Graduate Research Symposium 2026

Poster Session Programming

11:00a.m. – 12:00p.m.

Department

Developmental and Cell Biology

Distinct Roles of AMPK Isoforms in Controlling Nutrient Dependent Primordial Germ Cell Quiescence in C. elegans

Shabnam Moghareh, Grad Student Lara-Gonzalez Lab

Germline development begins early in embryogenesis with the specification of primordial germ cells (PGCs). In several organisms, PGCs undergo noncanonical quiescence at the G2 cell cycle stage. In Caenorhabditis elegans, this quiescence is nutrient dependent. Upon hatching into the first larval stage (L1) without food, the two PGCs remain quiescent, while food triggers cell cycle re-entry and proliferation. AMPK, an energy-sensing kinase, is required to maintain PGC quiescence during starvation. Loss of AMPK results in inappropriate proliferation in the absence of food. C. elegans expresses two isoforms of the AMPK catalytic subunit, named AAK-1 and AAK-2. However, the tissue-specific roles of AAK-1 and AAK-2 and their downstream mechanisms remain unclear. To determine the requirement for AAK-1 and AAK-2 in maintaining PGC quiescence, we used an auxin-inducible degron (AID) system enabling tissue-specific depletion. We tagged the endogenous copies of AAK-1 and AAK-2 with mChilada and the AID degron using CRISPR-Cas9. These tagged proteins were functional, although partially compromised. We found that in arrested L1 larvae, AAK-1 is strongly expressed in PGCs, with signal also detected in the neighboring somatic gonad precursor cells (SGPs). In contrast, AAK-2 appears to be expressed broadly but is excluded from PGCs. Using the AID system for tissue-specific degradation, we found that AAK-1 functions to promote quiescence in the germline, as germline-specific depletion of AAK-1 is sufficient to trigger inappropriate PGC proliferation during starvation. In contrast, germline-specific depletion of AAK-2 does not disrupt quiescence. Furthermore, somatic depletion of AAK-1 does not disrupt quiescence when AAK-2 is present but leads to inappropriate PGC proliferation in an aak-2 null background. However, somatic depletion of AAK-2 in an aak-1 null background results in inappropriate PGC proliferation. Together, these results suggest the existence of two AMPK-dependent pathways that ensure PGC quiescence: a germline, cell-autonomous pathway that works through AAK-1; and a somatic, cell non-autonomous pathway that works primarily through AAK-2. We are currently investigating whether TORC1, a cell proliferation

mediator whose inhibition by AMPK promotes quiescence, works in a cell-autonomous or cell-non-autonomous manner. Overall, our work reveals how somatic and germline AMPK signaling pathways coordinate to regulate germline quiescence.

Enabling lead optimization of sphingosine mimetics to overcome drug resistance

Sarah Valles, Postdoc Edinger Lab

Targeted therapies and chemotherapies are toxic and fail within 3-12 months. Drug resistance arises from tumor heterogeneity, new mutations, and epigenetic changes that reduce drug uptake or bypass targeted pathways. Drug-like sphingosine (SO) mimetics can be a part of new strategies to kill drug-resistant tumor cells while minimizing drug toxicity and the development of drug resistance in patients. SH-BC-893, the conformationally constrained FTY720 analog, exhibits broad anti-cancer effect without these liabilities. Rigorous target deconvolution revealed that SO and 893 bind five structurally related proteins: PPP2R1A, (PP2A scaffolding subunit), and four importins (KPNB1, TNPO1, IPO5, IPO7). SH-BC-893 binds proteins in these parallel pathways to enact its anti-cancer program: to activate the tumor suppressor PP2A and inhibit importin-mediated nuclear trafficking of oncogenic cargo. However, pharmacological limitations make 893 a lead compound rather than a clinical drug candidate. Here, we work to enable the structure-driven design of optimized drug-like sphingosine mimetics by mapping the 893 binding site on PPP2R1A and importins to identify shared structural features that can be used to guide rational, structure-driven design of next generation safe and effective cancer therapeutics capable of overcoming drug resistance.

Genome-wide analysis of mRNA expression and modifications profiles in FSHD cell lines

Negar Mojgani, Grad Student Mortazavi Lab

Myogenesis is orchestrated by precise pre- and post-transcriptional regulation. Dysregulation of these mechanisms drives RNA metabolic defects that contribute to many muscle diseases, causing structural instability, metabolic dysfunction, and loss of muscle mass and function. RNA modifications are widespread regulators of gene expression, influencing alternative splicing, polyadenylation, RNA stability, and other co- and post-transcriptional processes that shape protein levels and phenotype. In skeletal muscle, reversible modifications such as m6A and m5C, along with irreversible changes including A-to-I editing, pseudouridylation, and 2′-O-methylation, regulate myogenesis, homeostasis, and regeneration. We used ONT bulk long-read direct RNA sequencing to characterize the epitranscriptomic landscape of control and engineered facioscapulohumeral dystrophy (FSHD) human skeletal myotubes. Preliminary analysis of FSHD targets identified more than 20 genes with disease-specific differential modification patterns at individual sites, with ZSCAN4 and H3Y1 exhibiting markedly distinct modification profiles between mutant and control samples. Ongoing analyses will investigate transcriptome-wide modification differences, including non-FSHD disease-specific profiles, and will leverage genomic DNA isolated from these conditions for Fiber-seq to generate a more comprehensive view of overlapping epigenomic and epitranscriptomic changes between control and mutant states.

Epidermal and ECM Damage Following Pinch Injury Restricts Dendrite Regeneration in Drosophila

Mia Brantley, Grad Student Thompson-Peer Lab

Neuronal dendrites can be injured by a number of insults, but the cellular mechanism by which dendrites respond to tissue injury and undergo repair is poorly understood. Much of the field’s progress has evaluated dendrite regeneration following laser injury. While precise, laser injury does not accurately model the real-world damage to surrounding tissue that would accompany neuronal injury. Here, we modify a pinch injury technique to injure both the dendrites and their surrounding tissues in Drosophila melanogaster larvae, more similar to what is observed in real-world injury. We refined this technique such that only half of a sensory neuron’s dendrites are injured, leaving the other half uninjured. Our data indicate that both dynamic and stable dendritic arbors regrow dendrites following pinch injury. Neurons primarily engage in compensatory regeneration whereby new branches are added on the uninjured half of the arbor. Comparing the regenerative response following pinch versus laser injury revealed that dendrites preferentially regrew into areas where the surrounding tissue was left intact, and not into areas where the surrounding tissue was damaged by pinch. These results prompted us to evaluate the damage sustained to surrounding tissue. In examining non-neuronal tissues after pinch injury, we found damage to epidermal cells and the

ECM, but not glia. We also observed a robust immune response on the pinched half of the arbor. We conclude that the sustained damage to surrounding tissue and the initiation of an immune response create a non-permissive environment for dendrite regeneration following pinch injury.

PFKM governs metabolic shifts throughout skeletal muscle differentiation

Melissa Campos, Grad Student Albrecht Lab

Metabolism is known to influence cell identity, but the underlying mechanisms remain unclear. Here we reveal spatiotemporal dynamics of phosphofructokinase 1 (PFK1), a key glycolytic enzyme, within the skeletal muscle lineage. The expression of PFKM (the muscle isoform of PFK1) is low in muscle stem cells and increases during differentiation. Mechanistically, Wnt signalling rapidly induces lysosomal degradation of PFKM through a methyl arginine degron motif, which gets selectively methylated by the protein arginine methyltransferase (PRMT1) and delivered to lysosomes through microautophagy. PFKM degradation shifts glucose metabolism from glycolysis to the pentose phosphate pathway. PFKM overexpression increases glycolysis and promotes differentiation into terminally differentiated myofibres. On the other hand, PFKM knockdown blunts differentiation, which can be rescued by supplementation with the downstream glycolytic intermediate 3-phosphoglycerate. In sum, our findings highlight the importance of compartmentalized metabolism in cell fate decisions.

UHRF1 as an epigenetic driver of tumorigenicity and immune evasion in triple negative breast cancer

Marina Suarez Pizarro, Grad Student Benavente Lab

Ubiquitin-like with PHD and RING Finger Domains 1 (UHRF1) is a multidomain epigenetic regulator essential for the maintenance of DNA methylation and repressive histone marks during cell division. As a downstream effector of the RB/E2F pathway, frequently deregulated in cancer, UHRF1 is aberrantly overexpressed in several malignancies, including triple-negative breast cancer (TNBC). Clinical datasets reveal that high UHRF1 expression correlates with poor overall survival in TNBC, underscoring its clinical relevance. Using CRISPR/Cas9-mediated gene editing, we demonstrate that UHRF1 loss significantly impairs TNBC cell tumorigenicity. UHRF1-deficient TNBC cells exhibited reduced clonogenicity, migration, and invasion in vitro. In orthotopic xenograft models, tumors with low UHRF1 expression displayed significantly reduced growth and metastatic potential compared to controls. Transcriptomic profiling revealed that UHRF1 loss led to widespread depression of immune-related genes, suggesting that UHRF1 orchestrates an epigenetically repressed, immune-evasive state. To validate these findings in an immunocompetent context, Uhrf1 knockout (KO) TNBC cell lines were generated from mouse 4T1 cells. Consistent with human models, Uhrf1 KO cells displayed reduced tumorigenic potential and formed significantly smaller tumors in Balb/cJ mice. Importantly, Uhrf1 KO tumors showed increased infiltration of activated cytotoxic T cells (CD8+ CD44hi) and plasmacytoid dendritic cells (pDCs), alongside a higher cytotoxic-to-regulatory T cell ration, indicating an enhanced anti-tumor immune landscape following loss of UHRF1-mediated repression. Together, these results establish UHRF1 as a master epigenetic regulator linking the RB/E2F axis to tumor progression and immune modulation in TNBC. Targeting UHRF1-dependent chromatin regulation may represent a promising strategy to simultaneously suppress tumor growth and overcome immune evasion in aggressive breast cancers.

