2023 Columbia University Postdoctoral Research Symposium

SESSION ONE

1 **Chang, Hyun-Kyung**
Postdoctoral Research Fellow, Obstetrics & Gynecology
"LentiMPRA Detects Functional Non-coding Variants Affecting Longevity"

Human longevity is heritable, and associated genome-wide association (GWA) studies have successfully identified associated variants. However, the majority of risk variants reside in non-coding regions, and they have limited predict ability for how longevity variants affect cell type- specific regulatory interactions. To address this, we use lentivirus-based massively parallel reporter assays (lentiMPRA), a high-throughput, to measure the functional regulatory activity of longevity variants in several cell type. In particular, we employed lentiMPRA in fibroblast and endothelial cell to comprehensively screen cell type-specific regulatory activity. We anticipate large scale screen for functional non-coding longevity-associated genetic variation and the role of regulatory elements across diverse cell types.

2 **Bal, Elodie**
Associate Research Scientist/Scholar, *Institute for Cancer Genetics*
"Super-Enhancer Hypermutation Alters Oncogene Expression in B-cell Lymphoma"

Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin lymphoma and remains incurable in ~40% of patients. Coding-genome sequencing efforts identified several genes/pathways altered in this disease, including new potential therapeutic targets. However, the non-coding genome of DLBCL remains largely unexplored. Here we show that active super-enhancers (SEs) are highly and specifically hypermutated in 92% of DLBCL samples, display signatures of activation-induced cytidine deaminase (AICDA) activity, and are linked to genes encoding B-cell developmental regulators and oncogenes. As evidence of oncogenic relevance, we show that the hypermutated SEs linked to the BCL6, BCL2, and CXCR4 proto-oncogenes prevent the binding and transcriptional downregulation of the corresponding target gene by transcriptional repressors, including BLIMP1 (BCL6) and the steroid-receptor NR3C1 (BCL2 and CXCR4). Genetic correction of selected mutations restored repressor DNA-binding, downregulated target gene expression, and led to the counter-selection of cells harboring corrected alleles, indicating oncogenic dependency from the SE mutations. This pervasive SE mutational mechanism reveals a new major set of genetic lesions deregulating gene expression, which expands the involvement of known oncogenes in DLBCL pathogenesis and identifies new deregulated gene targets of therapeutic relevance.

3 **El-Ashmawy, Mariam**
Postdoctoral Clinical Fellow, Hematology-Oncology
"Novel Multi-RAS(ON) Inhibitor Efficacy in KRAS-Amplified Gastroesophageal Cancer"

Introduction: KRAS amplification (copy number gain of wild type allele) exist in 17% of gastroesophageal cancers (GEC) and 1.6% of all cancers. The unique KRAS-amplified state

is difficult to target therapeutically due to adaptive signaling in the PI3K pathway and increased pAKT levels after MEK inhibition. We test novel muti-RAS(ON) inhibitor (RMC-042; Revolution Medicines, Inc) against unique KRAS-amplified GEC models.

Methods: Human GEC cell lines (n=5) and patient-derived GEC organoids (n=5) each with genetically-annotated KRAS-amplification, KRAS-G12D mutation, or wild-type KRAS treated with drug.

Results: In response to RMC-042, KRAS-amplified GEC lines exhibit inhibitory concentrations (IC50) in the hundreds of nM range, and KRAS-G12D cell lines were 100-fold more sensitive (IC50 < 5nM). In KRAS-amplified GEC, RMC-042 potently and swiftly inhibits pAKT and pERK levels in vitro, an on-target effect which persists for up to eight weeks of drug treatment. Compared to treatment with MEK inhibitor trametinib, we observe that RMC-042 results in less adaptive signaling induction of pAKT, which further supports the use of RAS(ON) inhibitors in KRAS-amplified tumors. Whole transciptome analysis at 24 hours revealed deep suppression of RAS signaling pathway genes after treatment with RMC-042 across all cell lines, as well as enriched interferon response gene set in KRAS amplified GEC. In vivo pharmacodynamic and tumor growth assessed in xenografted KRAS-amplified GEC (n=10 mice) show early tumor regression and inhibition of pERK and pS6 by immunostaining after three doses of RMC-042.
Conclusion: Overall, we show efficacy of RMC-042, a RAS-GTP(ON) inhibitor, in preclinical in vitro and in vivo KRAS-amplified GEC models.

4 **Bompolaki, Maria**
Postdoctoral Research Scientist, Psychiatry
"The Pathophysiology of Hippocampal Dysfunction in Mice Exposed to Early Life Stress and Patients with a Stress-Related Psychiatric Illness"

Early life adversity is a major risk factor for emotional and cognitive dysfunction and psychiatric illness throughout the life. We recently found that hippocampal neurogenesis, which is highly susceptible to the effects of psychosocial stress, sustains cholinergic hippocampal inputs and maintains working memory throughout life. Mice without adult neurogenesis had a major reorganization of cholinergic hippocampal innervation and memory deficits. Our current studies reveal that mice exposed to early life stress also develop life-long hippocampal cholinergic dysfunction and working memory deficits. Animals exposed to early life stress exhibited decreased cholinergic signaling in the hippocampal dentate gyrus while exploring a maze and a decline in spontaneous alternations (a measure of non-reinforced working memory). Deficits in cholinergic signaling and working memory performance were accompanied by increased dentate gyrus theta power (a measure of synchronous activity) and were completely rescued by pharmacologically increasing cholinergic tone. We then examined working memory in children affected by nonverbal learning disability, a persistent developmental deficit that has been associated with early life adversity. We found that children with nonverbal learning disability have deficits in working memory and increased baseline resting state connectivity between the cholinergic basal forebrain and the hippocampal dentate gyrus. This finding is reminiscent of our anatomical studies in mice and highlights the power of translational research to probe the mechanisms of how stress impacts mental health.

5 **Jafariyan, Amirhossein**

Postdoctoral Research Fellow, Chemistry

"Live Yeast Dressings Engineered via Synthetic Biology to Deliver Protein Factors to Diabetic Wounds"

Synthetic biology is ushering in a new era where cells act as a new class of therapeutics, continuously generating therapeutic factors. While topical delivery of wound-healing protein factors is a predominant strategy for treating chronic ulcers, new approaches are needed to optimize the effectiveness of the delivered factors by improving their bioavailability, continuous dosage, reduced cost, and widespread availability all over the globe. Here, we used synthetic biology to develop a new modular platform technology for continuous on-site production and delivery of wound-healing factors via the topical application of engineered living yeast as a hydrogel dressing. Saccharomyces cerevisiae was engineered to secrete biologically active epidermal growth factor (EGF), leptin (LEP), and stromal cell-derived factor (CXCL12) in high titers. It was formulated as a hydrogel dressing that accelerated and enhanced the quality of healing of excisional wounds of different sizes in streptozotocin-treated diabetic mice.

6 **Miura, Akihiro**

Postdoctoral Research Fellow, Medicine (Columbia Center for Human Development)

"Conditional Blastocyst Complementation of a Defective Foxa2 Lineage Efficiently Promotes the Generation of Whole Lungs"

Millions worldwide suffer from incurable lung diseases. Lung transplantation is the only option for terminally ill patients. Despite the technological advances in bioengineering and regenerative medicine, none of the technology achieved the entire lung generation. Identifying lineages, which target all lung cell types, would facilitate the design of the entire lung generation. Given that Forkhead boxA (Foxa2) is the marker for mesendoderm that may give rise to both lung epithelium and mesenchyme, we performed Foxa2 lineage tracing analysis using Foxa2Cre/+; RosatdTomato/+ mice. We confirmed that the Foxa2 lineage labeled the entire lung epithelium and most lung mesenchyme, including endothelium. Because the Fgf7-, Fgf10-Fgfr2 signaling is a critical mitogen for lung epithelium and mesenchyme, we generated the mice harboring Fgfr2 gene depletion in the Foxa2 lineage. We found that those mice showed lung agenesis phenotype throughout lung development. We injected mouse-induced pluripotent stem cells (iPSCs) into the host blastocysts to rescue this phenotype. Almost 100% of the lung epithelium and about 90% of the mesenchyme were significantly complemented with donor iPSCs alone. These results suggest that Foxa2 promoter-driven Fgfr2 depletion causes defects in the lung epithelium and the mesenchyme that promotes whole lung generation efficiently. Our studies offer future autologous transplantation therapies using iPSC in the near future.

7 **Vilfranc, Chrystelle**

Postdoctoral Research Fellow, Epidemiology

"The Hair Tales of Pregnant Women of Color in New York City"

Exposure to endocrine disrupting chemicals (EDCs) can have negative fetal and maternal health outcomes, including increased risks of fetal growth disruption and breast cancer. Notably, women of color are the largest consumers of personal care products, a main source of EDC exposure. The Let's Reclaim Our Ancestral Roots (Let's R.O.A.R) Study proposed a behavioral intervention during pregnancy to promote reduced use of phthalate-containing hair care products (HCPs). Here, we conducted a qualitative study through educational sessions and semi-structured focus groups to evaluate the factors that influenced the hair journey and product choices of women of color at various stages of life. Of the 47 individuals who were eligible, consented, and enrolled in the study, 31 participated in an English or Spanish educational session discussing the adverse implications of using phthalate-containing HCPs. In a brief post-session focus group, we gathered feedback on the sessions and learned more about their hair journey. We imported session transcripts into NVivo 12 to analyze the data through thematic analysis and coding. We framed questions to capture the participants' unique hair journeys from birth to current pregnancy and identified two main periods: before gaining agency over their hair care and product choices and after agency. We identified three dominant themes: (1) influencers, which included individuals or entities that impacted their hair experiences, (2) products, which involved all conversations of hair products, and (3) culture, which discussed the influence of culture on their hair journeys. These three themes intersected with each other and impacted the participants' sense of self differently at each period of the hair journey. The data reveals the importance of intervening prior to participants gaining agency of their hair choices. This study provides context of psychosocial and sociodemographic narratives of identity and culture and will be integral to the successful translation of intervention results.

