there is an urgent need for the therapeutic interventions to mitigate CH. To address this question, we have employed multiomics approach using promoter capture HiC, single cell RNA (scRNA) sequencing and single nuclear ATAC (snATAC) sequencing in mouse and human bone marrow (BM) samples to understand the molecular underpinnings of CH. We have used HSPCs from cre inducible models of *Tet2-/-* and the *Dnmt3aR878H+* mutant mice along with *Mx-Cre* control to obtain Lineage-Kit+ cells for our scRNA sequencing and snATAC sequencing. We have observed a significant expansion of LT-HSCs that is relevant to both age context and the mutation status. From our analysis we have also observed differentially expressed genes (DEGs) commonly regulated in *Tet2-/-* and *Dnmt3aR878H+*. Overall, we have been able to detect 44 upregulated and 53 downregulated DEGs some of which are essential for maintaining LT-HSCs. Thus, our study can provide valuable insights into how two epigenetic modulators with opposite functions can cause the same causality in clonal hematopoiesis providing important regulators of aging.

14. Marwa Elamin

Sodium Channel Dysfunction in Dup15q Syndrome, a Genetic Form of Epilepsy and Autism

Marwa Elamin[1], Aurelie Dumarchey[2], Christopher Stoddard[2], Tiwanna M. Robinson[1], Christopher Cowie[1], Dea Gorka[2], Stormy J. Chamberlain[2], and Eric S. Levine[*1]*

[1]Neuroscience, [2]Genetics and Genome Sciences, University of Connecticut School of Medicine, Farmington, CT, US

Dup15q is a neurodevelopmental disorder caused by maternal duplications of the 11.2-13.1 region of the long arm of chromosome 15. Children with an isodicentric supernumerary chromosome that carries two extra copies of the 15q11.2-q13.1 suffer from profound autism and refractory epileptic seizures among other symptoms. Patch-clamp recordings of iPSC-derived Dup15q neurons reveal intrinsic and synaptic hyperexcitability phenotypes at different time points of in vitro development. Significant increases in inward sodium current and action potential amplitude were observed in Dup15q neurons. Dysfunction in sodium channels is known to contribute to several familial forms of autism and epilepsy. The goal of this study is to investigate the role of voltage-gated sodium channels in the development of Dup15q hyperexcitability phenotypes.

15. Yamin Liu

Controlled Delivery of Anti-SASP Ruxolitinib Promotes Aged Bone Healing by Modulating Macrophage M1 to M2 Transition

Yamin Liu[1], Kara Spiller[2], Liisa T. Kuhn[1]

[1]Department of Biomedical Engineering, University of Connecticut (UConn) Health; [2]Drexel University, Philadelphia, School of Biomedical Engineering, PA, USA.

Delayed bone healing in the elderly is known to be associated with age-related impairment in macrophage polarization. With aging, macrophages become senescent and develop a senescence-associated secretory phenotype (SASP). Ruxolitinb (RUX), an anti-SASP drug, is a promising therapeutic candidate for improving the outcome of aging-related delayed bone healing, but it is associated with serious adverse effects. In this study we used a novel immunomodulatory drug delivery system, a transient barrier layer (TBL) made of calcium phosphate, to deliver a low dose of RUX *in vitro* and *in vivo* to tested if localized timed delivery of RUX using a TBL could modulate aged macrophage M1 to M2 transitions and promote bone healing and repair in old mice.

Mouse bone marrow-derived macrophages (BMMs) from adult (3 months) and old (25 months) female mice were used for *in vitro* studies. BMMs were plated on TBL with immediate RUX (imm-RUX; in culture media) or delayed RUX (del-RUX; under the TBL) followed by M1 stimuli for 24h and then culture for 6 days. Del-RUX significantly elevated M2 (Ccl17) gene expression on D6 without changing the M1 gene expression on D1 compared to cells without RUX. In comparison, imm-RUX significantly reduced the M1 markers on D1, but only caused a small increase of M2 markers on D6. *In vivo* studies, a TBL coated 3D printed calcium phosphate/polylactide-co-glycolide scaffold with imm-RUX, or del-RUX, was placed in a critical-sized calvarial defect in old female mice. After 9 weeks, del-RUX increased calvarial bone defect repair compared to imm-RUX as quantified by microcomputed tomography. In summary, TBL offers a convenient way to control the timing of the anti-SASP drug, RUX, to effectively modulate old mouse M1 to M2 macrophage transitions and to promote bone healing in aged mice. These studies highlight the importance of timed local delivery of drugs that affect the SASP in bone healing.