Environmental Pollutant Benzo[a]pyrene Disrupts the Meiotic Spindle in Mouse Eggs

Kathleen Leon Parada, Grad Student Luderer Lab

Benzo[a]pyrene (BaP) is released as a by-product from the incomplete combustion of organic materials. Sources include combustion of tobacco/cannabis, fossil fuels, wood, or grilled foods. The finite pool of oocytes within the ovary is established from primordial germ cells (PGCs) during prenatal development. The Luderer laboratory demonstrated that prenatal exposure to BaP significantly reduced the number of germ cells, leading to premature ovarian failure, increased oxidative stress in surviving eggs, and reduced fertility in adulthood. The meiotic spindle consists of microtubules that attach to chromosome kinetochores to ensure that each egg receives half of the chromosome set in which euploidy is reestablished upon fertilization. The meiotic spindle plays a pivotal role in preserving egg quality and genetic integrity. We hypothesized prenatal exposure to BaP would significantly reduce the quantity and quality of surviving oocytes by disrupting spindle integrity. To test this hypothesis, we orally dosed

pregnant F0 dams to 0, 0.033, or 2 mg/kg/day of BaP from 6.5-15.5 dpc. Eggs were collected from F1, post-natal day 35-45 mice. Spindle length, metaphase plate length, spindle volume, chromosome alignment, and ploidy were assessed using IMARIS software. The number of eggs collected from BaP-exposed mice did not differ from controls (GEE, $p>0.05$). Spindle length (GEE, $p=0.005$) and spindle volume (GEE, $p=0.002$) were significantly increased in BaP-exposed mice compared to control mice. Compared to control mice, percent aneuploidy was significantly higher in mice exposed to 0.033 mg/kg/day BaP (Kruskal Wallis, $p=0.034$). Chromosome alignment and metaphase plate width was not altered by BaP exposure (GEE, $p>0.05$). Prenatal BaP exposure compromised egg quality by disrupting spindle characteristics that are implicated in reduced developmental competence. This exposure also undermines the genetic integrity of eggs at a human relevant dose, which raises an important question of BaP effects on long-term reproductive, maternal, and prenatal health.

Enhancer Synteny Confers Phenotypic Robustness During Mammalian Development

Joshua Alcantara, Grad Student Kvon Lab

Developmental genes are controlled by multiple cell-type-specific enhancers that frequently exhibit functional redundancy and are often located at considerable distances from their target genes. Despite extensive conservation of enhancer positioning across species, whether the relative arrangement of enhancers (enhancer synteny) is functionally important for robust gene activation during development remains unclear. Using genome editing at the Gli3 locus, which is critical for limb development, we generated a series of knock-in mice with altered enhancer arrangements, including duplications and positional swaps of limb enhancers. The positions of these enhancers are highly conserved across all amniotes, suggesting functional importance. Surprisingly, none of these rearrangements caused detectable changes in Gli3 expression or limb morphology. By contrast, when enhancer rearrangements were introduced into a sensitized genetic background with reduced Gli3 baseline expression, all configurations produced limb abnormalities. This reveals that enhancer synteny is critical for robust gene activation under conditions of genetic stress. Chromatin accessibility profiling across limb cell populations showed that rearranged enhancers maintain their accessible chromatin architecture, suggesting that disruption of enhancer synteny specifically impairs proper enhancer-promoter interactions rather than intrinsic enhancer activity. Together, our findings demonstrate that enhancer synteny confers phenotypic robustness to genetic and environmental perturbations, providing a functional explanation for the widespread conservation of enhancer positioning at developmental loci across species. Funding sources: NICHD (F31 HD118819-01); Department of Education (GAANN).

Investigating the Importance of Mitotic Timing in Embryogenesis

Johnny Vertiz, Grad Student Lara-Gonzalez Lab

The successful completion of embryonic development depends on the coordination of mitotic divisions with tissue morphogenesis. During mitosis, cells must spend enough time to properly align their chromosomes before segregating them equally during anaphase. Although mitosis is typically the shortest phase of the cell cycle, the biological significance of its short duration and how it contributes to tissue development remain unclear. Here, we utilized C. elegans to understand how prolonged mitotic timing affects embryogenesis. For this, we employed a two-pronged genetic approach using strains carrying mutations in the key regulators of mitotic timing, namely the anaphase-promoting complex / cyclosome (APC/C) and its co-activator CDC-20. Specifically, we employed a temperature sensitive allele of emb-30 to delay APC/C activation, and a CDC-20 T32S mutant to delay its ability to bind and activate the APC/C. Relative to controls, CDC-20 T32S delayed anaphase by ~160 sec, while the emb-30 allele delayed anaphase by ~210 sec. Using live imaging microscopy, we determined that CDC-20 T32S caused embryonic arrest at a late, post-morphogenic stage, whereas emb-30 mutant induced arrests at both pre- and post-morphogenic stages. These findings led us to hypothesize that mitotic timing control is crucial for late embryogenesis events. To address this, we engineered transgenic C. elegans lines where a pan-somatic tissue promoter (eft-3) was used to drive a wild-type copy of CDC-20 in mid and late development. This transgene fully rescued the lethality of the CDC-20 T32S mutant, confirming that its lethality does not stem from defects in early development. We are currently employing tissue specific promoters to drive wild-type CDC-20 and determine which tissue is primarily affected by prolonged mitotic duration. Strikingly, our preliminary data revealed that mechanical compression via agar pads

suppresses the lethality of CDC-20 T32S mutant embryos, suggesting that prolonged mitosis may perturb the biomechanical properties of the embryo, such as the assembly and/or function of the actin cytoskeleton. Overall, our work has the potential to unravel how mitotic timing and mechanical processes within the embryo are coordinated to ensure proper embryonic development.

Embryonic origins of tendon heterogeneity: looking to the eye for differences in tissue-specific attachments

Cameron Miller, Grad Student Schilling Lab

Tendons attach muscles to multiple tissue types to accommodate diverse structural and functional needs. Vertebrate tendon fibroblasts (tenocytes) are distinguished by their expression of scleraxis (scx), an early tenocyte fate determinant transcription factor, but differences in the genetic programs between tenocytes of varying attachment types remain largely unexplored. We have investigated this heterogeneity through a transcriptomic analysis of craniofacial tendons in zebrafish. The extraocular muscles (EOMs) that control eye movements exemplify functional tissue heterogeneity, since they feature both hard tissue attachments to the skull as well as unique soft-tissue tendon attachments to the eye (EOM-eye tendons). The EOM-eye tendons are resilient to injury, atrophy with aging, and various muscular dystrophies, suggesting distinct modes of muscle attachment regulation. Single-cell RNA sequencing using sorted mCherry positive cells taken from heads of Tg(scxa:mCherry) embryos at 72 hpf, coupled with quantitative in-situ hybridization chain reaction in whole embryos, identified novel EOM-eye tenocyte subpopulations. These tenocyte clusters differ in their extracellular matrix (ECM) components associated with distinct mechanical properties, and express key retinoic acid (RA) synthesis genes (aldh1a2 and rdh10a) and RA signaling effectors (pitx2), implicating RA signaling in their development. Consistent with this hypothesis, blocking RA signaling pharmacologically with DEAB uniquely prevents the formation of EOM-eye tendons, without affecting other tendon attachments. Conversely, elevated RA levels cause expansion of tenocyte number at EOM-eye tendon insertions. These results suggest differences in gene regulatory networks involved in EOM tenocyte cell specification at hard versus soft tissue attachments. Studies are ongoing to investigate the temporal dynamics of RA involvement in EOM-eye tendon specification and growth. We are pursuing genetic knockouts of ECM modulators and transcription factors highly differentially expressed in the EOM-eye tenocyte population to further identify tissue specific mechanisms of tendon development.

Parallel actions of sphingosine mimetic SH-BC-893 overcome and avoid drug resistance across cancer classes Cameron Geller, Grad Student Edinger Lab Drug resistance accounts for over 90% of cancer-related deaths, arising through diverse mechanisms including tumor heterogeneity, epigenetic reprogramming, and upregulation of compensatory oncogenic pathways. Here, we show that the multifaceted actions of the synthetic sphingosine analog SH-BC-893 (893) could make it effective against drug resistance across cancer types. Consistent with its ability to simultaneously activate PP2A and inhibit a subset of importins, 893 is active in over 500 human cancer cell lines. When subjected to a resistance-selection protocol, cells treated with standard-of-care (SOC) drugs rapidly developed resistance, shifting IC50 at least 20-fold within one month. 893-treated cells, under the same protocol, failed to show even a 5-fold shift over three months. Combination therapies offer only temporary benefit in a subset of patients and frequently cause dose-limiting toxicity. There is a critical unmet need for strategies that overcome multiple resistance mechanisms without triggering toxicity. Consistent with its broad activity across diverse mutational backgrounds, SOC-resistant cell lines remained sensitive to 893 at levels comparable to drug-naïve parental cells. 893 synergized with several SOC agents, and co-administration of sub-therapeutic 893 with SOC slowed the emergence of SOC resistance. Three established routes to multi-drug resistance (polyploid giant cancer cell formation, ABC transporter upregulation, and disrupted apoptosis) all failed to limit 893 activity. Together with multiple published studies showing 893 is well tolerated at the anti-neoplastic dose, these findings suggest that 893's capacity to simultaneously engage multiple structurally related target proteins represents a new form of polypharmacology capable of addressing cancer drug resistance, justifying lead optimization of these novel agents that leverage the anti-neoplastic actions of the natural compound sphingosine.

Phosphoregulation of CDC20 as alternative mitotic duration control pathway in human cells mitosis

Anh Nguyen, Lara-Gonzalez Lab

Proper cell division relies on the precise coordination of mitotic progression to ensure faithful chromosome segregation and genomic stability. The anaphase-promoting complex/cyclosome (APC/C) and its co-activator cell division cycle protein 20 (CDC20) play central roles in triggering anaphase onset, and their activity is tightly regulated by both the Spindle Assembly Checkpoint (SAC) and cyclin-dependent kinase (CDK)-mediated phosphorylation. Studies in multiple model organisms have demonstrated that phosphoregulation of CDC20 at specific phosphorylation residues can modulate mitotic timing independently of the SAC. These studies have also identified conserved phosphorylation sites on CDC20. However, the relative importance of the SAC versus CDC20 phosphoregulation on mitotic timing in human cells remains understudied.

To address this question, we generated a doxycyline-inducible CDC20 system in HeLa cells. For this, we expressed wild-type CDC20 (WT), a non-phosphorylatable mutant (3A: T55A, T59A, and T70A), a MAD2-binding deficient mutant (R132A), or a combined MAD-2 binding deficient and non-phosphorylatable mutant (3A/R132A). After induce expression of CDC20 and mutants using doxycycline, mitotic duration was observed using live-cell time-lapse imaging.

Expression of non-phosphorylatable 3A mutant significantly shortened mitotic duration relative to WT, suggesting that phosphorylation of three threonine sites contribute to delaying mitotic progression. Disruption of SAC-mediated inhibition through the MAD2 binding deficient mutation (R132A) also reduced mitotic duration relative to both WT and 3A, serving as an internal positive control. Notably, the combined 3A/R132A mutant produced a greater reduction in mitotic duration than either single 3A or R132A alone. This suggests that CDC20 phosphoregulation and SAC-mediated inhibition through MAD2 provide partially independent restrain on APC/C-CDC20 activation and anaphase onset.

Together, these findings support a model in which two parallel pathways promote normal mitotic duration control in HeLa cells. We are currently expanding this analysis to cell lines exhibiting different baseline mitotic durations. Overall, these efforts will enhance our mechanistic understanding of mitotic timing control, which may have implications for the development of novel strategies for targeting mitotic duration in cancer.