8 **Heeter, Karen**

Postdoctoral Research Fellow, Lamont-Doherty Earth Observatory (Tree Ring Lab)

"The North American Temperature Atlas: A Climate Field Reconstruction for Investigating the Effects of Temperature on Past Droughts Prior to Anthropogenic Forcing"

Spatially-resolved climate reconstructions are opportune for analyzing spatial anomaly patterns and characterizing regional-scale trends resultant from climate change. To date, few fine-scale spatially-resolved paleo-temperature datasets exist in the Northern Hemisphere, especially those with representation of locations south of 40oN. Here, we present the North American Temperature Atlas (NATA), a 0.5x0.5o gridded dataset of reconstructed warm season mean and maximum surface air temperatures, developed from a continental-scale synthesis of density and blue intensity tree-ring chronologies collected over the last four decades. This publicly-available paleoclimate record is the first of its kind for the North American continent and will provide novel past temperature estimates which can be used to examine (1) past and current spatiotemporal patterns of temperature variability, (2) the timing and direction of temperature rate changes through time, (3) the relationship between temperature and drought events across time and space, (4) the occurrence and impacts of compound climatic extreme events, and (5) the role of ocean-atmosphere circulation on low frequency temperature variability and the sensitivity of temperature changes to radiative forcing. Upon completion, the NATA will fundamentally advance the knowledge and understanding of the relationships between drought and warm-season temperature at annual to centennial timescales and allow for the comparison of these relationships at varying spatial scales. Further, the fine resolution of this 0.5o gridded dataset will uniquely allow for the

differentiation and comparisons of regional-scale, shorter-term climate variability from long-term global changes.

9 **Hwang, Youngmin**
Postdoctoral Research Scientist, Medicine
"Reactive Oxygen Species (ROS)-induced Remodeling of NOTCH3+ Human Airway Progenitors and Goblet Cells by NRF2 Activation"

"Ions, one of the exogenous reactive oxygen species (ROS), are an unavoidable environmental stimulus generated by various devices such as TVs, PCs, hair dryers, air conditioners, and various electrical appliances. However, the association of ion stimuli with airway remodeling and COVID-19 has been unclear. We hypothesized that ion stimulation would not influence the cellular differentiation of human airway epithelial cells. To test this hypothesis, differentiated human tracheal epithelial cells (hTECs) cultured in vitro by air-liquid-interface (ALI) culture condition were exposed to ions with an ion generator (IG) (1x106 ions/cm3 for 2 or 24 hrs). The sufficient ion concentration was measured by changing the distance between IG and hTECs.

We found that ion exposure to hTECs was sufficient to induce a moderate level of ROS activations in hTECs with little cytotoxic effects. By flow cytometry analyses of ROS in hTECs, the ROS level was increased (IG 24hr + 3 days post culture) and recovered (IG 24hr + 7 days post culture) to normal (No IG). qPCR and immunostaining analyses confirmed that ion exposure to the hTECs increased NRF2, a master regulator of ROS signaling, and NOTCH3 expression, a marker of parabasal cells. Interestingly, goblet cell marker (MUC5AC) expression was significantly decreased after IG treatment (IG 24hr) but increased at 7 days after IG treatment, coinciding with the increased SPDEF expression, the transcription factor in inducing MUC5AC. Conversely, club cell (SCGB1A1), multiciliated cell (FOXJ1, DNAI1), and ACE2 expression, a receptor of SARS-CoV2, were significantly decreased. These results indicate that contrary to our hypothesis, ion exposure influenced airway remodeling by the ROS-mediated differentiation program in NOTCH3+ parabasal cells to expand MUC5AC+ goblet cells at the cost of SCGB1A1+ club cell and ACE2+ FOXJ1+ multiciliated cells. These results also suggest ions induce airway remodeling, possibly altering airway infection efficiency against SARS-CoV2 by losing ACE2."

10 **Lin, Zhexi**
Postdoctoral Research Scientist, Chemical Engineering
"Metal-Modified Molybdenum Nitride as Selective Catalysts for Ethanol Reforming and Dehydrogenation"

The transformation of biomass feedstocks into renewable fuels and chemicals has shown promise for solving the current energy and environmental challenges. The growth of bioethanol production brings about an abundance of ethanol, which can be further upgraded into value-added products to benefit the process economics. Among the many approaches for upgrading bioethanol, two pathways are particularly promising: (1) ethanol reforming to produce renewable hydrogen and (2) ethanol dehydrogenation to produce acetaldehyde, an industrially important intermediate.

This study combines machine learning predictions, UHV model surface experiments, and powder catalyst evaluation to demonstrate metal-modified Mo2N as promising catalytic materials for ethanol reforming and dehydrogenation. Using Mo2N as a substrate significantly reduces the cost of Pt while maintaining the high reforming selectivity. Fe- and Cu-modifications of Mo2N selectively enhance the C-H bond scission of ethanol to produce value-added acetaldehyde. The implementation of machine learning predictions accelerates catalyst discovery. The insights from this study shed light on the rational design of efficient catalysts to upgrade abundant bioethanol into renewable fuels and chemicals.

11 **Nadezhdin, Kirill**
Postdoctoral Research Scientist, Biochemistry and Molecular Biophysics
"Structural Mechanisms of TRPM7 Channel Activation and Inhibition"

The TRP channel TRPM7 is a master regulator of the organismal balance of divalent cations that plays an essential role in embryonic development, immune responses, cell mobility, proliferation, and differentiation. TRPM7 is implicated in neuronal and cardiovascular disorders, tumor progression and emerged as a new drug target. In this work, we use cryo-EM, functional analysis, and molecular dynamics to uncover the mechanisms of TRPM7 gating and inhibition. Our structural analysis of TRPM7 with a gain-of-function mutation reveals the molecular basis for its constitutive activity, with local transformations of S6 and TRP helix underlying channel opening. A similar channel opening is induced by the agonist naltriben that binds at the intersubunit interfaces and causes dramatic conformational rearrangements of the intracellular domains. The highly potent and selective inhibitors VER155008 and NS8593 dock into the vanilloid-like site and stabilize the closed state of TRPM7. The discovered mechanisms provide foundations for understanding the molecular basis of TRPM7 channelopathies and drug development.

12 **Tanaka, Junichi**
Postdoctoral Research Fellow, Medicine (Columbia Center for Human Development)
"Generation of Salivary Glands via Conditional Blastocyst Complementation"

Cell-based therapy for generating salivary glands is a promising next-generation therapy for patients suffering from dry mouth, rampant caries, fungal infections due to Sjögren's syndrome, or hyposalivation by the side-effect of radiotherapy for head and neck cancers. However, none of the technology achieved the entire salivary gland generation because the exact origin of the salivary gland has also been enigmatic. Previous studies reported that Fgfr2 is crucial for salivary gland formation and Shh-lineage or Pitx2-lineage labels entire salivary glands, but we found that neither Shh lineage-driven nor Pitx2 lineage-driven Fgfr2-driven knockout mice showed the agenesis phenotype. Based on these results, we speculated the existence of a prospective lineage crucial for the primary bud formation of the salivary gland or specification mechanism mediated by Fgfr2. After performing extensive lineage tracing analyses with different Cre drivers for depleting the Fgfr2 gene, we discovered that the Foxa2 lineage is crucial for salivary gland formation. Foxa2Cre/+; RosaLSL-tdTomato/+ lineage-tracing mice showed partial lineage labeling in the oral epithelium at E8.5 and entire labeling in the primordial oral epithelium at E11.5. This labeling was not observed in Pitx2- nor Shh-driven lineage tracing mice. This observation was further supported by analyzing the deposit scRNA-seq of embryonic mice. Indeed, Foxa2-driven Fgfr2 knockout mice of Foxa2Cre/+;

Fgfr2flox/flox; RosaLSL-tdTomato/+ caused salivary gland agenesis phenotype at E18.5. To rescue this phenotype, we injected GFP+ mouse iPSCs into blastocysts of Foxa2Cre/+; Fgfr2flox/flox; RosaLSL-tdTomato/+ mice. Donor GFP+ mouse iPSCs complemented the salivary gland agenesis phenotype and generated salivary glands. Histological analysis revealed that all salivary gland epithelial cells were derived from host GFP+ cells. Taken together, Foxa2 is a critical lineage for initiating salivary gland through Fgfr2-mediated developmental process. Foxa2 lineage is also promising for generating iPSC-derived salivary glands.

13 **Vasciaveo, Alessandro**

Associate Research Scientist/Scholar, Systems Biology

"Using Network Biology and Treatment-resistant In Vivo Models to Study Lethal Prostate Cancer"

Prostate cancer (PC) is the most common malignancy in males and, although localized disease has a favorable prognosis, metastasis often leads to high mortality rates as current treatments are not curative. Hormonal therapy, called Androgen Deprivation Therapy (ADT), is the standard of care for advanced PC, but it frequently leads to the emergence of a lethal phenotype, known as Castration Resistant Prostate Cancer (CRPC), which is treatment-resistant and often gives rise to an aggressive variant with features of neuroendocrine differentiation, called Neuroendocrine PC (NEPC). The authors used genetically-engineered mouse models of CRPC to identify molecular drivers of NEPC using a forward genetic screening approach and Sleeping Beauty (SB) transposon system. They generated a mouse strain in which the SB transposase expression is under the control of a tamoxifen-inducible Cre allele driven by the prostate-specific Nkx3.1 promoter, resulting in tumors with an accelerated growth phenotype and histological features of NEPC. They performed an integrative analysis of DNA-Seq and RNA-Seq data to identify Master Regulator (MR) proteins associated with NEPC cell identity and the upstream genomic events responsible for the treatment-resistant phenotype. The study resulted in a regulatory network of NEPC-associated candidate MR proteins which were not yet associated with lethal prostate cancer. Computational analysis of patient-specific MRs activity conservation between human and mice cohorts aligned NEPC patients to mice tumors with NE differentiation features, validating the study's clinical relevance. The authors are in the process of validating these results in vitro using the latest CRISPR technology.

14 **Withall, Jennifer**

Postdoctoral Research Fellow, Nursing

"Identifying Reuse and Redundancies in Respiratory Flowsheet Documentation: Implications for Clinician Documentation Burden"

Documentation burden is experienced by clinical end-users of the electronic health record. Flowsheet measure reuse and clinical concept redundancy are two contributors to documentation burden. In this paper, we described nursing flowsheet documentation hierarchy and frequency of use for one month from two hospitals in our health system. We examined respiratory care management documentation in greater detail. We found 59 instances of reuse of respiratory care flowsheet measure fields over two or more templates and groups, and 5 instances of clinical concept redundancy. Flowsheet measure fields for physical assessment observations and measurements were the most frequently documented

and most reused, whereas respiratory intervention documentation was less frequently reused. Further research should investigate the relationship between flowsheet measure reuse and redundancy and EHR information overload and documentation burden.