16. Yuan Gui

Calponin 2 harnesses metabolic reprogramming to determine kidney fibrosis

Yuan Gui[1], Dong Zhou[1]

[1]Division of Nephrology, Department of Medicine, UfDu\Conn Health, Farmington, CT

The formation of a deleterious tissue microenvironment is determined by mechanical forces amid kidney fibrosis. This process also requires metabolism for energy generation and consumption. However, how cellular mechanics and metabolism are connected remains unclear. Our proteomics revealed that actin filament binding and cell metabolism are top dysregulated biological events in a fibrotic kidney. As a prominent actin filament-associated regulatory protein, Calponin 2 (CNN2) predominantly expressed in fibroblast and knockdown of CNN2 preserved kidney function and alleviated fibrosis. Global proteomics profiled that knockdown of CNN2 enhanced the activities of the key enzymes and regulators of fatty acid oxidation (FAO) in the diseased kidneys. Selective inhibition of carnitine palmitoyltransferase 1α in FAO pathway accelerated lipid accumulation in tubules and extracellular matrix deposition but were restored by knocking down CNN2. In patients, serum CNN2 levels is correlated with extent of lipid content. Bioinformatics and chromatin immunoprecipitation showed that CNN2 regulates FAO pathway through estrogen receptor 2. *In vitro*, exogenous CNN2 repressed FAO in tubular epitheliums. Our results suggested that balancing cell mechanics and metabolism are crucial to develop therapeutic strategies to halt kidney fibrosis.

17. Manoshi Gayen

Therapeutic potential of CX3CL1 derived peptides

Manoshi Gayen[1], Riqiang Yan[1]

[1]Department of Neurosciences, University of Connecticut Health Center

Extensive neuronal and synaptic degeneration contribute to the memory impairment, cognitive dysfunction, and behavioral deficits in Alzheimer disease (AD). Programmed cell death is a well synchronized process required for normal neuronal development and physiological functioning of neurons. Various forms of cellular stress and inflammatory cascades in neurological diseases cause abnormal activation of cell death pathways including apoptosis. Fracktalkine, CX3CL1 is a chemokine predominantly expressed on the neuronal cell surface while its only receptor CX3CR1 is expressed by microglia. The crosstalk mediated by CX3CL1/CX3CR1 axis was suggested to regulate adult neurogenesis. However, our recent studies have shown that the intracellular C terminal domain of CX3CL1 (CX3CL1-ct), that does not interact with the receptor, has an inherent ability to induce various signaling pathways such as the IGF1 pro-survival pathway that led to attenuation of neuronal apoptosis. Here, we explore the therapeutic potential of synthetic peptides designed from CX3CL1-ct sequence to trigger neurogenesis and compensate for the neuronal loss in AD.

18. Amnah Siddiqa

Trackable and scalable LC-MS metabolomics data processing using asari

Shuzhao Li[1], Amnah Siddiqa[1], Maheshwor Thapa[1], Shujian Zheng[1]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT

Metabolomics holds the promise to measure and quantify small molecules comprehensively in biological systems, and LC-MS (liquid chromatography coupled mass spectrometry) has become the leading technology in the field. Significant challenges still exist in the computational processing of data from LC-MS metabolomic experiments into metabolite features, including provenance and reproducibility of the current software tools. We present here asari, a new open-source software tool for LC-MS metabolomics data processing. Asari is designed with a set of new algorithmic framework and data structures, and all steps are explicitly trackable. It offers substantial improvement of computational performance over current tools, and is highly scalable.

19. Shyam Kishor Sah

Mutations in ASAH1 and PHLDA3 promote keloid-like characteristics in hiPSC-derived organotypic 3D skin constructs

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Keloid scarring is a chronic inflammatory skin disorder characterized by aberrant activation of fibroblasts, which leads to excessive and prolonged deposition of extracellular matrix and to fibrotic scar tissue that extends beyond the boundary of the original wound. There is increasing evidence for a genetic predisposition for keloid formation. By linkage analysis and whole-exome sequencing, we identified $ASAH1_{L401P}$ and $PHLDA3_{Q108*}$ variants in families with inherited keloid susceptibility. We then introduced the $ASAH1_{L401P}$ and $PHLDA3_{Q108*}$ variants into human induced pluripotent stem cells (hiPSCs) from peripheral blood mononuclear cells using CRISPR/Cas9 editing and differentiated homozygous mutant and wild-type hiPSCs into

fibroblasts. *ASAH1* is an acid ceramidase that degrades ceramides into sphingosine and fatty acids. *PHLDA3* acts as a tumor suppressor. Here we study the effects of the $ASAH1_{L401P}$ and $PHLDA3_{Q108*}$ mutations in hiPSC-derived fibroblasts. We found that ASAH1 activity was diminished in $ASAH1_{L401P}$ fibroblasts compared to wild-type fibroblasts. In contrast, no changes were found in total ceramide content of $ASAH1_{L401P}$ fibroblasts. We next generated *in vitro* 3D skin equivalents using rat tail type I collagen matrix containing iPSC-derived fibroblasts and keratinocytes. This organotypic culture was raised to the air-liquid interface and fed from below to induce epidermal differentiation for 14 days. hiPSC-derived keratinocytes containing $ASAH1_{L401P}$ and $PHLDA3_{Q108*}$ -specific mutations formed a thicker multilayered epidermis and cornified layer at the surface of the epidermis on a dermis consisting of iPSC-derived fibroblasts, similar to those using normal human keratinocytes and human keloid-fibroblasts. Moreover, abundance of keloid-specific gene transcripts such as periostin, TGFβ1, TGFβR1, α-SMA were significantly higher in cells harvested from *in-vitro* 3D skin culture consisting $ASAH1_{L401P}$ and $PHLDA3_{Q108*}$ mutations as compared to wild-type. These finding will help to understand keloid pathogenesis and may be useful for development of potential therapeutics.