Cyclin B3 (CCNB3) accelerates mitosis in cancer cells

Amrutha Kizhedathu, Postdoc Lara-Gonzalez Lab

Antimitotic drugs have been the cornerstone of cancer chemotherapeutics; however, toxicity remains a major concern as they target both normal and cancer cells. Thus, there is widespread interest in uncovering cancer-specific mitotic vulnerabilities. One promising avenue is targeting genes that normally regulate meiosis but are aberrantly expressed in cancer cells. Here, we have identified Cyclin B3 (CCNB3), a conserved B-type cyclin, as one such gene. Cyclin B3 binds and activates its partner Cyclin Dependent Kinase (Cdk) and is crucial for promoting the metaphase-to-anaphase transition in meiosis I oocytes, although it does not play a significant role in somatic divisions. Recent studies, however, have shown that CCNB3 is also expressed in a subtype of sarcomas in bone and kidneys. Even though the expression of CCNB3 in these cancers is associated with poorer outcomes for patients the mechanism remains unclear. Our study explores how the expression of CCNB3 aids the progress of cancers. We find that while the expression of CCNB3 mRNA is virtually undetectable in normal cell lines, cancer cell lines express high levels of CCNB3 mRNA. Next, we evaluated the effect of CCNB3 depletion through either siRNA or CRISPR-mediated knockout and found that it led to a 3-8-fold increase in mitotic duration in cancer cells, while normal cells remained unaffected. On the other hand, overexpression of CCNB3 in accelerated mitosis on average, from 51 minutes to 25 minutes. Finally, CCNB3 depletion in cancer cells led to a higher incidence of mitotic defects such as unaligned and lagging chromosomes and it significantly reduced their growth rate. Taken together, these data suggests that CCNB3 is selectively overexpressed in cancer cells to accelerate and better the quality of mitosis. This dependency on CCNB3 could be exploited as a cancer specific vulnerability to aid therapeutics targeting mitosis.

Molecular Biology and Biochemistry

Elucidating the role of SAPS3 in tumor development

Shelly Lee, Grad Student Kong Lab

Cancer cells rely on metabolic reprogramming to sustain growth and survival in environments where nutrients and oxygen are often limited. In contrast to normal cells, which reduce growth and conserve resources under these conditions, tumor cells actively reprogram metabolic pathways to support biosynthesis, redox balance, and energy homeostasis. While much of the field has focused on metabolic enzymes involved in glycolysis and glutamine metabolism, less is known about how upstream regulatory proteins that coordinate these adaptive shifts. One such candidate is SAPS3 (PPP6R3), a regulatory subunit of the PP6 phosphatase complex, which may play a broader role in controlling this metabolic reprograming in cancer cells. Supporting this idea, analysis using TCGA/GTEx datasets reveals elevated PPP6R3 expression across multiple cancer types, including breast, colorectal, liver, and pancreatic cancers, leading to a potential role for SAPS3 in driving tumor progression. In addition to this, our previous proteomic analysis identified SAPS3 among the proteins enriched in the AMPK signaling pathway, indicating that SAPS3 may influence metabolic adaptation, at least in part, through this pathway. Based on these observations, we hypothesize that SAPS3 drives metabolic reprogramming in cancer, enabling cells to shift into metabolic states that support biosynthesis and adaptation to fluctuating nutrient conditions. However, its functional contribution to metabolic reprogramming and tumor progression remains poorly understood.

Effects of APOE4 and TREM2R47H mutations on the gut microbiome in the hAbKI Alzheimer's mouse model

Sayali Mulay, Postdoc Whiteson & Mapstone Lab

Background: Alzheimer's disease (AD) pathology is increasingly linked to dysregulation of the gut-brain axis, yet the specific interplay between amyloid pathology and major genetic risk factors remains poorly understood. While the APOE4 allele and TREM2R47H variants are critical genetic modifiers of AD risk, their influence on the gut microbiome in the presence of humanized amyloid-beta remains uncharacterized. This study utilizes UCI MODEL-AD consortium's knock-in mouse model to investigate how these genetic risk factors, individually and in combination, alter the microbiome across the lifespan.

Methods: This study utilized a total of 286 mice across four distinct genotypes: hAbKI WT, hAbKI, hAbKI-APOE4, and the triple-mutant. Cohorts were balanced by sex and analyzed at four critical time points: 4, 12, 18, and 24 months. Gut microbiome composition was assessed via shotgun metagenomic sequencing.

Results: Preliminary analysis of the microbiome reveals that multi-gene interactions significantly drive microbial community structure in an age-dependent manner. Unlike the stable diversity observed in hAbKI WT controls, alpha diversity (Shannon index) in the triple-mutant hAbKI-APOE4-TREM2 genotype displayed significant temporal changes. Diversity significantly differed from baseline at 4, 12, and 18 months, peaking at 12 months. These changes were not significant at 24 months. Taxonomic profiling further demonstrates shifts in the relative abundance of dominant phyla during these intermediate time points, suggesting that APOE4 and TREM2 mutations induce a transient but profound change in the composition of gut microbiome.

Conclusion: These findings provide preliminary evidence that APOE4 and TREM2 mutations differentially modulate the gut microbiome in an amyloid-dependent context, characterized by a specific window at 12 and 18 months. This transient fluctuation highlights a potential critical period where peripheral mechanisms may contribute to AD susceptibility.

Mechanisms and Therapeutic Potential of DRAK2-Targeted Metabolic Reprogramming for Regulatory T Cell Induction for Treating Multiple Sclerosis

Sarah Lee, Grad Student Walsh Lab

Multiple sclerosis (MS) is an autoimmune disease where autoreactive T cells attack the central nervous system (CNS). Current therapies make patients vulnerable to infection and cancer during treatment, emphasizing the need for safer approaches. Previous study shows that DAP kinase-related apoptosis-inducing protein kinase 2 (DRAK2)-deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE) without any defect in T cell recruitment to CNS upon infection. Drak2-/- T cells show increased regulatory T cell (Treg) numbers upon activation, and mouse in vivo data demonstrates

that the increased number of Treg is responsible for EAE resistance in Drak2-/- mice. Drak2-/- T cells also showed higher mitochondrial membrane potential, which indicates the alterations in mitochondrial metabolism in the absence of DRAK2. Drak2-/- T cells also exhibited dramatically reduced intracellular lipid droplets, which may be due to the change in metabolic pathways. Previous studies have demonstrated that DRAK2 inhibits autophagy by inactivating Unc-51 Like Autophagy Activating Kinase 1 (ULK1), which is critical for the initiation of autophagy in an early phase. Our protein analysis showed that Tripartite Motif containing 5 (TRIM5), a selective autophagy receptor, was also considerably low in Drak2-/- T cells, demonstrating the role of DRAK2 in selective autophagy. DRAK2 also seems to regulate the balance between AMPK-mTOR pathways, which are critical for Treg-Teff (effector T cell) cell expansion after T cell receptor (TCR) signaling. However, further in-depth investigation is needed to fully understand how DRAK2 regulates AMPK-associated metabolism to determine T cell fate. Eventually, these findings suggest that DRAK2 is a promising novel therapeutic target, while highlighting the role of immunometabolism in promoting regulatory T cell generation as an innovative therapeutic strategy for MS.

A Deletion of Drak2 Induces Regulatory T Cell Differentiation via Metabolic Reprogramming of Fatty Acid β-Oxidation

Madison Nguyen, Grad Student Walsh Lab

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease caused by autoreactive T cells targeting neuronal myelin. There is a need for more targeted MS therapeutics, such as improving regulatory T cell (Treg) expansion and suppressive function. One regulating factor of Treg proliferation is metabolic reprogramming, which shifts how cells rely on metabolic pathways for energy post-activation. Tregs tend to be less glycolytic, favoring fatty acid β-oxidation (FAO) and oxidative phosphorylation (OXPHOS). Here, we identify the death-associated protein kinase-related apoptosis-inducing protein kinase 2 (DRAK2) as a target involved in metabolic reprogramming of FAO to drive conventional T cell (Tconv) vs. Treg expansion. A DRAK2 deficiency was previously implicated in resistance against experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. However, Treg depletion in DRAK2-deficient mice restored EAE sensitivity. We also found that Drak2-/- Tregs and Tconvs had upregulated lipid homeostasis that became normalized during FAO inhibition. Since myelin-reactive Drak2-/- Tregs are essential to EAE resistance, further understanding metabolic targets associated with DRAK2 can lead us towards T cell-targeted MS treatments.

Decoration of Self-Assembling 2D Crystal Lattices for CryoEM of Small Proteins

Kristal Brandon, Grad Student Gonen Lab

Cryogenic electron microscopy (cryoEM) is a highly valuable structural analysis technique due to its lack of need for protein crystals, ability to study proteins in near-native states, and observe protein dynamics. However, these benefits cannot be applied to small proteins due to the size limit of cryoEM. As a majority of a cell's proteome is smaller than the theorized size limit of approximately 40 kDa, it is pertinent to develop effective strategies to overcome this size limitation. Two-dimensional protein crystals have been utilized to study small proteins via cryoEM, but growing 2D crystals is often quite challenging. To address the difficulty of growing 2D crystals, three proteins that self-assemble into 2D crystal arrays were developed in a previous study. This project aimed to test whether these self-assembling 2D crystal constructs could be decorated with small proteins and retain their ability to form 2D crystal lattices. These fusion lattices could have many applications including structure determination by cryoEM.

Fighting Evolution with Evolution: Training Phages to Overcome Bacterial Resistance

Kayla Raygoza, Grad Student Whiteson Lab

Bacteriophage (phage) therapy is a promising strategy for treating antimicrobial-resistant bacterial infections. However, widespread implementation remains limited by two major challenges: the narrow host range of many phages and the rapid evolution of bacterial resistance. Here we use directed evolution to engineer phage communities with enhanced activity against resistant bacterial populations.

We generated phage-resistant mutants (PRMs) from three AMR clinical isolates of Stenotrophomonas maltophilia through repeated phage exposure. Preliminary genomic analysis identified parallel mutations across independently evolved PRMs, including genes encoding putative phage receptors such as the hydroxamate siderophore receptor FhuE/B12 transporter and a mechanosensitive channel protein. Kirby–Bauer assays revealed collateral sensitivity with antibiotics: SM50 PRMs became susceptible to Trimethoprim-

sulfamethoxazole (SXT), SM22 PRMs became susceptible to Minocycline, and SM71 PRMs became susceptible to both SXT and Minocycline.
The PRMs were subsequently used as training strains for directed phage evolution. In as few as five passages, evolved phage communities displayed substantially improved activity against resistant bacterial populations compared with the ancestral three-phage cocktail. Preliminary results show that training efficiency was enhanced when ancestral bacteria were included alongside the target PRMs during the training process. Ongoing work is evaluating differences between ancestral and evolved phage communities in resistance suppression, bacterial clearance, and host range. Community and isolate-level genomic analyses are also being performed to determine which phages and genetic changes contribute to the expanded activity observed following training. This work demonstrates that directed phage evolution is a rapid and effective strategy for overcoming bacterial resistance and supports the development of pre-adapted phage therapeutics for antimicrobial-resistant infections.