15 **Akita, Keitaro**

Postdoctoral Research Fellow, Cardiology

"Prediction of Cardiac Death in Patients with Hypertrophic Cardiomyopathy Using RNA-Sequencing of Plasma Small Non-coding RNAs"

Background:
Hypertrophic cardiomyopathy (HCM) causes cardiac death, whereas no clinical model is available to predict cardiac death in HCM. The underlying molecular mechanisms underpinning cardiac death in HCM also remain uncertain. We aimed to develop a plasma small non-coding RNA-sequencing (sncRNA-Seq)-based model for predicting cardiac death in patients with HCM and to determine signaling pathways dysregulated in those who subsequently experience cardiac death.

Methods:
In this prospective, multi-center cohort study, we conducted RNA-Seq of 3740 plasma sncRNAs on 390 patients with HCM. The outcome was cardiac death defined as a composite of heart transplant, heart failure death, and sudden cardiac death. We developed a sncRNA-Seq-based prediction model with linear discriminant analysis to predict cardiac death using data from one institution (training set, n=271). We tested its predictive ability in samples from other institutions (test set, n=119). In the test set, we performed the log-rank test between high- and low-risk groups, which was determined by the prediction model. We also performed pathway analysis of microRNAs significantly associated with cardiac death.

Results:
During a median follow-up of 2.7 years, 9 patients in the training set (3.3%) and 8 in the test set (6.7%) experienced cardiac death. The area under the receiver-operating-characteristic curve of the prediction model for cardiac death was 0.73 (95% confidence interval 0.55–0.90). In the test set, the high-risk group (n=74) had a significantly higher rate of developing cardiac death (log-rank P=0.03) compared to the low-risk group (n=45). In the pathway analysis, both known pathways (e.g., TGF-β) and novel pathways (e.g., Hippo) were dysregulated with false discovery rate <0.01 in patients who subsequently experienced cardiac death.

Conclusions:
This study is the first to demonstrate the ability of plasma sncRNA-Seq for predicting cardiac death in HCM. Patients who subsequently experienced cardiac death exhibited dysregulation of both known and novel pathways.

16 **Ali, Alessandra**

Postdoctoral Research Scientist, Radiology

"Real-time Assessment of the Intracranial Accumulation of Therapeutics Using 3D Optical Imaging"

Glioblastoma (GBM) is the most common malignant primary adult brain tumor with a poor prognosis and median survival of fewer than 2 year. Focused ultrasound (FUS) combined with microbubbles is an emerging method that can potentially enable drugs to reach the infiltrating GBM cells outside the natural BBB-permeable core. Our goal is to develop a preclinical modality to evaluate in real-time the intracranial accumulation of potential therapeutics for brain tumor. We hypothesize that 3-dimensional optical/CT of near-infrared (NIR) fluorescent dyes labeled therapeutics can be used as a preclinical tool to estimate its tumor accumulation over time. To validate the real-time 3D optical/CT we used Cy7 labeled albumin (Cy7-albumin) as a therapeutic surrogate to test this technique for future use with optically labeled therapeutics. Mouse serum albumin was labeled with Cy7 near-infrared (NIR) fluorescent dye according to manufacturer instructions. DEFINITY microbubbles were prepared according to manufacturer instructions. FUS was applied to the mouse right cerebral hemisphere. Localization of the ultrasound beam with the brain was achieved using stereotactic localization of skull landmarks with a brain atlas-guided FUS. Cy7-albumin was injected iv immediately after FUS application. 3D Optical/CT images were acquired 8, 24, 40, 52, 120 min and 24 h after Cy7-albumin administration using MILabs 3D optical scanner (MILabs, Netherland). To confirm BBB opening, mice were injected iv with 1% Evans Blue dye immediately after FUS. Mice were sacrificed 24 hours after FUS. Brains were dissected and imaged for Evans Blue. We demonstrated that 3D optical/CT imaging detected in real-time the location and the level of Cy7-albumin in the brains of the mice during 24 h after FUS+Cy7-albumin injection.3D optical/CT is a promising way to evaluate real-time concentration of NIR-labeled therapeutics. Therefore, 3D optical/CT in conjunction with Cy7-labeled therapeutic candidates can play a valuable role in preclinical setting to follow / evaluate in real-time the intracranial accumulation of the therapeutic administered dose.

17 **Amissah, Emma**

Postdoctoral Research Fellow, Environmental Health Sciences

"The Relationship between Maternal Dietary Patterns during Pregnancy in Women with Gestational Diabetes Mellitus and Infant Appetitive Feeding Behavior at 6 Months"

Early dietary exposure may influence infant appetitive feeding behavior and their later health. Maternal diabetes in pregnancy is associated with an increased risk of obesity in the offspring. We, therefore, examined third-trimester dietary patterns of women with gestational diabetes, their offspring's appetitive feeding behavior at 6 months of age, and relationships between these. We used data from a prospective cohort of women with gestational diabetes and assessed maternal dietary patterns at 36 weeks' gestation using principal component analysis; infant appetitive feeding behavior at 6 months of age using the Baby Eating Behaviour Questionnaire; and relationships between these using general linear modeling and chi-square tests. In 325 mother-infant dyads, we identified three distinct maternal dietary patterns: 'Junk,' 'Mixed,' and 'Health-conscious.' The maternal 'Health-conscious' pattern was inversely associated with 'enjoyment of food' in their sons (β −0.24, 95% CI −0.36 to −0.11, p = 0.0003), but not daughters (β −0.02, 95% CI −0.12 to 0.08, p = 0.70), and was positively associated with 'slowness in eating,' (β 0.13, 95% CI 0.02 to 0.24, p = 0.01). Third-trimester dietary patterns in women with gestational diabetes may have sex-specific effects on infant appetitive feeding behavior at 6 months of age.

18 **Bergman, Maja**

Postdoctoral Clinical Fellow, Psychiatry

"Acceptance and Mindfulness-Based Exposure Therapy (AMBET) for PTSD Following Sudden Cardiac Arrest Survival: An Open Trial of a Novel Treatment Protocol"

"Background:
It is estimated that over 30,000 sudden cardiac arrest (SCA) survivors with PTSD are discharged from U.S. hospitals each year. SCA-induced PTSD has been associated with increased mortality and cardiovascular disease risk, but no psychotherapeutic treatment has been tested in this population. Exposure therapy is gold standard treatment for PTSD, but current therapy protocols are not specific to unique disease courses for SCA survivors and have high dropout rates. Mindfulness-based interventions are typically well-tolerated and have shown promise in reducing PTSD symptoms from other events.

Objective:
To determine the safety, feasibility, and preliminary efficacy of a novel SCA-specific psychotherapy protocol that combines mindfulness and exposure-based interventions to reduce symptoms and improve health behaviors in patients with PTSD following SCA.

Methods:
An open feasibility pilot study was conducted with a small sample (N=11) of SCA survivors diagnosed with PTSD. The intervention consisted of eight 90-minute individual sessions that were delivered via synchronous video sessions. PTSD symptoms were assessed using the Clinician Administered PTSD Scale for DSM-5 (CAPS-5) at baseline, mid-point, post-treatment, and three months following completion of the treatment protocol.

Findings:
Out of 11 enrolled participants, ten completed the treatment protocol. Satisfaction was high, no adverse events were reported, and clinically and statistically significant reductions were observed on PTSD symptoms across all clusters with large effect sizes.

Conclusions: In this open trial, the AMBET protocol was safe, feasible, and potentially efficacious in reducing PTSD following SCA. The treatment was well-liked and tolerated by patients with very low attrition.

19 **Bloom, Paul**

Postdoctoral Research Scientist, Child and Adolescent Psychiatry

"Does Missingness Matter? Identifying Factors Impacting Smartphone-based Research on Adolescent Suicidal Thoughts and Behaviors"

Intensive longitudinal research—including experience sampling and mobile sensor monitoring—may identify proximal risk factors for mental health crises including suicidal thoughts and behaviors (STB). Missing data complicates interpretations. Clarifying whether clinical factors predict missingness could help inform missing data management strategies and development of just-in-time interventions. Adolescents ages 13-18-years-old (N=182) reporting depressive, anxiety, and/or substance use disorders were enrolled in the study. 65% were experiencing current suicidal ideation, with 28% reporting a past year attempt. STB and

symptom assessments were completed at baseline, 1-, 3-, and 6-month follow-up assessments. Additionally, mobile sensor data (e.g., mobility, key inputs) and daily mood were acquired during the entire follow-up on the Effortless Assessment Research System smartphone app. Multilevel logistic regression indicated that missingness of daily mood (56.2% of observations) increased over time from baseline, on weekends, and during summers. However, neither STB nor depressive or anxiety symptoms associated with the percentage of missing daily mood assessments. Mood was not associated with likelihood of missingness on the prior or subsequent day although it was autocorrelated across successive days. Passive mobile sensor measures, in particular estimates of bedtimes and wake times, predicted when daily mood surveys would be missing on a day-to-day basis. Overall, results suggest that study design choices and technical factors contribute more strongly to missingness than do clinical factors. Together, these findings may guide future analyses and interpretation in the context of adolescent STB.

20 **Castellano, Joan**

Postdoctoral Research Scholar, Medicine (HICCC)

"Aneuploidy Impact in Lung Basal Cell Differentiation and Squamous Cell Carcinoma Development"

Aneuploidy is a nearly universal characteristic of solid tumors. Some aneuploidy alterations are present at a high frequency since early stages of cancer development. In the case of lung squamous cell carcinoma (LUSC), loss of chromosome arm 3p and gain of chromosome arm 3q are present in 80% and 60% of the stage I TCGA cases respectively. Basal cells of the lung are progenitors of different mature cell types residing in the bronchi and bronchiolar regions of the lung. Additionally, the presence of basal cell markers such as KRT5 or P63 in LUSC tissues indicates that this cell type is the cell of origin of LUSC. Using a CRISPR/CAS9 approach our lab engineered immortalized isogenic airway lung epithelial cells to harbor the 3p loss or the 3q. Our project aims to characterize the effect of chr3 alterations in basal cells in vitro using 3D models (organoids). For this purpose, we induced differentiation in lung organoids with different aneuploidy backgrounds and characterized at histology and molecular level. We found that WT organoids tend to generate squamous organoids with a higher degree of cellular organization in the basal layers when compared to the aneuploid counterparts. Additionally, expression of maturation markers such as MUC5B or SCGB1A1 was detected by immunofluorescence in WT but not in aneuploid organoids. Finally, detection of altered expression of KRT14, NOTCH1 and TGFB1 was observed in aneuploid organoids, indicating that possible alterations in theses pathways might affect organoids differentiation. In summary, our project is uncovering how chromosome 3 alterations affects basal cell differentiation as an early event in the development of lung squamous cell carcinoma.