20. Cesar R. Zoni

Donor cause of death in heart transplantation and its effect on post-transplant survival – A UNOS database review

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With the number of cardiac-related deaths rising each year, the number of heart transplantations have not increased due to a donor deficit. To help bridge this deficit, this study analyzed causes of donor death (CoD) as a predictor of short and long-term survival outcomes of heart transplantation patients. This study evaluated the United Network for Organ Sharing registry for all adult heart transplant recipients and their adult donors from 1987 to 2022. Recipients were grouped based on their donor's cause of death: gunshot wound (GSW), intracranial bleed/stroke (IB/S), anoxia, and other causes. Chi-square test and Kaplan-Meier estimates were used to compare recipient survival. Proportional hazards model analyzed survival adjusting for potential demographic and clinical confounders. Of the 45,304 adult donors, 18.8% died from GSW, 28.9% died from IB/S, 16.0% died from anoxia and 36.3% died from other causes. IB/S donor's CoD had the worse survival at 30 days, 1 year, 5 years and 10 years. Proportional hazards models identified donor CoD-IB/S and GSW as factors decreasing survival. Other risk factors were older age of donor or recipient and race/ethnicity. Women and recipients of women's hearts had better survival. Patients with IB/S donor's CoD had worse survival outcomes in unadjusted model. In the adjusted model by age, race/ethnicity, and gender; IB/S and GSW donor's CoD were associated with worse survival outcomes. Donor's CoD was a risk factor that modified post-heart transplant survival in this study.

21. Kevin Child

Identification of the role of alternative splicing during human heart organogenesis

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Congenital Heart Defects (CHDs) are among the most common congenital abnormalities affecting approximately 1% of births worldwide. Most investigations attempting to identify genetic causes for CHDs have focused on gene expression during fetal heart development and later. Although, recent evidence has suggested the importance of gene expression dynamics during heart organogenesis in the first eight weeks of human development classified into 23 Carnegie Stages (CS). Previous studies have revealed a unique splicing program conserved across multiple species in both brain and heart that differs from several other developing tissues. Our lab analyzed samples spanning the period from CS 13 to 23 including triplicates of 8 different CSs during heart organogenesis. From gene co-expression analysis we found enrichment for a module which encompasses RNA splicing during this period. To investigate the alternative splicing patterns during human heart organogenesis I reanalyzed the short-read bulk RNA sequencing from these early heart samples. From this analysis I have identified thousands of alternative splicing events which include a set of genes involved in heart development and function. I have also identified several novel 5' and 3' UTR regions which have been further validated using Nanopore long-read sequencing from these same samples. From this analysis a novel TBX5 transcript was identified which contains an unannotated 5' UTR upstream from the canonical 5' UTR which appears to be highly conserved and constrained. This novel 5' UTR in TBX5 could play an important role in post-transcriptional regulation affecting TBX5 localization and/or translational efficiency. As TBX5 mutations have already been associated with Holt-Oram Syndrome this region could be valuable for inclusion in future patient screening. Together this analysis will be beneficial for explaining the previously undiagnosed genetic causes of CHDs.

22. Sungryong Oh

Optimizing a model of human heart development with CRISPRi system

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Mammalian embryonic development is controlled by well-orchestrated gene expression regulatory networks. The core components of these developmental regulatory networks, specifically transcription factors, are highly conserved across mammalian species. However, there are noticeable differences in organ structure and function across species necessitating differences likely in regulatory networks during early patterning. Several studies have suggested that these inter-species differences might be driven by variability of gene regulatory sequences. Among them, heart developmental regulatory networks are thought to have decreased conservation of regulatory genomic regions across species. For this reason, human model system of heart is highly required to clearly understand human embryonic heart development. Here, I optimized heart organoids as well as cardiomyocytes mono-layer system derived from human pluripotency stem cells. In particular, our organoid system shows several cavity-like compartmentalized sections. Our models also show clearly distinguished gene expression patterns, thereby expected to highly represent the human embryonic heart formation. In addition, I optimized inducible CRISPRi-system (dCas9-KRAB) in both organoids and mono-layer cardiomyocytes. This inducible system gives me an opportunity to precisely understand the function of human-specific gene regulatory regions at accurate developmental stages. Using these comprehensive approaches and models, I will uncover human-specific heart gene regulatory regions and identify their function through our model system.

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