Investigating the impact of actively secreted pyruvate on the metastatic potential of colorectal cancer
Katherine Pasterczyk, Grad Student Kong Lab
An estimated 90% of cancer deaths can be attributed to metastatic disease, the process by which cancer cells detach from the primary tumor and spread via the blood or lymphatic system to other sites within the body. In preliminary studies comparing key differences in secreted metabolites between patient-matched primary (SW480) and metastatic (SW620) colorectal cancer cell lines, we identified a significant increase in secreted pyruvate coming from metastatic cells. The secretion of pyruvate by metastatic cells is both active and selective, and not a byproduct of dysfunctional metabolism. This phenomenon is striking, as pyruvate is a highly valuable metabolite for the cell, residing at the center of many catabolic reactions. In in vivo xenograft models, SW620 tumors contain more pyruvate than SW480 tumors, suggesting this secretion is also apparent in vivo. These metastatic cells experience an increase in the monocarboxylate transporter SLC16A6, and SLC16A6 KO reduces pyruvate secretion in vitro. Furthermore, SLC16A6 KO reduces cancer cell infiltration to the lung in in vivo tail vein injection models. This phenomenon of increased transporter expression and pyruvate secretion in metastatic cells may be apparent in other human and murine cancer models. Currently, I am determining the mechanism(s) by which this increase in secreted pyruvate contributes to metastatic potential through in vitro and in vivo pyruvate tracing experiments, conditioned media and co-culture experiments, as well as syngeneic mouse models. Collectively, this study identifies a potential key metabolic adaptation that promotes cancer progression.

Fat and Furious: How CD1a-mediated Inflammation Protects and Harms
Julio Ayala Angulo, Grad Student Nicholas Lab
Obesity is characterized by adipose inflammation dominated by Th17 cells. Standard mouse models poorly recapitulate human T2D. We discovered CD1a, a lipid antigen-presenting molecule absent in mice, mediates protective T cell responses in male obesity mouse models. We hypothesized that CD1a lipid antigen presentation drives inflammation in response to High Fat Diets (HFD). We used human CD1a expressing mice (hCD1a) and fed them a HFD 45% fat for up to 34 weeks. As expected, hCD1a HFD mice developed higher levels of inflammation in adipose tissue. However, hCD1a HFD mice had lower fasting insulin levels and HOMA-IR as early as week 6 on diet despite comparable fasting blood glucose.They also had higher gonadal fat pad weight despite overall weight remaining comparable to WT HFD. We demonstrate that CD1a mediates robust T cell infiltration into adipose tissue under HFD concurrent with protection from insulin resistance, suggesting a protective role for CD1a-mediated T cell inflammation. These data may explain limitations of traditional mouse models. This work identifies CD1a-lipid interactions as novel therapeutic targets and suggests immunomodulation strategies that enhance rather than suppress specific T cell responses in metabolic disease.

Hmgb2 regulation of virus-specific CD4+ T cells during chronic viral infection

Jamie-Jean De La Torre, Grad Student Tinoco Lab

CD4+ T cells are required to mediate an effective immune response during chronic (Cl13) Lymphocytic choriomeningitis viral (LCMV) infection via their helper functions that support cytotoxic CD8+ T cells and B cell responses. During acute infection, viral clearance results in differentiation of functional memory T cells, while chronic infection leads to exhaustion. At late stages of infection, effector CD4+ T cells differentiate into Tfh cells that provide survival signals to exhausted CD8+ T cells while also stimulating antibody production by B cells. High mobility group box 2 (HMGB2) is a DNA-binding protein that is highly expressed in T cells, but the role of HMGB2 in virus-specific CD4+ T cells is unknown. We hypothesize that HMGB2 modulates the differentiation and exhaustion phenotype of virus-specific CD4+ T cells. To test this, we infected WT and Hmgb2-/- (KO) mice with Cl13 and tracked the kinetics of LCMV-specific CD4+ T cells. KO hosts had significantly decreased virus-specific CD4+ T cells and had higher viral titers in the blood, lung, brain and kidneys of KO hosts compared to WT by late infection. LCMV-specific CD4+ T cells in Hmgb2-/- mice showed increased expression of inhibitory receptors, reduced pro-survival molecule expression, and reduced markers of Tfh differentiation at 30dpi. To evaluate cell-intrinsic roles, we co-transferred congenically marked WT and Hmgb2-/- LCMV-specific CD4+ T cells into WT hosts and tracked their kinetics during Cl13 infection. Hmgb2-/- CD4+ T cells also had increased inhibitory receptor expression and reduced TCF1 levels, a Tfh-associated marker. However, KO co-transferred cells were present at higher numbers compared to WT cells, unlike in Hmgb2-/- mice, possibly due to extrinsic factors present in WT hosts. Together, these results indicate that HMGB2 is key in regulating the differentiation and survival of virus-specific CD4+ helper cells, including Tfh cells, that are crucial in resolving chronic viral infection.

Identifying Biomarkers of Alzheimer's Disease through a Comparative Analysis of Mouse and Human Microbiomes

Gina Faraci, Postdoc Whiteson Lab

Background: The gut microbiome has been implicated in Alzheimer's disease (AD) through interactions along the gut-brain axis. In previous studies, we identified gut taxa in mouse models associated with AD in humans, but direct cross-species microbiome comparisons were limited. Recently published shotgun metagenomics datasets now enable these comparisons to identify conserved taxa relevant for model evaluation and biomarker discovery.

Methods: We analyzed publicly available microbiome sequencing data from individuals with preclinical AD (Ferreiro et al. 2023) alongside cecal microbiomes of 4- and 12-month-old hAβ-KISWE/IB;APOE4;hMAPT (MAD2) and hAβ-KI;APOE4;hMAPT (MAD1) mice. Both datasets were processed using reference-based classification and de novo metagenome assembly, followed by alpha and beta diversity analyses, random forest (RF), linear mixed-effects (LME) modeling, and differential abundance (DA) analysis.

Results: Of the 100 most abundant taxa, 45% of species and 64% of genera were shared between humans and mice. RF and LME modeling showed that the DA genera of Clostridium, Alistipes, and Turicibacter were associated with the separation of the mouse genotypes. We generated 1,148 high quality metagenome-assembled genomes (MAGs) from the human samples. RF analysis identified 10 MAGs representing 9 species. Five taxa were DA between preclinical and healthy microbiomes, and all five lack cultured representatives.

Conclusion: The DA mouse genera have been previously associated with AD in humans, supporting the relevance of our model. Significant differences between preclinical and healthy human microbiomes were only identified at the MAG level, highlighting the importance of including "unknowns" in AD microbiome studies. Lastly, the observed overlap between mouse and human microbiomes supports these cross-species analyses for AD research.

scRNA-seq of pituitary from letrozole-induced PCOS mouse model reveals abnormal prolactin secretion

Gabriela De Robles, Grad Student Nicholas Lab

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among reproductive-aged women with a complex pathophysiology. Ovarian hormonal dynamics are regulated via the secretion of the gonadotropin-releasing hormone (GnRH), which stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland. High serum levels of LH in PCOS patients indicate an increase in GnRH levels, but the mechanisms within the anterior pituitary at a cellular level are unclear. We performed single-cell mRNA sequencing (scRNA-seq) on the pituitaries of the letrozole-induced (LET) mouse model of PCOS to profile the functional genomics of endocrine cell populations relative to serum hormone levels. The population percentage of lactotropes significantly decreased; in contrast, two gonadotrope populations significantly increased in the LET mice compared to the placebo-treated mice. Consistent with these changes, an increasing trend of serum LH levels and a decreasing trend of serum prolactin (PRL) levels were observed in the LET mice. A gene set enrichment analysis (GSEA) on the DEGs between the two gonadotrope clusters in the LET mice also confirmed a variation in biological pathways linked to ribosomal subunits, transcription regulation, and the regulation of steroid metabolic processes. Based on the scRNA-seq results of the anterior pituitaries of LET mice, alterations in the proportions of hormone-secreting cells may be a response to hyperandrogenism, in which functionally diverse gonadotropes could contribute to heterogeneity in reproductive phenotypes. Changes in the lactotrope population and PRL serum levels also support further research on the lactation hormone and its role in PCOS. PRL is involved in metabolic homeostasis and reproduction, and understanding its dysregulations may provide additional insight into the pathophysiology of PCOS.

May the flow be ever in your favor: A microfluidic chip for cervical cancer chemotherapy and radiation studies

Daniela Gaebler, Grad Student Hughes Lab

Cervical cancer is the fourth most common cancer in women worldwide, with approximately 660,000 new cases each year. While HPV vaccination and screening have improved prevention, outcomes for advanced or recurrent disease remain poor. Standard treatments - cisplatin-based chemotherapy and radiotherapy - are frequently limited by resistance and recurrence. A key bottleneck in improving these therapies is the lack of preclinical models that faithfully capture the complexity of the tumor microenvironment.

To address this, we developed a vascularized micro-tumor (VMT) model using a microfluidic chip - a miniaturized platform that precisely controls fluid flow through microscale channels - to recreate the cervical cancer microenvironment in the lab. Within this chip, functional micro-vessels form spontaneously and are co-cultured alongside cancer cells and surrounding stromal tissue, producing a three-dimensional (3D) model that more closely mimics how tumors behave in the body. Critically, these micro-vessels enable controlled delivery of nutrients, drugs, and immune cells directly into the tumor mass - something conventional 2D cell culture cannot replicate.

We validated the cervical cancer VMT using established tumor markers and demonstrated that cisplatin treatment resulted in a significant reduction in tumor cell proliferation, underlining the model's ability to capture clinically relevant drug responses in a physiologically relevant microenvironment. Building on these findings, ongoing work aims to integrate radiation therapy into the 3D VMT platform to evaluate combined treatment responses in a vascularized context. This approach is supported by complementary 2D studies demonstrating radiation-induced DNA double-strand breaks and reduced cell proliferation, with enhanced effects observed upon combination with cisplatin, as well as by established clinical evidence for concurrent chemoradiation. Ultimately, this cervical cancer VMT platform has the potential to advance our understanding of cervical cancer biology by enabling physiologically relevant 3D studies of treatment response and tumor-stroma interactions, supporting the long-term optimization of therapeutic strategies.