21 **Chang, Chia-Yuan**

Postdoctoral Research Scientist, Psychiatry

"Adult-born Neurons Maintain Hippocampal Cholinergic Inputs and Support Working Memory during Aging"

Adult neurogenesis has been linked to the maintenance of normal hippocampal functions and its impairment has been implicated in disorders of stress, memory, and cognition.

However, a systems-level understanding of how low levels of ongoing neurogenesis contribute to major brain functions over time is lacking. To address this gap, we conducted a connectivity analysis of inputs into the dentate gyrus (DG) in mice without neurogenesis (NG-). We found that NG- mice developed a slow-emerging deficit in the spontaneous alternation task, indicating working memory impairments. This was accompanied by a selective reorganization of the cholinergic projection to DG. Specifically, the somatotopic organization of medial septal cholinergic hippocampal inputs was lost after 5 months without neurogenesis, as neurons that normally project to the ventral hippocampus also innervated the dorsal hippocampus. Thus, more cholinergic neurons projected to the dorsal DG overall. Despite increased cholinergic connectivity, the levels of acetylcholine (ACh) in the dorsal DG were significantly lower in NG- mice. Behavioral pharmacology studies revealed that functional deficits in ACh signaling preceded delayed changes in cholinergic innervation and ACh levels. With assistance of fluorescent acetylcholine sensor and fiber photometry, we observed slower and attenuated ACh responses upon task exposure before behavioral, anatomic, and biochemical changes emerged. Our findings suggest that adult neurogenesis is important for maintaining normal cholinergic signaling. The slow structural reorganization of cholinergic innervation, decline in baseline ACh, and accompanying working memory deficits are likely a failed compensatory response to functional disruption of cholinergic signaling. Importantly, a chemogenetic increase in cholinergic signaling rescued behavioral deficits in animals long after anatomic and biochemical changes took place. Our findings support that ongoing neurogenesis throughout life is necessary to maintain normal septohippocampal circuit function and optimal working memory performance. The results have important implications for understanding age and stress-related cognitive decline and for treatment development.

22 **Copperi, Francesca**
Postdoctoral Research Scientist, Institute of Human Nutrition
"Dorsal Raphe Nucleus Melanocortin Signaling Regulates Energy Homeostasis"

The Dorsal Raphe Nucleus (DRN) was recently shown to be an important regulator of energy expenditure and food intake. Previous scientific work have shown that GABAergic and glutamatergic DRN neurons are modulated by changes in energy balance and in turn generate opposite effects on feeding: while GABAergic neurons increase food intake, glutamatergic neurons suppress food intake. Within the DRN, melanocortin 4 receptor (MC4R), a key player in the regulation of metabolism, is enriched in GABAergic neurons with minimal expression in glutamatergic neurons. Previous investigations from our group have shown that DRN MC4R is a pivotal regulator of food intake and mood. However, whether this effect is specifically modulated by GABAergic neurons is unclear. In this study, we characterize the role of DRN MC4R GABAergic neurons. Through a combination of both chemogenetic and genetic approaches, we were able to show that short term activation of DRN MC4R GABAergic neurons is able to increase food intake and decrease depressive behavior in mice. Additionally, long term loss of MC4R in DRN GABAergic neurons causes a significant body weight gain and accumulation of fat mass, while improving depression by regulating serotonin levels. Overall, our data point to a pivotal role of DRN MC4R GABAergic neurons in the regulation of food intake, energy homeostasis and mood.

23 **Curiel Garcia, Alvaro**

Postdoctoral Research Scientist, Medicine (HICCC)

"Ras-dependent Activation of BMAL2 Regulates Hypoxic Metabolism in Pancreatic Cancer"

To identify novel drivers of PDA malignancy, we employed a regulatory network analysis, which accurately infers the activity of transcription factors and other regulatory proteins based on the integrated expression of their positive and negative target genes. This highly validated approach enables the identification of the most hyper-activated and hyper-repressed regulatory proteins (i.e., the "master regulators (MRs)") that drive phenotypic distinctions. We applied this technique to a set of 200 laser capture microdissected human PDA samples as well as 45 low-grade precursors for which we had matched histopathological, clinical, and epidemiological annotation.

We identified the MRs associated with four malignancy phenotypes: precursors vs. PDA (initiation), low-grade vs. high grade histopathology (progression), survival post resection, and association with KRAS activity. Integrating across these phenotypes, the top MR of PDA malignancy was found to be BMAL2, a member of the PAS family of bHLH transcription factors.

Although the canonical function of BMAL2 is linked to the circadian rhythm protein CLOCK, gene set enrichment analysis highlighted a potential role in hypoxia response. We previously demonstrated that PDA in humans and in the genetically engineered "KPC" mouse model is hypovascularized, hypoperfused, and profoundly hypoxic, with a partial oxygen pressure <1mmHg. Given the close homology of BMAL2 to HIF1B (ARNT) and its potential to heterodimerize with HIF1A, we investigated whether BMAL2 plays a role in the hypoxic response of PDA. Indeed, BMAL2 activity was induced in response to hypoxia and inhibited following treatment with multiple RAF, MEK, and ERK inhibitors, validating its computationally-inferred association with RAS activity. Strikingly, knockout or knockdown of BMAL2 in human PDAC cells led to defects in viability and invasion in the setting of hypoxia. BMAL2 knockout cells lost the ability to induce glycolysis upon exposure to severe hypoxia and this was associated with a loss of expression of the glycolysis enzyme LDHA. Strikingly, knockout of BMAL2 led to a complete loss of HIF1A stabilization in response to hypoxia, consistent with the stabilizing role of HIF1A heterodimerization partners such as HIF1B. By contrast, HIF2A was further upregulated under hypoxia in the setting BMAL2 loss. We conclude that BMAL2 is a key master regulator of hypoxia responses in PDA that serves as a molecular switch between the disparate metabolic roles of HIF1A- and HIF2A-dependent hypoxia responses. This will be further validated in ongoing metabolomic studies.

24 **Dong, Rui**

Postdoctoral Research Scientist, Neurology

"For the Case-only Design How Rare Must a Disease Be to Control Type I and II Errors?"

The case-only design can be a powerful approach to identify gene-gene and gene-environment interactions for rare diseases. However, it has not been investigated how rare a disease must be for the case-only design to be a valid approach. Through extensive simulations studies, we investigated the rare disease assumption and show that for diseases with prevalence <0.05 the case-only design has well controlled type I error and is substantially more powerful to

detect interactions than the case-control design, but for higher disease prevalences both type I and II errors are inflated. For a case-only study (N=10,000) where the disease prevalence is 0.2, and the genetic variant has a MAF=0.2 and a main effect odds ratio (OR)=1.2 and the environmental exposure has a prevalence of 0.1 and a main effect OR=2.0, under the null of no interaction for alpha=0.05 the type I error is 0.07. For the same scenario, but where there is an interaction (OR=1.2) the power to detect an interaction is 0.49, while for a case-control study (N=10,000 cases and 10,000 controls) the power is 0.85. For a disease prevalence of 0.04 instead of 0.2 the power for the case-only design is 0.88 and for the case-control design 0.55. We will also demonstrate the behavior of the case-only design when there are no main effects or only one main effect. Although, the case-only design is a powerful method to detect interactions for rare diseases, for many complex traits their prevalence is too high for this method to be beneficial.

25 **Feng, Xiangsong**
Postdoctoral Research Scientist, Biochemistry and Molecular Biophysics
"A Method of High-resolution Time-resolved Single-particle Cryo-EM for Capturing Short-lived Intermediates in Biomolecular Reactions"

In the past decade, single-particle cryogenic electron microscopy (cryo-EM) has made indispensable contributions to the elucidation of the structural foundation of life processes in the cell. Reconstructions achieved in our lab reached resolutions in the range of 2.5 – 3.5 Å, that allowed building of detailed atomic models. However, important structural information is missing about short-lived intermediates (10 – 1000 ms), i.e., shorter than the minimum time required (several seconds) with the standard blotting method. Several time-resolved cryo-EM (TRCEM) methods that have been developed previously are typically ineffective in micro-mixing so unavoidably the reaction cannot be initiated uniformly among all reactants. Some other problems of existing microfluidic devices are unexpected blockages, protein adsorption in the mixing and reaction channels, which nonspecifically change the stoichiometry of the reaction of interest.

To overcome these problems, our work has focused on the development of a novel PDMS-based microfluidic chip assembly with an efficient three-dimensional Splitting-And-Recombination (SAR)-based micromixer, reduced protein adsorption in the microchannels by coating them with a SiO2 layer, stable flow control in the capillary tubing, and reproducible performance of the micro-sprayer. We are applying this improved method to the capturing of short-lived states during the reaction (e.g. E. coli 70S ribosome recycling, 70S ribosome association, eukaryotic translation termination, and other processes on molecular machines). As one example, the mechanism of HflX-mediated recycling of the E. coli ribosome has been elucidated by using our TRCEM method. We successfully captured three intermediate states of the 70S-HflX complex, presenting the time course of the recycling of the 70S ribosome by HflX in the presence of GTP. The high speed of this process makes it impossible to study it by the normal pipetting-blotting method. Our results demonstrate the power of this improved design in elucidating the mechanisms of translation, one of the most fundamental processes in biology.