Neurobiology and Behavior

Spatial representations are preferentially encoded in superficial layers across primary sensory and association cortices

Wing Ning, Grad Student McNaughton Lab

The brain creates an internal representation of the world by extracting knowledge about the context (where) and content (what) of experiences. The indexing theory postulates that the hippocampus (HPC) generates unique contextual codes that associatively link to sensory content stored in the neocortex. Recent studies have shown that place cell sequences are also observed across multiple neocortical regions, including retrosplenial cortex, posterior parietal cortex (PPC), primary and secondary motor cortices. However, the relative coding of context versus content information in superficial (L2/3) versus deep (L5/6) cortical layers remains elusive. To investigate this, we developed a treadmill behavioral paradigm for head-fixed mice that integrates virtual reality (VR) environments with discrete tactile cues. On recording day, mice experienced two distinct familiar VR contexts and two novel tactile cues. The novel tactile cues were swapped in position, allowing us to decouple cue activity from location-specific activity in a double dissociation design. Using high-density (512-channel) silicon probes, we recorded neural activity across superficial and deep layers of the primary somatosensory cortex (S1), PPC, and dorsal CA1 of HPC (N=10 mice). As expected, population vector correlation analyses showed that S1 had higher tactile cue discrimination, whereas PPC exhibited greater selectivity for spatial position, similar to HPC. Interestingly, laminar analyses revealed greater global remapping in superficial compared to deep layers in both S1 and PPC, indicating more distinct spatial representations. Cue detection was primarily encoded in PPC superficial layers, whereas S1 deep layers showed a trend toward stronger cue detection. PPC exhibited higher lifetime sparsity and information scores relative to S1. Experience within a session increased lifetime sparsity and information in both cortical regions. These increases occurred across successive VR context and cue swap transitions and were observed in superficial and deep layers. Together, our data reveal that spatial representations are preferentially encoded in superficial than deep layers in both primary sensory and association cortices, and that experience refines cortical representations across layers.

Is it "just a phase"? How adolescent THC exposure affects decision making in adulthood

Selen Dirik, Grad Student Mahler Lab

With increased accessibility, public attitudes on cannabis use are shifting, with a growing perception that THC, the main psychoactive drug in cannabis, is safer than other abused drugs. However, human correlational studies suggest a relationship between cannabis use early in adolescence in particular, and long-term cognitive and emotional outcomes, including incidence of psychiatric disorders like depression, addiction, and anxiety. Preclinical studies of adolescent THC exposure (AdoTHC) also consistently report long-lasting brain and behavioral changes, including alterations in cognition, emotional regulation, and motivation-related processes. This suggests that AdoTHC may alter the adolescent development of circuits underlying reward and threat processing, and top-down regulation thereof. Since these are the same circuits thought to underlie maladaptive decision making characteristic of several psychiatric disorders, we aim here to further characterize the specific psychological processes impacted by AdoTHC, and the altered neural substrates responsible for them.

We subjected male and female rats to our established, well-characterized AdoTHC treatment protocol during postnatal days 30-43; corresponding with early-to-mid adolescence. Following a THC washout period, the rats are then trained in a series of behavioral tasks aimed at quantifying reward seeking, risk avoidance, and pursuit of reward in the face of danger during adulthood. Ultimately, these findings may shed light on whether and how adolescent THC use causally impacts behavioral and brain processes related to psychiatric disease.

Cluster-based Metric of Early Tau-PET Aggregation is Associated with Plasma P-tau217 in Cognitively Unimpaired Older Adults with Subthreshold Amyloid-beta

Leah Varghese, Grad Student Yassa Lab

Early detection of AD is essential for timely intervention and improved patient outcomes. Plasma p-tau217, which reflects soluble, phosphorylated tau, is one of the most promising biomarkers for early AD detection. However, previous studies show weak or absent

correlations between p-tau217 and tau-PET in cognitively unimpaired (CU) older adults. Although tau initially aggregates in focal clusters driven by local seeding and cell-specific vulnerability, traditional methods average tau-PET signal within a region, potentially washing out this early signal. We tested whether a cluster-based tau-PET approach could better detect focal tau deposition in the entorhinal cortex (EC), revealing stronger relationships with plasma p-tau217 in CU older adults. We analyzed 142 CU older adults from ADNI with paired plasma p-tau217 (Janssen) and 18F-Flortaucipir-PET. SUVR maps were thresholded at 1.3, and clusters identified using MATLAB's bwconncomp.EC tau cluster volume (total cluster volume/cluster count, normalized by EC volume) and mean SUVR were calculated. EC tau cluster volume correlated with plasma p-tau217 in both Aβ+ and Aβ- subgroups, whereas EC mean SUVR was significant only in Aβ+. Across continuous amyloid burden, the association was strongest at subthreshold levels (<20 Centiloids) and weakened at higher amyloid, likely because at high amyloid burden, tau aggregation spreads outside the EC, and p-tau217 becomes a more global tau phosphorylation marker rather than regional. Our findings suggest plasma p-tau217 tracks Aβ-modulated entorhinal tau detectable by cluster-based tau-PET in CU adults with subthreshold Aβ. P-tau217 was not related to tau-PET clusters in Aβ- individuals with little to no measurable Aβ, where clusters likely reflect primary-age-related tauopathy or measurement noise. These findings highlight the potential utility of plasma p-tau217 for detecting and monitoring tau responses in anti-tau therapeutic trials in individuals with subthreshold amyloid, who represent a key preclinical window for intervention.

Evaluating the Impact of SARS-CoV-2 Infection on Alzheimer's Disease Pathology Across Distinct Mouse Models

Latifa Zayou, Grad Student Lane Lab

INTRODUCTION

The COVID-19 pandemic underscored the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on worsening the severity of Alzheimer's disease (AD) neuropathology and disease progression. To further investigate the long-term effects of SARS-CoV-2 infection in a more clinically relevant AD model, we extended these studies to the MODEL-AD2 (MAD2) mouse model expressing humanized Aβ, tau, and APOE4.

METHODS

Aged 5xFAD and wild-type (WT) mice were infected with a mouse-adapted SARS-CoV-2 (MA10), and extensive characterization of molecular and cellular changes within the brains was carried out. In parallel, MAD2 mice were infected with MA10 and evaluated at later time points to determine the impact of infection on AD-related neuropathology.

RESULTS

MA10 infection induced acute viral pneumonia. Viral RNA was undetectable within the brains of infected 5xFAD and WT mice, and there was no evidence of glial activation or neuroinflammation. MA10 infection did not affect amyloid beta (Aβ) plaque volume or numbers in 5xFAD mice compared to uninfected mice. Spatial transcriptomics revealed altered expression of genes associated with homeostatic function in neurons, glia, and vascular endothelial cells. Preliminary analyses of MA10-infected MAD2 mice suggest infection-associated alterations in amyloid pathology at later time points following infection.

DISCUSSION

Collectively, these findings demonstrate that while MA10 infection did not affect AD neuropathology in 5xFAD mice, there were numerous downstream effects on gene expression associated with resident central nervous system cell functions that may impact neurologic disease. Preliminary findings in MAD2 mice suggest that the long-term effects of SARS-CoV-2 infection on AD-related neuropathology may be influenced by disease model, genetic background, and stage of disease progression.

Microglial Expression of TREM R47H And APOE In a Chimeric Tauopathy Mouse Model

Kimiya Mansour, Grad Student Blurton-Jones Lab

Microglia, the resident immune cells of the central nervous system, are implicated in the onset and progression of neurodegenerative diseases such as Alzheimer's Disease (AD) and other tauopathies. Recent studies have highlighted the TREM2-APOE signaling axis as a critical pathway regulating microglial activation and AD-pathogenesis. While loss of function mutations in TREM2, including the R47H and R62H variants, have been shown to impair microglial response to amyloid depositions, it remains largely unknown how these variants impact microglial interactions with tau. Furthermore, although APOE4 has been repeatedly identified as the highest AD genetic risk factor, APOE is most abundantly expressed by astrocytes

within the brain. Therefore, it remains largely unclear how microglial APOE4 impacts disease progression and tau formation.
To examine how microglial TREM2 and APOE variants impact tau fibrillary tangles in AD and other tauopathies, we utilized CRISPR gene editing to engineer an isogenic set of TREM2 WT or R47H and APOE3 or E4 iPSC lines, then transplanted iPSC-microglia into xenotolerant, tau-accumulating P301S neonate mice. Using an inhibitor resistant approach, mice were fully chimerized with human APOE-3, APOE-4, or APOE3-TREM2 R47H microglia until 9 months of age. Preliminary data suggests unique engraftment patterns and altered APOE expression profiles in xMG expressing TREM2 R47H in both wild-type and P301S mice. Our ongoing work aims to define and characterize the interplay between these two genetic risk factors governing microglial dysfunction and shaping the progression of tauopathy.

Predicting Tau Positivity using Gray Matter Diffusion Weighted Imaging

Julie Loritsch, Grad Student Stark Lab

Background: Early detection of histopathological hallmarks of Alzheimer's disease (AD) is critical for accurate diagnosis. Current approaches are limited in identifying tau pathology without PET imaging, which is costly and not widely accessible. Diffusion-weighted imaging (DWI) is a non-invasive method that provides insights into the microstructure of brain regions. Prior studies have utilized structural MRI and diffusion tensor imaging (DTI) metrics in white matter for classifying tau positivity. In this work, we extend these efforts by combining gray matter diffusion-derived metrics, structural MRI measures, and demographic information to predict tau positivity. Methods: Data was obtained from Standardized Centralized Alzheimer's & Related Dementias Neuroimaging (SCAN) and Alzheimer's Disease Neuroimaging Initiative (ADNI). Diffusion data was preprocessed using QSIPREP, from which ten diffusion-derived metrics were computed, including measures from DTI, neurite orientation dispersion and density imaging (NODDI), and mean apparent propagator MRI (MAP-MRI). Analysis included 219 amyloid-β positive participants with available multi-shell DWI and tau PET data (mean age: 76.63±8.02 years; 53.0% female, 39.3% tau positive). Features derived from demographic, structural MRI, and diffusion data were screened using ridge regression for feature selection. Four machine learning classifiers (decision tree, logistic regression, random forest, and gradient boosting) were trained to predict tau positivity. Models were evaluated using individual metric types as well as combined diffusion and structural features. Model performance was assessed with and without five-fold cross-validation on the full dataset. Results: Logistic regression and gradient boosting achieved the highest performance, with five-fold cross-validation accuracies of 0.772 and 0.762 respectively, and full dataset accuracies exceeding 0.95. Models incorporating a combination of diffusion-derived metrics performed better than structural or demographic metrics, with a combination of diffusion and structural increasing accuracy slightly. Regional analyses identified the middle temporal cortex as the most consistently selected feature across metric types. Conclusion: Diffusion demonstrates strong potential for predicting tau positivity, particularly when combined with structural MRI. These findings suggest that temporal lobe microstructural changes occur alongside tau accumulation and can be captured using diffusion-based metrics. These findings support diffusion MRI as a promising, non-invasive alternative for detecting tau pathology and improving the accessibility of AD diagnosis.

iPSC-Microglia Replacement for the Treatment of GRN-related Frontotemporal Dementia

Jean Paul Chadarevian, Postdoc Blurton-Jones Lab

Frontotemporal dementia (FTD) is the second leading cause of dementia in people under 65. Among the known genetic contributors, progranulin (GRN) haploinsufficiency is the second most prevalent cause of familial FTD (FTD-GRN) and the leading cause of early onset dementia. Therapeutic strategies have previously sought to increase GRN levels using viral vectors or peripheral infusion. However, poor biodistribution, immune reactivity, and peripheral toxicity have remained persistent challenges. Alternatively, iPSC-microglia (iMG) transplantation offers a novel cell-based strategy. Microglia are the innate immune cell of the brain and most prevalent source of progranulin within the CNS. To examine the therapeutic potential for microglial transplantation for FTD-GRN, we generated a xenotolerant, progranulin-deficient model (hGrnR493X) that allows for the long-term engraftment of human microglia into the brain. Importantly, onset of initial FTD-GRN symptomology in patients is highly variable, ranging from 45-80 years of age. Therefore, we utilized an inhibitor resistant

approach to assess widespread engraftment of healthy donor microglia in aged, 7-month-old hGrnR493X mice after symptomology onset. We found in sacrificed 10-month-old mice, microglia transplantation significantly lowered lysosomal content throughout the brain. However, CSF1Ri-mediated microglia replacement was necessary to also reverse widespread astrogliosis and microglial activation. Biochemical analysis further validated microglial transplantation and replacement significantly reduced multiple markers of inflammation and elevated GRN levels within the brain and periphery. Ongoing experiments will further elucidate the impact of human microglial transplantation on other cell populations within the brain. Together, these results provide preliminary evidence that iMG transplantation and replacement could be developed as a novel therapy for FTD-GRN and likely other neurodegenerative diseases.