26 **Gong, Zhen**

Postdoctoral Research Scientist, Biochemistry and Molecular Biophysics

"Crystal Structure of LGR Ligand α2/β5 from *C. elegans* with Implications for the Evolution of Glycoprotein Hormones"

A family of leucine-rich-repeat-containing G-protein-coupled receptors (LGRs) mediate diverse physiological responses when complexed with their cognate ligands. LGRs are present in all metazoan animals. In humans, the LGR ligands include glycoprotein hormones (GPHs) chorionic gonadotropin (hCG), luteinizing hormone (hLH), follicle stimulating hormone (hFSH) and thyroid stimulating hormone (hTSH). These hormones are αβ heterodimers of cystine-knot protein chains. LGRs and their ligand chains have co-evolved. Ancestral hormone homologs, present in both bilaterian animals and chordates, are identified as α2β5. We have used single-wavelength anomalous diffraction (SAD) and molecular replacement to determine structures of the α2β5 hormone from Caenorhabditis elegans (Ceα2β5). Ceα2β5 is unglycosylated, as are many other α2β5 hormones. Both Hsα2β5, the human homolog of Ceα2β5, and hTSH activate the same receptor (hTSHR). Despite having little sequence similarity to vertebrate glycoprotein hormones, apart from the cysteine patterns from core disulfide bridges, Ceα2β5 is generally similar in structure to these counterparts; however, its α2 and β5 subunits are more symmetric as compared with α and β of hCG and hFSH. This quasi-symmetry suggests a hypothetical homodimeric antecedent of the α2β5 and αβ heterodimers. Known structures together with AlphaFold models from the sequences for other LGR ligands provide representatives for the molecular evolution of LGR ligands from early metazoans through the present-day GPHs. The experimental Ceα2β5 structure validates its AlphaFold model, and thus also that for Hsα2β5; and interfacial characteristics in a model for the Hsα2β5:hTSHR complex are similar to those found in an experimental hTSH:hTSHR structure.

27 **Haim-Nachum, Shilat**

Postdoctoral Research Fellow, Psychiatry

"Does Disconfirmatory Evidence Shape Safety-and Danger-Related Beliefs in Trauma-Exposed Individuals?"

Recent accounts of predictive processing in posttraumatic stress disorder (PTSD) suggest that trauma-exposed individuals struggle to update trauma-related hypotheses predicting danger, which may be involved in the etiology and the maintenance of this disorder. Initial research supports this account, documenting an association between trauma-exposure, impaired expectation updating, and PTSD symptoms. Yet, no study to date has examined biased belief updating in PTSD using a scenario-based approach. Here, we examined the predictive processing account among trauma-exposed (n = 180, Mage = 47.11) and non-trauma-exposed individuals (n = 41, Mage = 44.12) using a modified Trauma-Related version of the Bias Against Disconfirmatory Evidence task. The task presents both danger-and safety-related scenarios highly relevant for trauma-exposed individuals. For each scenario, participants viewed several explanations and rated their plausibility. Their ability to update their initial interpretation following new-contradictory information was assessed. Results revealed a significant association between belief updating and PTSD symptoms, which was evident for disconfirming both safety and danger scenarios. Current findings support initial evidence that

individuals with higher PTSD symptoms show a higher resistance to update their beliefs upon new disconfirmatory evidence.

28 **Hangai, Sho**
Postdoctoral Research Scientist, Surgery
"An In Vivo CRISPRi Screen That Enables Identification of the Optimal Combination Drug Targets for Antibody Therapeutics"

Antibody therapeutics are becoming to play a pivotal role in systemic therapy for cancer patients. The mechanism of action of these antibodies is mainly antibody dependent cell cytotoxicity (ADCC) mediated by Natural Killer (NK) cells. However, antibody drugs are mostly used in combination with cytotoxic chemotherapeutic agents and possible synergy with other targeted therapies that takes into account the mechanism of action of the antibodies has not been fully explored yet. Here, we are developing an in vivo CRISPR screening platform to discover optimal partners of antibody drugs in the context of the human immune system. We utilize herceptin, an anti-human HER2 antibody and one of the most widely used antibody drugs for establishing proof of concept of this screening. An inducible CRISPR interference (CRISPRi) is employed to mimic clinical settings. Since ADCC requires engagement of NK cells, we use HLA-matched humanized mice. As for library, we will target three different gene modules:genes that are expressed by cancer cells and are known to inhibit NK cell cytotoxicity, DNA damage response (DDR) pathway genes that potentially activate innate immune response and enhance NK cell cytotoxicity, and genes that clinical trial are ongoing to target and hence should have immediate clinical impact. This novel screening platform should enable us to identify the optimal combination drug targets for antibody therapeutics at scale in the context of the human immune system. We will introduce the recent progress in development and optimization of this screening system. We will also discuss the possibility of combining this screening with spatial analysis by employing optical readouts.

29 **Haq, Imdadul**
Postdoctoral Research Scientist, Neurology
"An In Vitro Microglia Model for Studying Functional Genomics of Neuroimmunological Diseases"

Microglia, resident immune cells of the central nervous system (CNS) play critical role in brain development, homeostasis, and neurological diseases. Recent discoveries in genome-wide association studies implicated the importance of microglia in the pathophysiology of neurodegeneration. However, the translation of these genetic discoveries is hindered by the current lack of robust human microglia in vitro model system and standardized protocols for functional genomics assessment. While induced pluripotent stem cell-derived microglia (iMG) hold potential as a cellular model, the current differential protocols are variable, lengthy and give inefficient, mixed yields, limiting our ability to utilize modern gene editing tools in this model (e.g., CRISPR-Cas9) for elucidating the functional consequences of disease-relevant genes and variants. Here we developed a comprehensive toolkit for studying microglia functional genomics in vitro. This toolkit involves a two-step differential protocol that efficiently produces induced pluripotent stem cell-derived microglia (iMG) with improved purity and reduced time requirements.

Through scRNA-seq and mass spectrometry-based protein profiling, we determined that our iMG resemble human adult and fetal microglia but differ from human monocytes. Our newly formed iMG also displayed functional capabilities such as Ca2+ transients, migration, and phagocytosis of CNS substrates such as synaptosomes, myelin, and Aβ1-42. Additionally, our iMG cultures responded appropriately to inflammatory toxins like lipopolysaccharides. Overall, our data demonstrate that the iMG produced through our optimized protocol is pure and possesses many characteristics of human brain microglia. The protocol also includes a sequential harvesting and cell freezing step to allow for scalable production of iMG for high-throughput assays. Additionally, we have incorporated a drug-inducible CRISPR-ON/OFF system into these newly established microglia model, enabling researchers to investigate gene function temporally. The toolkit also provides an online searchable platform for gene expression, proteome, and cytokine/chemokine secretion profiles of the newly established iMG model system: https://jasonchungngo.shinyapps.io/IPSC-derived-Microglia/

30 **Hu, Lucas Zhongming**
Postdoctoral Research Scientist, Systems Biology
"'Elucidating Compound Mechanism of Action and Polypharmacology with a Large-scale Perturbational Profile Compendium"

Precision cancer medicine is predicated on the ability to identify drugs whose mechanism of action (MoA) matches one or more key tumor dependencies. Although the structure of many target proteins is available and their binding affinity to specific compounds can be experimentally assessed, drug efficacy and toxicity are infrequently determined based on single target affinity. Rather, the pharmacologic property of drugs are often the result of its complex polypharmacology, as mediated by both unknown or poorly characterized off-target effects (i.e., lower-affinity binding proteins) as well as by tissue-specific secondary effector proteins (i.e., non-binding) that are undetectable by traditional assays. MoA elucidation typically relies on in vitro affinity binding assays tailored to specific protein classes, such as kinases or metabolic enzymes, which cannot reveal critical high- and low-affinity binding targets among other protein classes, as well as context-specific downstream effectors. The goal of this study is to address critical unresolved questions in cancer pharmacology by characterizing the proteome-wide MoA of oncology drugs as a critical, yet highly elusive step necessary to fully understand their clinical efficacy and toxicity. To elucidate proteome-wide, drug-mediated changes in protein activity (drug MoA), we performed network-based analyses of genome-wide RNA-seq profiles representing the response of patient-matched cell lines to a comprehensive repertoire of clinically relevant drugs. More specifically, we generated genome-wide drug perturbation profiles from 23 cancer cell representing high-fidelity models of patients in clinical cohorts representing distinct tumor subtypes, using > 700 oncology drugs. This represents the largest resources of functionally annotated, clinically-relevant, genome-wide perturbational profiles for clinically relevant drugs, named PanACEA. VIPER-based analysis of this resource effectively elucidated the effect of each individual drug on the activity of ~6,500 regulatory and signaling proteins, in each of the 23 distinct represented cancer subtypes. Analyses of these data, using a graph theory approach, helped elucidate critical functional relationship between individual drugs and group drugs into functionally-distinct modules within each cancer context, thus providing critical insight into both drug MoA and polypharmacology. These analyses also effective drug inhibitors for critical cancer

dependencies represented by transcription factors and co-factors, considered undruggable, that were experimentally validated. Finally, integrative analysis of PANCEA predictions and Cancer Dependency Map data significantly improved our understanding of cancer-related drug pharmacology, thus providing a unique resource for cancer related studies, which has been used in key clinical and pre-clinical trials, both published and in review.

31 **Kesner, Jordan**
Postdoctoral Research Scientist, Medicine & Systems Biology
"Noncoding Translation Mitigation"

Translation is pervasive outside of canonical coding regions, occurring in lncRNAs, UTRs, and introns, especially in aging, neurodegeneration, and cancer. Notably, the majority of tumor-specific antigens are results of noncoding translation. While the resulting polypeptides are often nonfunctional, translation in noncoding regions is nonetheless necessary for the birth of new coding sequences. The mechanisms underlying the surveillance of translation in diverse noncoding regions and how escaped polypeptides evolve new functions remain unclear. Intriguingly, functional polypeptides derived from annotated noncoding sequences often localize to membranes. Here, we integrate massively parallel analyses of over 10,000 human genomic sequences and millions of random sequences with genome-wide CRISPR screens, accompanied by in-depth genetic and biochemical characterizations. Our results show that the intrinsic nucleotide bias in the noncoding genome and in the genetic code frequently results in polypeptides with a hydrophobic C-terminal tail, which is captured by the ribosome-associated BAG6 membrane protein triage complex for either proteasomal degradation or membrane targeting. In contrast, canonical proteins have evolved to deplete C-terminal hydrophobic residues. Our results reveal a fail-safe mechanism for the surveillance of unwanted translation from diverse noncoding regions and suggest a possible biochemical route for the preferential membrane localization of newly evolved proteins.

32 **Kwon, Soon Bin**
Postdoctoral Research Scientist, Neurology
"Exploring the Relationship between Cerebrospinal Fluid Output and changes Ventricular Size for Shunt Dependency Prediction"

Acute hydrocephalus often complicates brain injury including and subarachnoid hemorrhage (SAH), requiring emergent placement of an external ventricular drain (EVD). The EVD allows rapidly accumulated blood and cerebrospinal fluid (CSF) to exit, immediately relieving increased pressure on the brain. There is great variability in the management of EVDs across centers and conflicting recommendations in the literature about when to wean EVDs. Accurately determining ventriculoperitoneal shunt (VPS) dependency or EVD liberation as early as possible can minimize duration of drainage and length of stay at intensive care unit. Our central hypothesis is that temporal information related to CSF hydrodynamics is reflective of intracranial dynamics that contains information about shunt dependency.