CSF1R inhibitor-resistant model for CNS-wide microglia replacement strategies

Jasmine Nguyen, Grad Student Blurton-Jones Lab

Recent advances in cell-based therapies have demonstrated therapeutic safety and efficacy for a variety of diseases. However, significant challenges remain in their development for the treatment of neurological disorders. Preclinical studies have explored microglial replacement strategies utilizing bone marrow transplantation and CSF1R inhibitor (CSF1Ri) treatment. However, these approaches often require highly invasive strategies and result in engrafted cells that remain transcriptionally and functionally distinct from microglia. To assess less-invasive microglia replacement strategies capable of preserving microglial ontogeny, we developed a Csf1r-G793A knockin mouse. We found that G793A mice develop typical microglial densities and peripheral hematopoietic populations, which exhibit broad CSF1Ri resistance enabling widespread microglia replacement following direct intraparenchymal injection or bone marrow transplantation. Furthermore, we demonstrate that widespread microglia replacement can be achieved via less-invasive intracisternal delivery of CSF1Ri-resistant murine and induced pluripotent stem cell (iPSC)-derived human microglia. These findings demonstrate that G793A mice provide a robust and broadly applicable source of engraftable donor microglia and peripheral macrophages for the preclinical investigation of microglia replacement strategies.

Hugs or Drugs? How Ventral Pallidal Cell-types Modulate Social-or-Heroin Choice

Erica Ramirez, Grad Student Mahler Lab

Opioid addiction is a major societal problem, and we still have few tools available to prevent or treat it in those who suffer—especially tools derived from an understanding of the basic neurobiology of addiction. Part of the reason for this failure to translate neuroscience findings into the addiction clinic may be a lack of translationally relevant models that incorporate complexities of the disorder, such as social factors that can protect against or exacerbate addiction. Indeed, social factors play key roles in the initiation and progression of addiction, and the COVID-19 pandemic laid this bare, with marked changes in drug use, overdose, and addiction occurring in the early 2020s.

Here, we address this knowledge gap by asking how GABA neurons in the ventral pallidum (VP), a brain region known to play a key role in food and drug reward, are involved in social reward and the social modulation of drug reward. In transgenic GAD1-Cre Long Evans male and female rats, we chemogenetically inhibited or stimulated VP GABA neurons during social behaviors and during an operant social/drug self-administration task in which rats chose between social and drug rewards. Specifically, we targeted inhibitory and excitatory DREADDs (hM4Di and hM3Dq) to VP GABA neurons, allowing bidirectional control during behavior. Wildtype and DREADD-expressing animals were socially isolated from their same-sex sibling cage mate for one week before assessments of: (1) social conditioned place preference, (2) social play and investigation, (3) social communication via ultrasonic vocalizations, (4) operant social contact self-administration, and (5) choice of social versus heroin reward. Additional experiments using c-Fos immunohistology and fiber photometry assessed VP GABA and non-GABA neuronal activity during social behavior.

Our findings demonstrate that chemogenetic manipulation of VP GABA neurons modulates social reward seeking, social behavior, and communication in rats in a domain- and sex-specific manner. These results highlight an underappreciated role for the VP in social behavior and provide insight into how social factors modulate opioid reward and seeking.

Spatial Transcriptomic Imaging Reveals Viral Encephalitis Induces Altered Myeloid Responses To Amyloid Plaque Pathology In 5xFAD Mice

Dominic Javonillo, Grad Student Lane Lab

Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by the aggregation of extracellular amyloid-beta (Aβ) plaques, neurofibrillary tangles containing hyperphosphorylated tau protein, and chronic neuroinflammation. Recent studies revealed key immunological mechanisms within the central nervous system (CNS) that contribute to AD pathology. Additionally, analyses of human AD datasets have also associated viral encephalitis exposure (i.e., viral-induced neuroinflammation) with the development of AD and dementia, highlighting the need to better understand how viral encephalitis and neuroimmune mechanisms within the brain may impact AD pathologies such as Aβ plaque deposition.

Methods: To determine how coronavirus-induced encephalitis may impact established Aβ plaque deposition, we intracranially infected 5xFAD mice with the neurotropic JHM coronavirus. At 12 days post-infection, collected mouse semi-brains to perform immunohistological staining, confocal microscopy, flow cytometry, and spatial transcriptomic imaging.

Results: Histological staining of Aβ plaques revealed compacted dense-core plaques of JHMV-infected 5xFAD mice at 12 days post-infection, despite minimal changes in overall Aβ plaque load within the brain. Furthermore, we observed robust peripheral monocyte/macrophage infiltration into the brains of JHMV-infected 5xFAD mice, in addition to CD4+ and CD8+ T cell infiltration in brain regions correlating with compact dense-core Aβ plaques. To determine the transcriptional impact of acute viral encephalitis on cells within the 5xFAD mouse brain, we investigated gene expression within myeloid cells and other CNS cell types using spatial transcriptomic imaging. Utilizing differential gene expression and pathway analysis, we found that myeloid cells exhibited an increased proportion of myeloid cells demonstrating down-regulated disease-associated (DAM) pathways involving Aβ clearance, response to lipids, and macrophage activation within the post-encephalitis 5xFAD brains.

Conclusion: Together, these findings suggest an attenuated myeloid cell response to Aβ plaque burden in 5xFAD mice following acute viral encephalitis. Future experiments aim to further dissect inflammatory mechanisms between infiltrating myeloid cells, T cells, and the progression of Aβ and tau pathology. Data derived from these experiments will further elucidate the viral-induced neuroimmune mechanisms that affect AD pathology and o#er an opportunity to determine how these neuropathologic changes, such as subsequent neuronal damage, occur

A Multimodal MRI Approach to Longitudinal Vascular and Microstructural Change in a Canine Model of Alzheimer's Disease

Claire Illeck, Grad Student Stark Lab

Background. Domestic canines naturally develop amyloid-β pathology, neurodegeneration, and cognitive decline, making them a powerful translational model for Alzheimer's disease (AD). In addition, their shorter lifespan also enables efficient longitudinal characterization of age-related brain changes. Arterial spin labeling (ASL) estimates cerebral blood flow (CBF), while diffusion MRI characterizes tissue microstructure via water diffusion. We tested whether vascular and microstructural MRI markers change systematically in a longitudinal, randomized trial of calcineurin inhibitor tacrolimus and NFAT inhibitor Q134R versus placebo in middle-aged beagles.

Methods. Forty-three beagles (36 female; baseline age 6.47±1.10 years) were randomized to tacrolimus (n=15), Q134R (n=14), or placebo (n=14) and underwent annual 3T MRI for five years. Diffusion MRI yielded tensor (FA, AD, RD) and NODDI (NDI, ODI, FWF) metrics (processed with MRtrix3 with canine-optimized ANTs-based registration). ASL was processed with BASIL (M0 calibration, partial volume correction) to derive CBF. ROI values were extracted from the Johnson atlas in six a priori regions (hippocampus, caudate nucleus, posterior compositus and posterior cingulate gyri, precruciate and orbital gyri). Longitudinal effects were tested with linear mixed-effects models (subject random intercepts; baseline age; timepoint linear/quadratic; pooled across treatment groups). Robustness was assessed with an exact within-subject timepoint-label permutation test (720 permutations) using mean absolute deviation (MAD) from fitted trajectories, providing an agnostic measure of longitudinal change across linear and quadratic effects. Treatment effects are currently being analyzed.

Results. CBF declined across all six regions, with significant longitudinal change in five regions ($p<0.05$) and a trend in posterior cingulate ($p=0.078$) (Fig. 1). Diffusion metrics showed region- and metric-specific longitudinal change (Fig. 2). RD was most consistent (5/6 regions, $p\leq0.0028$), and NODDI metric changes were significant in multiple regions (NDI 4/6; ODI 4/6; FWF 4/6; $p\leq0.011$), including a decrease in hippocampal NDI across the study. FA and AD showed additional regional longitudinal changes (each 4/6 regions; $p<0.05$).
Conclusion. Aging beagles show widespread perfusion decline alongside coordinated microstructural change across cortical and subcortical regions. ASL and diffusion MRI provide complementary information for tracking vascular and tissue change longitudinally in a naturalistic animal model. These trajectories provide quantitative imaging measures to test whether interventions alter AD-relevant age-related patterns.

The Abi3S212F Alzheimer's Disease risk variant induces amyloid beta plaque remodeling and disrupts microglia-plaque dynamics
Claire Butler, Postdocs Green Lab
The microglial gene ABI3 has been implicated in Alzheimer's disease (AD) risk, with the ABI3S209F variant being associated with increased risk for developing Late-Onset AD. However, the functional impact of human variants remains poorly understood. To address this, we generated a mouse model that has the same amino acid switch at the equivalent position in the mouse genome, S212F.
To determine the effects of the Abi3S212F variant on amyloid pathology and microglial responses, male and female 5xFAD and 5xFAD/Abi3S212F mice were aged to 4, 12, and 18 months, representing early, middle, and late disease stages. Amyloid burden was evaluated using thioflavin S (dense-core plaques) and the OC antibody (fibrillar Aβ). 5xFAD/Abi3S212F mice exhibited reduced dense-core plaque deposition in cortex from 12 months and in subiculum at all ages, while fibrillar Aβ was unchanged or selectively increased at 12 months. Soluble Aβ levels were unaltered between 5xFAD and 5xFAD/Abi3S212F genotypes, suggesting age-dependent redistribution of Aβ from compact plaques into more fibrillar, potentially more, neurotoxic species.
Microglial responses to plaques were assessed by IBA1, CD68, and PU.1 staining. Microglial density and volume were preserved until 18 months, when both were significantly reduced in 5xFAD/Abi3S212F mice, disproportionate to plaque loss. At this stage, microglial activation (CD68+) and number of plaque-associated microglia were markedly decreased in cortex and subiculum. These findings suggest that Abi3S212F microglia may have deficits in recruitment, proliferation, reactivity to plaques and/or be more vulnerable to Aβ-induced cell death.
Supporting an effect on vulnerability to cell death, unbiased proteomics identified a specific interaction between Abi3S212F and Gasdermin D (GSDMD), the key effector of pyroptosis. Consistent with this, 18-month 5xFAD/Abi3S212F mice showed increased GSDMD+/IBA1+ microglia in the subiculum.
Together, these results suggest the Abi3S212F variant results in a loss of function while also revealing a novel Abi3S212F -GSDMD interaction that may promote plaque-associated microglial death. Collectively, our findings demonstrate that Abi3S212F alters plaque composition, impairs microglial-plaque dynamics characterized by deficits in microglial survival and compaction activity. Ultimately providing new insight into how ABI3 variants may contribute to AD progression.

Digital Cognitive Phenotyping Identifies Imminent Risk in Preclinical Alzheimer's Disease
Casey Vanderlip, Grad Student Stark Lab
As Alzheimer's disease (AD) diagnosis shifts toward biological definitions, clinicians increasingly encounter cognitively unimpaired individuals with elevated β-amyloid (Aβ). However, amyloid positivity alone provides limited insight into when cognitive decline will begin, complicating counseling, monitoring, and trial enrollment. We tested whether patterns of performance on a brief, self-administered digital cognitive assessment could be used to identify a high-risk cognitive phenotype that predicts imminent decline and tau progression in preclinical AD. We analyzed baseline digital cognitive performance in 1,146 Aβ+ and 538 Aβ− participants from the A4 and LEARN studies, followed for up to 4.6 years. Using unsupervised k-means clustering, we derived cognitive phenotypes from task-level performance across six computerized tasks. We evaluated associations with meaningful cognitive decline on the Preclinical Alzheimer's Cognitive Composite (PACC; ≥0.5 SD within-person decline), clinical progression, baseline tau PET burden, longitudinal tau accumulation, and plasma pTau-217 using Cox proportional hazards models and linear mixed-effects

models. Clustering baseline digital cognitive performance split participants into four phenotypes. One phenotype, encompassing 18% of Aβ+ individuals, concentrated risk for rapid cognitive decline, clinical progression, and tau spread. Relative to Aβ− participants, this group experienced rapid cognitive decline, with a mean 240-week PACC decline of −4.11 points (Fig1, CI −5.17 to −3.09) and nearly tripled risk of meaningful cognitive decline (HR 2.89; CI 2.26–3.77). Over follow-up, more than half progressed to mild cognitive impairment, corresponding to a nearly fivefold increase in hazard of clinical progression (HR 4.83; CI 3.61–6.44). This phenotype mapped directly onto AD biology, showing greater baseline medial temporal tau burden and accelerated neocortical tau accumulation over time (Fig2, all $p<0.05$ vs other phenotypes). Plasma pTau-217 and the digital phenotype captured complementary risk signals; individuals positive for both exceeded a fourfold hazard of meaningful cognitive decline (Fig3, HR 4.40; CI 3.32–5.84). Using a brief digital cognitive assessment, we identified a latent phenotype consisting of a subgroup of Aβ+, cognitively unimpaired individuals who are at disproportionately high risk for near-term cognitive decline and tau spread. When combined with plasma pTau-217, this scalable approach substantially improves prognostic precision, with direct implications for trial enrichment, monitoring strategies, and early therapeutic decision-making.

Evaluating Acute Psilocybin Treatment in Reversing Trauma-Induced Phenotypes in Rats

Briana Morales, Grad Student Mahler Lab

Psychedelic-assisted therapy is a re-emerging treatment that shows clinical promise in alleviating symptoms of psychiatric disorders including post-traumatic stress disorder (PTSD). Our research sought to determine if psilocybin reduces hyperreactivity to a mild stressor in previously “traumatized” rats—a behavioral phenomenon called stress-enhanced fear learning (SEFL).

The SEFL paradigm begins with the rats being exposed to unpredictable shocks in a specific context (Context A). Rats are then extinguished of the contextual “trauma” association for the next 4 days where they are placed in Context A for 30 minutes with no shock deliverance. On the same days as extinction, rats also underwent a one-hour habituation in a separate “Treatment Context.”

5 days after shock exposure, rats are given either psilocybin (1 mg/kg) or saline and left in the “Treatment Context” for an hour immediately after administration. Looking at some of the acute behavioral effects of psilocybin in this hour, females that received psilocybin had a significant increase in their head-twitch response, a measure of serotonergic psychedelic potency, compared to females that received saline. This was an increase not observed in males. Following treatment, rats were returned to their home cages for 2 days.

Lastly, rats underwent a SEFL test where they are placed in a novel context (Context B) and delivered one shock. Rats that received shock exposure in Context A had significantly higher freezing levels to the Context B shock compared to rats that did not receive these previous shocks. We found that psilocybin attenuated this increased freezing response during the SEFL test in females, but not in males at this dosage.

Our findings indicate that psilocybin alters behavior both acutely and persistently in a sex-dependent manner. This study adds to a growing body of evidence suggesting that psychedelic therapy can be successfully modeled in rats, and demonstrates the behavioral procedures best suited for doing so.

Grid-cell-like entorhinal signals distinguish ambulatory path integration tasks that probe different strategies

Alina Tu, Grad Student Chrastil Lab

An animal’s ability to sense its own motion and infer its position and orientation in space, or path integration (PI), underlies successful navigation. In rodents, cells in the entorhinal cortex exhibit grid-like firing patterns thought to support PI by metrically representing space. Human neuroimaging studies have provided indirect evidence for these entorhinal mechanisms, but the relationship between entorhinal grid-cell-like signals and PI performance remains unclear. To address this gap, we recruited 67 young adults to complete two ambulatory PI tasks (Triangle Completion and Loop Closure) in immersive virtual reality, post-task strategy surveys, and a neuroimaging session to measure entorhinal grid-like activity. Both PI tasks assess one’s ability to walk back to the start location, but Triangle Completion involves walking two legs of a triangle first, before walking to the start. Loop Closure involves walking in a circle and stopping at the start. Behavioral results suggest that continuously updating the trajectory to the start (a homing-vector strategy) was more effective for Triangle

Completion, whereas summing path segments to locate the start (a configural strategy) yielded better results for Loop Closure. Given the configural-strategy advantage in Loop, we hypothesized that entorhinal grid-cell-like signals would correlate more strongly with Loop performance than Triangle, supporting the use of metric information for configural strategies. Neural findings revealed a significant correlation between the magnitude of the entorhinal grid-cell-like signal and Loop accuracy, but not Triangle performance. Furthermore, significant grid-cell-like signals were observed in low, but not high, Triangle performers and high, but not low, Loop performers, providing the first evidence of a neural distinction between PI tasks. Examining how strategy use moderates entorhinal involvement in PI offers new insight into human path integration ability.

Therapeutic Potential of Human Microglial Transplantation for the Treatment of Sanfilippo Syndrome (MPSIIIA)

Alina Chadarevian, Grad Student Blurton-Jones Lab

Sanfilippo Syndrome (MPSIIIA) is a pediatric lysosomal storage disease that leads to progressive neurodegeneration and shortened life span. MPSIIIA results from loss of function mutations in SGSH, a lysosomal enzyme critical for the degradation and recycling of heparan sulfates (HS). Recent evidence suggests that microglia express the highest levels of SGSH within the brain. Therefore, correction of SGSH mutations within microglia, or the transplantation of healthy microglia, could potentially increase levels of this enzyme, slowing or even preventing further neurodegeneration

To explore the potential for human microglia transplantation, we generated a novel xenotolerant mouse model of MPSIIIA via CRISPR-deletion of SGSH. Following histochemical and biochemical validation, SGSH KO pups were transplanted with PBS or human iPSC-microglia progenitor cells. After 4 months, mice were sacrificed, peripheral blood and liver collected and hemibrains collected for immunohistochemical and biochemical analysis and single nuclei RNA sequencing.

Initial analysis of 4-month-old SGSH-KO mice reveals a dramatic increase in lysosomal content, gliosis, lipid accumulation, and elevated levels of nonreducing end heparan sulfates (HS-NRE). Remarkably, transplantation of human microglial progenitors significantly lowers lysosomal accumulation and reduces lysosomal content within murine neurons adjacent to engrafted human microglia. Biochemical analysis further revealed that microglia transplantation significantly reduces disease-specific HS-NRE. Ongoing experiments will further examine mRNA and protein levels to elucidate the impact of human microglial transplantation on other cell populations within the brain.

Together, these results provide the first evidence that iPSC-microglia could potentially be developed as a novel therapy for MPSIIIA and likely other lysosomal storage diseases.

Ecology and Evolutionary Biology

Harnessing Cyanobacterial Secondary Metabolites as Novel Drug Scaffolds

Marie-Désirée Schlemper-Scheidt, Postdoc Avalon Lab

Natural products continue to represent a prolific source of structurally diverse and pharmacologically relevant compounds for drug discovery.[1] Since the 1970s, cyanobacteria have been recognized as rich producers of secondary metabolites exhibiting a broad spectrum of biological activities against human diseases.[1] This current study focuses on an environmental sample of cyanobacteria collected in the Republic of Cabo Verde in May 2023. The organic extract was subjected to fractionation by normal phase medium pressure liquid chromatography. All fractions were evaluated using a panel of bioactivity assays. Several fractions showed activity against Trypanosoma brucei, the etiologic agent of African sleeping sickness, as well as against Plasmodium spp., which cause malaria. Furthermore, the fractions were evaluated for anticancer activity in NCI H460 and D-283-med cell lines.

Here, we present the bioactivity-guided isolation as well as the mass spectrometry and nuclear magnetic resonance-based elucidation of secondary metabolites from this Cabo Verdean cyanobacterial extract. Our findings highlight the therapeutic potential of marine cyanobacteria as a source of novel bioactive compounds and support continued exploration of underexplored marine ecosystems for natural product-based drug discovery.

Exploring the Neuroprotective Potential of Microbial-Derived Metallophores in Zebrafish

Jeremiah Meloch, Grad Student Avalon Lab

Microorganisms, such as bacteria or fungi, have evolved complex mechanisms to survive in diverse and extreme environments. One such mechanism is the acquisition of metal ions, which are essential cofactors for numerous enzymatic processes. Many microorganisms produce small-molecule natural products known as metallophores, which chelate metal ions and facilitate both metal acquisition and local metal detoxification. These compounds mediate the uptake of essential metals while also helping to mitigate metal toxicity under conditions of metal excess. Cyanobacteria, ancient photosynthetic prokaryotes, produce a diverse array of these metallophores that can be isolated and evaluated for biological activity. In humans, dysregulation of metal ion homeostasis in the brain has been implicated in the progression of Parkinson's disease, driven by dopaminergic neuron degeneration induced by oxidative stress. Therefore, we sought to explore the neuroprotective potential of a diverse panel of metallophores in a dopaminergic degeneration zebrafish model.