Patients were divided into VPS-dependent (VPS+) and independent (VPS-) groups. The bicaudate index (BCI) was measured in all available computed tomography scans of patients. The change in BCI (ΔBCI) from different scans overtime was calculated. Relationship

between ΔBCI and CSF output was analyzed using Pearson's correlation over a 7-day period post EVD placement. Time-varying feature was extracted based on the analysis. Based on the relationship between ΔBCI and CSF output, we developed a K-nearest neighbor model to classify VPS. and VPS-.

A total of fifty-eight patients were included in the study. The correlation between ΔBCI and CSF output was negative on day-1 for both groups and stayed negative for 7 days for VPS+ group. For VPS- group, the correlation became positive starting from day-2 and stayed positive until day-7. The relationship between the two group was significantly different between day-4 to 6 (p<0.05). A weighted k-nearest neighbor model for classification had a sensitivity of 0.75, a specificity of 0.70, and an area under the receiver operating characteristic curve of 0.80.

This study showed relationship between ΔBCI and CSF output can be used as a biometric for VPS dependency prediction after SAH.

33 **Lee, Eunhyeong**
Postdoctoral Research Scientist, Pathology and Cell Biology
"Angiopoietin-2 Blockade Suppresses Growth of Liver Metastases from Pancreatic Neuroendocrine Tumors by Promoting T-cell Recruitment"

Pancreatic neuroendocrine tumors (PanNETs) with liver metastases are associated with poor survival, with liver metastases accounting for the majority of the mortality. Our study focused on identifying potential strategies to improve the management of PanNET liver metastases, and found that angiopoietin-2 (ANGPT2) was one of the most upregulated angiogenic factors in RNA-seq data from liver metastases of human PanNETs. We also identified that ANGPT2 expression correlated with poor survival, and was restricted to the endothelial cells of blood vessels in PanNET liver metastases. Additionally, upregulation of endothelial ANGPT2 had a consistent association with liver metastatic progression in patients and transgenic mouse models with PanNETs. Interestingly, ANGPT2 upregulation was also accompanied by suppression of T-cell infiltration and activation in human and mouse PanNET liver metastases, suggesting an immunosuppressive environment. Importantly, it was shown that pharmacologic inhibition and genetic deletion of ANGPT2 reduced the growth of PanNET liver metastases. This inhibition also promoted T-cell infiltration and activation and prolonged the survival of mice with metastatic PanNETs. Moreover, ANGPT2 blockade coincided with reduced plasma leakage and improved vascular integrity in metastases. Our findings suggest that ANGPT2 blockade may be a promising strategy for reducing the growth of liver metastases in PanNETs. By promoting T-cell infiltration and immunostimulatory reprogramming, ANGPT2 inhibition could help create an environment that is hostile to cancerous cells. Together, these findings provide evidence for ANGPT2 blockade as a strategy for promoting T-cell infiltration and immunostimulatory reprogramming to reduce the growth of liver metastases in PanNETs.

34 **Malik, Vikas**
Postdoctoral Research Scientist, Medicine
"Investigating the Restorative Functions of Sleep in Neural Stem Cells and Regeneration"

Despite sleep being an essential non-negotiable and conserved bodily function, human beings are the only mammals that actively avoid sleep in favor of other activities, e.g., work productivity or consumption of electronic media before bedtime, which delay sleep onset, reduce sleep duration, and compromise sleep quality. Women may need longer sleep duration than men and are more likely to suffer from anxiety and depression, which may also be related to their 40% higher prevalence of insomnia, although causal evidence is lacking and underlying mechanisms are unclear. We hypothesized that prolonged sleep restriction may negatively affect stem cells' regeneration potential by factors released in blood plasma, which act through a molecular cascade leading to defective differentiation of adult stem cells. Utilizing plasma samples from a clinical trial involving healthy women with a randomized crossover design of extended, objectively monitored sleep restriction (delaying bedtime by 1.5 hours over their regular schedule) that mimics "real-life" derangement of sleep duration (ClinicalTrials.gov NCT02835261), we performed metabolomic and proteomic profiling of the samples. We identified metabolites and proteins differentially enriched in adequate and restricted sleep conditions. Joint network pathway analysis showed enrichment of terms like long-term potentiation and depression, neurotrophin signaling, circadian entrainment, glutamatergic synapse and glutamine and glutamate metabolism under restricted sleep condition, highlighting a strong link between sleep restriction and neuronal development phenotypes. Using neural stem cells (NSCs) differentiated from human embryonic stem cells, we found treatment with metabolic candidates enriched in sleep restriction led to aberrant G1 phase of cell cycle, and defective differentiation of NSCs into neurons, astrocytes, and oligodendrocytes. We speculate that alterations in sleep can influence the daily dynamics of NSC divisions, and our study may shed light on how sleep shapes adult stem cell functions in neural development.

35 **Mihali, Andra**
Postdoctoral Research Fellow, Psychiatry
"Introspective Inference Counteracts Perceptual Distortion"

Introspective agents can recognize the extent to which their internal perceptual experiences deviate from the actual states of the external world. This ability, also known as insight, is critically required for reality testing and is impaired in psychosis, yet very little is known about its cognitive underpinnings. We developed a Bayesian modeling framework and a novel psychophysics paradigm to quantitatively characterize this type of insight while participants experienced a motion after-effect (MAE) illusion. Across two experiments with 44 participants total, we found that participants could compensate for the illusion when judging the actual direction of a motion stimulus. In a second experiment (N = 22 observers), a parametric choice of the test stimuli allowed us to fit Bayesian model variants jointly to the participants' responses and confidence reports. Confidence, reaction-time, and pupil-dilation data all showed signatures consistent with inferential adjustments in a Bayesian insight model. Our results suggest that people can question the veracity of what they see and make insightful inferences that incorporate introspective knowledge about internal distortions.

36 **Murphy, Michael**
Associate Research Scientist/Scholar, Medicine
"Defective Ribosomal Protein Paralog Switching Causes Neonatal Heart Failure"

Ribosomes are ubiquitous throughout the body and are composed of largely the same rRNAs and ribosomal proteins (RPs). Heart and skeletal muscle cells, however, express a vertebrate-specific paralog of RPL3, RPL3L. Recent case studies have highlighted compound heterozygosity for rare recessive missense mutations in RPL3L leading to neonatal heart failure in the absence of transplant, but the biochemical mechanisms leading to disease are unclear.

Knockout mouse studies have demonstrated that loss of RPL3L leads to an upregulation of RPL3, compensating for RPL3L function in young mice. Remarkably, knock-in homozygotes of one of the patient mutations, p.Arg161Trp, also led to compensation in mice. Analysis of patient heart tissue suggested that compensation was not taking place, creating a discrepancy between knock-in mouse and human patient. Thus we hypothesized that a subset of recessive mutations have dominant effects in genetic backgrounds expressing another mutant RPL3L allele.

To explore this, we generated cell lines expressing each of the six mutations identified in patients in an RPL3 knockdown background, and analyzed the cells for translational capacity, rRNA processing and protein localization. We found that Arg161Trp leads to compensation of RPL3 in cell line, whereas other mutations have toxic effects beyond that of an RPL3 knockdown alone. Mass spectrometry of interaction partners for RPL3L mutations also show similarities between toxic mutants compared to Arg161Trp.

Overall, we describe a nuanced picture of genetic interactions for a tissue-specific paralog with relevance in understanding the mechanism of neonatal heart failure with rare etiology.

37 **Nguyen, Trang**
Postdoctoral Research Scientist, Pathology and Cell Biology
"Loss of Function of CDK7 Is Synthetically Lethal with Fatty Acid Oxidation Inhibition in Glioblastoma"

CDK7 has been identified as a potential drug target for glioblastoma (GBM), a highly lethal primary brain tumor. However, resistance to therapy develops quickly, which may be facilitated by drug-induced reprogramming of metabolism. By combination of a transcriptome and metabolite screening analyses followed by carbon tracing (U-13C-Glucose, U-13C-Glutamine and U-13C-Palmitic acid) and extracellular flux analysis, we demonstrated that both genetic and pharmacological (YKL-5-124 and THZ1) CDK7 inhibition elicited substantial metabolic reprogramming. Specifically, CDK7 inhibition elicited an increase of oxygen consumption rate fueled by enhanced fatty acid oxidation (FAO) manifested by enhanced labeling of citric acid cycle intermediates from palmitic acid. Consistently, the combination treatment of CDK7 inhibitors with blockers of FAO (etomoxir) exerted substantial synergistic growth inhibition in patient derived xenograft as well as neurosphere GBM cultures, which was mainly driven by a collapse of oxidative energy metabolism. In turn, exogenous administration of adenosine triphosphate partially rescued from the cell death induced by the combination treatment. Finally, the combined administration of YKL-5-124 and etomoxir extended overall in an orthotopic patient-derived xenograft model of

GBM. In summary, these data support that simultaneous targeting of CDK7 and FAO might be a potential novel therapy against GBM.

38 **Qian, Jin**
Associate Research Scientist/Scholar, Medicine
"MDSC-targeted TFF2-MSA Synergizes with PD-1 Blockade Therapy in Advanced Gastric Cancer Models"

Recent studies revealed chemotherapy increases anti-PD1 response of gastric cancer (GC) by reducing tumor myeloid-derived suppressor cell (MDSC). However, a more potent MDSC-targeted treatment is needed to further improve anti-PD1 efficacy in advanced GC. Trefoil factor family 2 (TFF2), a partial agonist of CXCR4 and a secreted anti-inflammatory peptide, can decrease MDSCs. Here, we developed a novel peptide TFF2-MSA with an extended serum half-life by fusing murine TFF2 to murine serum albumin. Using a syngeneic mouse model of transplanted ACKP (Atp4b-Cre; Cdh1-/-; LSL-KrasG12D; Trp53-/-) GC cells, we investigated whether TFF2-MSA can synergize with anti-PD1 therapy by reducing MDSC accumulation and biogenesis. When the subcutaneously implanted ACKP tumors reached 150 mm3, TFF2-MSA or anti-PD-1 antibody or both was given to tumor-bearing mice. Intriguingly, while either TFF2-MSA or PD-1 antibody showed little benefit as a single agent (TGI 15% and 25% respectively, p>0.05), their combination dramatically suppressed ACKP s.c. tumor growth (TGI 78%, p<0.0001) and prolonged mouse median survival (64 days vs. 32.5 days in control) in a synergistic manner. Mechanistically, the combination therapy efficiently reduced intratumoral MDSCs by 67%, and profoundly increased tumor-infiltrating CD8+ T cells by 18 fold accompanied by increased TCF1+ stem-like T cells in TDLNs. In the bone marrow, the MDSC biogenesis from its progenitors was markedly decreased by TFF2-MSA or the combination, to a similar level of tumor-free mice. In an orthotopic model that ACKP-luc cells were implanted to stomach submucosa, the TFF2-MSA/PD-1 combo regimen eradicated GC in 80% mice compared to 0% in either monotherapy treatment. Finally, the combination significantly reduced 80% spontenous lung metastasis in s.c. xenograft resected mice (vs. control, p<0.0001), compared to minimal inhibition with either monotherapy (p>0.05). As a whole, our data indicated that targeting MDSCs using TFF2-MSA synergizes with PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of GC.

39 **Qu, Wenhui**
Postdoctoral Research Scientist, Pathology
"Examining Disease-associated Roles of Neurons in Alzheimer's Disease Using a Novel Stem-cell-based Human-mouse Chimeric Transplantation Model"

Alzheimer's disease (AD) and AD-related dementias (ADRD) are fatal neurodegenerative diseases that impair the cognitive function of more than 40 million people worldwide without effective treatments. Despite the high prevalence of AD and ADRD, molecular mechanisms of neurodegeneration are only partially understood. Emerging evidence supports the physiological relevance of using induced pluripotent stem cells (iPSC) to model AD and related dementias. This study sets out to determine cell-intrinsic changes in AD iPSC-derived neurons in vitro and in vivo. We have differentiated human iPSC-derived neural cells carrying the familial AD V717I (London) mutation in the amyloid precursor protein (APPV717I) into

mature neurons in vitro and employed whole transcriptomic profiling, immunostainings, and biochemical assays. We have also transplanted human AD iPSC-derived neural cells into the brains of adult immunocompromised mice followed by histological analysis. We then microdissected human grafts and employed single nucleus RNA sequencing (snRNA-seq) to better understand the transcriptomic changes in transplanted cells at single-cell resolution. Our results show cell-intrinsic changes in AD iPSC-derived neurons in vitro, including Aβ42- and TAU-related AD pathological changes, impairment of neurite outgrowth, and an increased vulnerability to oxidative stress. We successfully transplanted human iPSC-derived AD neural cells into the brains of adult mice and show the feasibility of modeling AD using this innovative in vivo human stem cell model. Histological and snRNA-seq analyses revealed reduced neuronal survival and transcriptional alterations of transplanted AD cells. Collectively, these data provide invaluable insights into pathological changes in AD cells and provide a novel stem-cell-based human-mouse chimeric transplantation model to further interrogate the molecular mechanisms of AD that may potentially open a new therapeutic avenue for treating AD.

40 **Raghuraman, Radha**
Postdoctoral Research Scientist, Pathology
"Impact of Locus Coeruleus Tau Pathology in Downstream Hippocampus on Spatial Memory"

The reason for tau emergence and accumulation in LC (Locus Coerelus) in young adults has been unclear despite the evidence of tau pathology originating rather in entorhinal cortex (EC). It is established that LC subserves a pivotal function in attention and memory, being the fulcrum for arousal and wakefulness/sleep regulation. APPNL-G-F, which have been shown to have physiological levels of APP in the brain were probed to pin down alterations if any, of electrophysiological properties of spatial cells in HPC (Hippocampus) region, as a result of tau spread in LC. Our preliminary data from single unit recording indicates that accumulation of tau facilitates impairments in spatial memory rendered by altered remapping that seems to be consistent with the changes in the environment as seen in the SLR (spontaneous location recognition) and NOR (novel object recognition) tasks. Altered firing properties is reflective of the limited accuracy in decoding spatial behavior mapping in neurons. Furthermore, the LFP (local field potential) measurements in the area CA1 in HPC region shows impairments in sleep recordings in comparison to control, tying it back possibly to the mechanistic deterrence in circadian rhythm brought about by the accumulation of tau tangles in LC. This in turn may substantiate EEG as a useful biomarker for early diagnostics of tauopathy in its vulnerability towards a specific brain region in the Alzheimer's condition and to identify changes not known thus far.

41 **Rodriques, Aaron**
Postdoctoral Research Scientist, Medicine
"Dysregulation of Cardiomyocyte Calcium Homeostasis with Overexpression of Heart Failure Proteome-Identified Galectin-1"

The calcium channel CaV1.2 mediates cardiac excitation contraction and β-adrenergic-driven increases in contractility. In heart failure (HF) abnormal calcium regulation occurs at CaV1.2

with consequences for contractility and arrhythmia. In our peroxidase-based screen of the cardiac CaV1.2 proteome microdomain in mice with HF, we identified enrichment of galectin-1 (gal-1).

We hypothesize that overexpression of gal-1 in cardiomyocytes reproduces the abnormalities of calcium handling noted in HF. We generated mice with cardiac-specific, tamoxifen-inducible overexpression of FLAG-tagged gal-1 through knock-in at the ROSA26 locus. After tamoxifen induction, we found a 3.5-fold increase in total gal-1 expression in homozygous knock-in mouse hearts compared to wild-type littermate controls. Though gal-1 mice had normal CaV1.2 currents, they also had larger calcium transients, greater diastolic calcium concentration, and more frequent extrasystolic events than wild-type mice after isoproterenol treatment. Transient analysis also identified a faster rate-constant of the calcium pump SERCA2, essential for myocyte relaxation.

There were not significant changes in protein levels of SERCA2 or the sodium/calcium exchanger in gal-1 mice, but there was, however, relatively high expression of S16/T17 phosphorylated- phospholamban (PLN) in gal-1 mice. Phosphorylation of PLN at S16/T17 increases SERCA activity. After examining calcium handling at the subcellular level, we found that, just as in cells of patients with HF, myocytes from gal-1 mice had more, and higher-peaking, spontaneous SR calcium release events compared to wild-type mice.

We find gal-1 mice have normal calcium currents but curiously increased SR calcium stores. The increased spontaneous calcium release events and extrasystoles are both hallmarks of calcium dysregulation noted in HF. One potential explanation for these phenomena is that excessive phosphorylation of PLN in gal-1 mice and subsequent overloading of the SR increases ryanodine open-probability and pathological calcium release.

42 **Saliba, Maelle**

Postdoctoral Clinical Fellow, Pathology and Cell Biology

"Delineating Invasive Mucinous Adenocarcinomas (IMA) Within the Spectrum of Pulmonary Adenocarcinomas with Mucinous Features (PAM): Refinement of Cytomorphologic Features and Correlation with Genomic Profiles"

"Background:
PAM encompass a heterogeneous group of adenocarcinomas with intracytoplasmic mucin and present as either purely mucinous or mixed tumors. IMA represent an uncommon and distinct tumoral subtype within PAM which classically displays low-grade cytology and frequently harbors KRAS oncogenic driver mutations. We aimed to refine the architectural and cytomorphologic features of IMA within the PAM spectrum while correlating with potential specific genetic alterations.

Methods:
Institutional records (2016-2022) were retrospectively reviewed for surgically resected PAM with available targeted next-generation DNA and RNA sequencing (NGS). PAM were divided into pure (mucinous features ≥90%) or mixed (mucinous component ≥10%) subgroups. High-grade architectural (≥20% solid, micropapillary, and/or complex glandular

patterns) and cytologic features (low versus high-grade) were evaluated and compared against molecular profiles.

Results:
Forty-eight PAM met the criteria, of which 32 were pure mucinous and 16 were mixed. NGS identified mutually exclusive mitogenic driver alterations in 83.3% (n=40) of cases and gene fusions in 10.4% (n=5) [Table 1]. Overall, KRAS mutations were present in 75.0% (n=36), with KRAS codon 12 mutations in 66.7% (n=32). Pure mucinous tumors were associated with low-grade architectural and cytologic features (90.6%, p<0.001) and were significantly more likely to harbor KRAS oncogenic driver mutations (93.8%, p<0.001), particularly affecting codon 12 (90.6%, p<0.001) [e.g. p.G12D (34.4%), p.G12V (28.1%), and p.G12C (15.6%)]. Inversely, mixed tumors were associated with high-grade architectural (87.5%, p<0.001) and cytologic features (62.5%, p<0.001) and significantly correlated with a KRAS wild-type status (62.5%, p<0.001). Fusions were identified in 41.7% (n=5) of the 12 KRAS wild-type cases.

Conclusion:
In general, pure and mixed PAM have divergent genomic profiles and a contrasting prevalence of high-grade features. Pure mucinous tumors fulfilling classic low-grade architectural and cytologic features of IMA harbor KRAS codon 12 driver mutations in the vast majority of cases. Integrating architectural and cytological features into the essential diagnostic components of IMA within the PAM spectrum could further increase the therapeutic relevance of these diagnoses by delineating tumoral subtypes with divergent biologic profiles and targetable alterations.

43 **Sariyeva, Mehriban**
Associate Research Scientist/Scholar, Neurology
"Clinical and Radiological Features of Intracerebral Hemorrhage in Pregnant and Postpartum Patients"

Objective:
To characterize intracerebral hemorrhage (ICH) in pregnant and postpartum patients.

Background:
ICH is a leading cause of maternal morbidity and mortality. Detailed data are limited regarding clinical and radiological features of ICH in pregnancy and postpartum.

Design/Methods:
We retrospectively reviewed medical records and brain imaging from pregnant or postpartum patients admitted to the neurological intensive care unit at one center with ICH between 1/01/2012 and 12/31/2021. We described clinical characteristics and ICH radiological features including presence of a culprit vascular lesion, and deep or lobar location in those patients without vascular lesions (primary ICH).

Results:
A total of 26 patients were identified (median age 34 years, IQR 6), of whom 11 (42%) were pregnant and 15 (58%) were postpartum. Among pregnant patients, 2 (18%) ICH occurred

in the 1st trimester, 6 (55%) in the 2nd, and 3 (27%) in the 3rd, with median gestational age of 22 weeks at time of the index event. Among postpartum patients,67 % of events occurred within 1 week after delivery.

Radiological features were as follows: 8 (31%) had a culprit vascular lesion identified (6 AVM, 1 aneurysm, 1 cavernous malformation), and 3 (11%) had hemorrhagic venous infarction. Of the 15 primary ICH, 10 (67%) were lobar (frontal, parietal or occipital); 2 (13%) were deep (cerebellar, brainstem, thalamus or basal ganglia), and 3 (20%) had both lobar and deep hemorrhage. Among patients with primary ICH, none had pre-existing hypertension, 4 (27%) had pre-pregnancy obesity, 1 (7%) had pre-gestational diabetes, and 9 (60%) had preeclampsia/eclampsia. Among patients with preeclampsia/eclampsia, 6 had lobar hemorrhages, 2 had deep hemorrhages and 1 had both lobar and deep hemorrhages.

Conclusion: Among pregnant or postpartum patients admitted to an academic medical center with ICH, the majority 10 (67%) had no culprit vascular lesion. These hemorrhages were predominantly lobar and occurred in patients with and without preeclampsia/eclampsia, suggesting an underlying pregnancy-related small vessel vasculopathy.

44 **Schilperoort, Maaike**
Postdoctoral Research Scientist, Medicine
"Efferocytosis Induces a Transient Burst of Glycolysis in Macrophages to Promote Lactate-driven Binding and Removal of Apoptotic Cells"

"Aim:
Resolving "M2-like" macrophages prevent chronic inflammation by clearing apoptotic cells through efferocytosis. These macrophages are thought to rely mainly on oxidative phosphorylation, but emerging evidence suggests a link between efferocytosis and glycolysis. To reconcile this apparent contradiction, we aimed to investigate molecular–cellular mechanisms involved in efferocytosis-induced macrophage glycolysis and its consequences.

Results:
We found that efferocytosis promotes a transient increase in macrophage glycolysis that is dependent on rapid activation of the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 (PFKFB2), which distinguishes this process from more-prolonged glycolysis in pro-inflammatory "M1-like" macrophages. Mice transplanted with activation-defective PFKFB2 bone marrow and then subjected to dexamethasone-induced thymocyte apoptosis exhibit impaired thymic efferocytosis, increased thymic necrosis, and lower expression of the efferocytosis receptors MerTK and LRP1 on thymic macrophages compared with wild-type control mice. In vitro mechanistic studies revealed that glycolysis stimulated by the uptake of a first apoptotic cell promotes continual efferocytosis through lactate-mediated upregulation of MerTK and LRP1. We next turned to a human-relevant model of inflammation resolution, i.e., the regression of atherosclerosis that occurs with marked plasma cholesterol lowering. Hypercholesterolemic mice with activation-defective PFKFB2 bone marrow show defective regression of atherosclerosis after cholesterol lowering, which coincided with a reduced clearance of apoptotic cells by lesional macrophages.

Conclusions:
Efferocytosis-induced macrophage glycolysis represents a unique metabolic process that sustains continual efferocytosis in a lactate-dependent manner. The differentiation of this process from inflammatory macrophage glycolysis raises the possibility that it could be therapeutically enhanced to promote efferocytosis and resolution in chronic inflammatory diseases, including atherosclerotic cardiovascular disease."

45 Withdrawn

46 Shin, Alice

Postdoctoral Research Scientist, Medicine (HICCC)

"LIN28B Promotes Cell Invasion and Colorectal Cancer Metastasis via the Induction of CLDN1 and NOTCH3"

Colorectal cancer (CRC) remains a significant public health concern, with a 5-year survival rate of only 14% for stage 4 patients. The RNA-binding protein LIN28B is overexpressed in more than 30% of CRC patients and is associated with poor prognosis. In this study, we uncover a novel mechanism by which LIN28B regulates colonic epithelial cell-cell junctions and CRC metastasis.

Using human CRC cell lines (DLD-1, Caco-2, and LoVo) with either knockdown or overexpression of LIN28B, we identified Claudin 1 (CLDN1) tight junction protein as a direct downstream target and effector of LIN28B. RNA immunoprecipitation revealed that LIN28B directly binds to and post-transcriptionally regulates CLDN1 mRNA. Further exploration of this LIN28B-CLDN1 interaction using in vitro assays and a novel murine model of metastatic CRC demonstrated its critical role in enhancing collective invasion, cell migration, and metastatic liver tumor formation. Bulk RNA-sequencing of metastatic liver tumors revealed the involvement of both the NOTCH and PI3K/Akt pathways as potential downstream effectors of the LIN28B-CLDN1 axis. Subsequent genetic and pharmacologic manipulations of NOTCH3 signaling confirmed its essential role in LIN28B-mediated invasion and metastatic liver tumor formation. Further protein analyses unveiled a functional relationship between NOTCH signaling and the PI3K/Akt pathway. Notably, the administration of Alpelisib, an FDA-approved PI3K inhibitor, significantly inhibited the formation of CRC liver metastases.

Our study reveals a novel LIN28B-CLDN1-NOTCH3 signaling axis in CRC metastasis and highlights the interconnected roles of NOTCH and PI3K/Akt pathways in this process. This discovery opens new avenues for therapeutic intervention in metastatic CRC, addressing an unmet need in an area where treatment advancements have been limited.

47 Thomas, Aline

Postdoctoral Research Scientist, Neurology

"Reduced Pace of Biological Aging Explains the Association of Diet with Dementia: Results from the Framingham Offspring Study"

Background:
Substantial evidence supports the benefice of healthy diet for brain aging; however, the underlying pathways remain largely unknown. Leveraging a unique cohort design with assessment of long-term dietary habits, DNA methylation data, and 10-year follow-up for dementia, we evaluated the mediating role of biological aging on the relationship of diet with dementia. We explored the specificity of this pathway for brain aging, as compared to overall aging, by investigating mortality.

Methods:
We included 1,525 participants (≥60y, without dementia at baseline [2005-2008]) from the Framingham Offspring Cohort. We evaluated long-term adherence to Dietary Guideline for Americans by averaging dietary scores across three assessments (1991-1995, 1998-2001, and 2005-2008). Using DNA methylation data at baseline, we measured the pace of biological aging through DunedinPACE epigenetic clock, which was the DNA methylation measure the most strongly associated with dementia in prior studies. Incidences of dementia and mortality were adjudicated from baseline until 2018. We performed mediation analyses to estimate the role of pace of biological aging on the relationship between diet and dementia or mortality.

Results:
Over the follow-up (maximum, 13.8y), 129 participants developed dementia and 432 died. Participants with a higher adherence to dietary guidelines had a slower pace of biological aging, and lower risks of dementia and mortality. A faster pace of aging was associated with increased risks of dementia and mortality. Biological aging mediated 15% of the association between diet and dementia (p=0.003), and 39% of the association of diet with mortality (p<0.001).

Conclusion:
Part of the association of healthier diet with lower risk of dementia is mediated by a slowing of global processes of biological aging. However, this pathway does not fully explain the association and the proportion mediated was 2-3 times smaller than what was observed for mortality, suggesting that other brain-specific mechanisms need to be further described.

48 **Wang, Qian**
Associate Research Scientist/Scholar, Ophthalmology
"The Lens-corneal Separation Requires Precision Control of Hippo-Yap Signaling"

Purpose:
The Hippo-Yap signaling pathway is implicated in tissue development and homeostasis. In the lens, Yap is required for the maintenance of the lens progenitor pool, but it is not clear whether this is due to Yap's function as a transcriptional activator or as part of the cell junction complex. In this study, we aim to investigate the role of Hippo-Yap signaling network and its mechanism in regulating murine lens development.

Methods:
We generated both conditional knockout and conditional transgenic mouse models using Le-cre, which is specifically expressed in the lens progenitors. Immunofluorescence was performed to characterize the lens phenotype.

Results:
Consistent with a previous study, genetic ablation of Yap resulted in substantial loss of the lens epithelium (LE) compartment at embryonic day 13 (E13). This LE defect could be rescued by expression of nuclear Yap (Yap5SA), which is resistant to serine phosphorylation induced by Hippo signaling. In addition, expression of nuclear Yap led to ectopic expression of LE markers in the lens fiber compartment in a cell autonomous manner, suggesting that the nuclear but not the membrane function of Yap is responsible for maintenance of the lens progenitor property. Interestingly, the Yap5SA-expressing lens remained connected to the surface ectoderm (which later develops into the cornea) and formed persistent lens stalk, indicating that Yap signaling is involved in the lens vesicle separation. Moreover, genetic ablation of Hippo signaling kinases mammalian STE20-like kinase (MST) and large tumor suppressor (LATS) recapitulated Yap gain of function (Yap5SA) lens stalk phenotype.

Conclusions:
Our study demonstrated that the nuclear function of Yap is both necessary and sufficient to maintain the lens progenitor pool. However, the level of active nuclear Yap must be tightly controlled by Hippo Signaling to ensure separation of the lens vesicle from the surface ectoderm. Suppression of Hippo signaling leads to the lens-corneal separation defect, which mimics Peters Anomaly in humans, suggesting that the Hippo-Yap signaling may underlie congenital lens disease.

49 **Yamamoto, Wataru**
Associate Research Scientist/Scholar, Biological Sciences
"Neuronal Mechanisms of Somersaulting in *Hydra vulgaris*"

Hydra vulgaris is a freshwater cnidarian that locomotes by somersaulting: an acrobatic maneuver performed by attaching the tentacles to the substrate and swinging the foot over the head to stand in a new position. Although *H. vulgaris'* somersaulting was already described as early as the 18th century, how a distributed nervous system of a few hundred neurons can exercise sensory-motor coordination to achieve such sophisticated behavior still remains a mystery.

To understand the neuronal mechanisms of somersaulting, we used *H. vulgaris* expressing the calcium indicator GCaMP6s, to image the entire neuronal and muscle activity during somersaulting. At the start of somersaulting, we found that the activity of the rhythmic potential 1 (RP1) circuit, an ensemble of synchronously firing neurons distributed throughout the body, significantly increased (RP1 burst: >0.5 Hz). During RP1 bursts, the activity of basal disc muscles and nematocytes on the tentacles also increased, which corresponds to the tentacle attachment and foot detachment of the somersaulting steps.

To elucidate the causal relationship between the RP1 circuit and somersaulting, we altered RP1 activity. Firstly, increasing medium osmolarity decreased RP1 activity (<0.5 Hz) and suppressed somersaulting. Secondly, targeted optical ablation of RP1 neurons using a two-photon laser also led to the suppression of somersault behavior. Our results indicate that the

RP1 circuit of *H. vulgaris* implements integrate-to-threshold decision-making to control body motion in precise order and timing, leading to somersaulting.

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