Systems Biology

mosaiQTL: A Python Framework for QTL Mapping in Multiparent Populations at Single-Cell Resolution

Ghassan Filimban, Grad Student Mortazavi Lab

Phenotypes are the product of genes interacting with the environment, and Quantitative trait loci (QTL) mapping in model organisms exploits structured genetic diversity among varied strains under controlled environmental conditions to link genomic variation to phenotypic differences. Gene expression is defined by gene regulatory architecture and heritable variants and is readily obtainable as a phenotypic readout at the cell-type level thanks to advances such as single-nucleus RNA sequencing (snRNA-seq). However, the mechanism by which genetic background and ancestral-defined regulatory architecture shape such a complex trait is poorly characterized. The mouse collaborative cross (CC) is a recombinant inbred multiparent population (MPP) panel derived from a genetically diverse set of eight founders, five of which are classical laboratory strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HILtJ) and three of which are wild-derived (CAST/EiJ, PWK/PhJ, and WSB/EiJ). Here, we present mosaiQTL, a multiomic tool to identify cell-type-specific QTLs (ct-QTLs) in multiple tissues from snRNA-seq profiles of a dataset consisting of the 8 CC founders, 7 F1 hybrid strains generated by crossing C57BL/6J dams with each of the remaining 7 founders, and 33 CC lines as a proof of concept. Our ongoing preliminary analyses incorporate kinship-structure correction and haplotype-resolved mapping to generate a fast, reliable Python implementation that improves on its R-based counterparts.

Differential methylation analysis of genetically diverse mice using Fiber-seq and pyDSS

Dayeon Cheong, Grad Student Mortazavi Lab

While short-read sequencing has historically mapped the differentially methylated patterns, it struggles to explain methylation states within complicated genomic loci and rearrangements. By contrast, long-read sequencing allows simultaneous capture of epigenetic modifications, especially with Fiber-seq being able to identify open chromatin, methylation, and hydroxymethylation. Hence, we have produced long-read sequencing data consisting of left and right cerebral cortex tissue of two female and two male replicates for each C57BL6/J (BL6) and CAST/EiJ (CAST) mouse strains, which are two of the Mouse Collaborative Cross (CC) Founder Strains and also the bridge samples of the IGVF (Impact of Genomic Variation on Function) Consortium. We have generated a total of 448.1 Gb (DNA Methylation, left cortex) and 531.2 Gb (Fiber-seq, right cortex) of long-read sequencing data (average coverage > 20x, average N50 > 30kb) using Oxford Nanopore Technologies.

In addition, we have developed pyDSS, which is a Python implementation of the BS-seq differential methylation workflow from the Bioconductor DSS (Dispersion Shrinkage for Sequencing data) package. Using this, we were able to perform a high-accuracy, production-ready Python implementation of differential methylation analysis, which identified differentially methylated regions (DMRs) that can be regulatory candidates for further functional validation and mechanistic studies by comparing the methylation levels across different genotypes and cortices. We are currently implementing FCAT(Fiber-seq read Cell type Annotation in Tissue), which is a tool that annotates individual Fiber-seq reads to cell types using multiome data, so that we can also identify DMRs across different cell types.

Mathematical, Computational & Systems Biology

GEMclear: High-Fidelity Spatial Proteomics for Deciphering Astrocyte Remodeling in Alzheimer's Disease

Yajin Mo, Grad Student Shi Lab

Protein function is fundamentally shaped by spatial context, yet most proteomic methods lose this information at subcellular resolution. Existing proximity labeling (PL) approaches enable in situ mapping of protein neighborhoods but often suffer from high background contamination, limiting their utility in complex tissues such as the brain. To address this challenge, I developed GEMclear, a high-fidelity spatial proteomics platform that integrates HRP-based proximity labeling with hydrogel embedding and electrophoretic clearing. This strategy selectively retains labeled proteins while removing unlabeled contaminants, substantially improving spatial specificity, signal-to-noise ratio, and proteome coverage in fixed cells and tissues. I apply GEMclear to investigate astrocyte remodeling in Alzheimer's disease (AD), where astrocytes transition from homeostatic to reactive states that contribute to neuroinflammation, synaptic dysfunction, and disease progression. I hypothesize that these transitions are driven by stage- and compartment-specific proteomic changes, particularly at synaptic interfaces and vascular endfeet. Using GEMclear, I aim to map subcellular astrocyte proteomes across disease stages, identify protein networks associated with neurotoxicity, and prioritize cell-surface and signaling proteins as therapeutic targets. Preliminary studies in AD mouse models demonstrate robust enrichment of astrocyte markers and detection of disease-relevant proteomic signatures. By extending this approach to human postmortem brain tissue and integrating computational tools to assess druggability, this work establishes a framework for linking spatial proteomics to therapeutic discovery. GEMclear provides a broadly applicable platform for understanding how protein organization drives cellular function in health and disease.

Mathematical and Mouse Models Identify T Regulatory Cell Influx as A Key Determinant of Acquired Resistance to PD-1 Immunotherapy

Rachel Sousa, Grad Student Lowengrub Lab

The immune system can eradicate cancer, but various immunosuppressive mechanisms active within a tumor curb this beneficial response. However, unraveling the effects of multimodal interactions between tumor and immune cells and their contributions to tumor control using an experimental approach alone is time- and resource-intensive. To identify the critical immunological features associated with tumor control and escape, we built a mechanistic, structurally identifiable mathematical model of the interactions between CD8+ T cells, Tregs, DCs, and tumor cells deeply rooted in current biological concepts. A distinguishing feature of our model is that it captures Treg accrual occurring after checkpoint blockade immunotherapy. After successfully fitting the mathematical model to experimental data from an immunogenic melanoma mouse model that shows acquired resistance to PD-1 immunotherapy and ensuring that the model was practically identifiable, we generated hundreds of parameter sets, each of which fits the data well and represents a unique 'virtual mouse' to capture variability across individuals. Our model indicates that the initial tumor and immune conditions instruct cancer control or progression and that there exist optimal initial ratios of immune cells that result in improved tumor control. The model further predicts that the Treg influx into the tumor is a key determinant of resistance to PD-1 immunotherapy. We validated all of these predictions experimentally. Overall, this integrated approach of modeling and experimental validation identified crucial determinants of resistance to PD-1 immunotherapy and can be used to guide the development of more effective therapeutic strategies.

Biomedical Engineering

Mitochondrial Transplantation as a Strategy to Reduce Noise-Induced Hearing Loss

Adam Ratajczak, Grad Student Djalilian Lab

Hearing loss is a prevalent and disabling condition with no curative treatment, highlighting the urgent need for new therapeutic strategies. Mitochondrial transplantation, the delivery of exogenous mitochondria into cells, has shown promise in mitigating mitochondria-related disorders. However, despite the central role of mitochondria in auditory function, this approach has not yet been explored for hearing loss.

Exogenous mitochondria were isolated from auditory cells and transferred into a new set of auditory cells following oxidative stress to evaluate their biological consequences. In vivo, isolated exogenous mitochondria were injected into the cochlea of mice, and their localization and impact on hearing after noise exposure were assessed.

Exogenous mitochondria from HEI-OC1 auditory cells were internalized in a dose-dependent manner without inducing toxicity. They enhanced bioenergetics, protected against oxidative stress, and reduced H_2O_2-induced apoptosis. In vivo, injected mitochondria localized to cochlear sensory cells, were well tolerated, and conferred protection against noise-induced hearing loss.

These findings provide the first evidence supporting the feasibility and therapeutic potential of mitochondrial transplantation in auditory cells. If validated in animal models and ultimately in humans, this strategy holds strong promise as a novel treatment for sensorineural hearing loss.

School of Medicine

Convergent DNA Methylation Abnormalities in Human Growth Disorders

Marie Wheeler, Grad Student Byun Lab

Loss-of-function mutations in DNMT3A, a DNA methyltransferase, or NSD1, a histone methyltransferase, cause overgrowth syndromes. Conversely, disruption of the DNMT3A domain that binds NSD1-deposited H3K36 dimethylation (H3K36me2) results in growth restriction. To investigate the molecular basis of these opposing growth outcomes, we generated isogenic human embryonic stem cells carrying growth syndrome–associated mutations in DNMT3A and NSD1. Unexpectedly, both overgrowth- and growth restriction–associated DNMT3A mutations led to DNA hypomethylation in a shared subset of active enhancers, implicating H3K36me2 in directing enhancer methylation maintenance. In contrast, bivalent promoters—marked by both active and repressive histone modifications—showed divergent DNA methylation changes: hypermethylation in growth restriction-associated DNMT3A mutants and hypomethylation in overgrowth-associated DNMT3A or NSD1 loss-of-function mutants. These findings identify locus-specific DNA methylation defects as a common molecular feature and nominate dysregulated DNA methylation at bivalent promoters as a potential driver of abnormal growth phenotypes.

1-methyladenosine is a tRNA-derived immunomodulatory metabolite secreted by mTORC1-active tumors in Tuberous Sclerosis Complex

Joohwan Kim, Postdoc Jang Lab

Patients with tuberous sclerosis complex (TSC) suffer from kidney angiomyolipoma, but the only available treatment, rapamycin, has cytostatic effects with serious adverse events, necessitating new therapeutic strategies. Through unbiased metabolomic and transcriptomic profiling and stable isotope tracing in tumor cells from TSC patients and mouse models, we identify tRNA methyltransferase 6 (TRMT6)-mediated 1-methyladenosine (m1A) tRNA modification as a conserved metabolic hallmark of TSC that modulates the tumor immune microenvironment. Mechanistically, mTORC1 activation by TSC1/2 mutations drives eukaryotic initiation factor 4A (eIF4A) to upregulate TRMT6 protein translation, thereby enhancing de novo m1A production on tRNA. Such m1A is secreted as a paracrine metabokine to reprogram macrophages toward immunosuppressive phenotypes via the adenosine receptor 3 (A3R)-ERK signaling. Pharmacological blocking of the TRMT6-m1A-A3R axis suppresses TSC tumor growth with decreased tumor-associated macrophages. Together, these findings establish mTORC1-driven m1A secretion as a previously unrecognized immunomodulatory mechanism, proposing this pathway as an immunotherapeutic target for treating TSC and other cancers with mTORC1 activation.

Regulatory T cells control anti-tumor microglia function in breast cancer brain metastasis

Isam Adam, Grad Student Lawson Lab

Breast cancer brain metastasis (BCBM) is linked with dismal outcomes. Current therapies fail due to their inability to cross the blood-brain barrier (BBB). Targeting immune cells in BCBM has garnered interest since immune cells naturally cross the BBB. Thus, there is a critical need to elucidate the immune microenvironment within BCBM. Regulatory T cells (Tregs) are immunosuppressive T cells implicated in several malignancies. However, their functions remain poorly understood in BCBM. Here we demonstrate that Tregs readily infiltrate the brain and suppress microglial responses to BCBM. Systemic Treg depletion in BCBM-bearing mice led to a massive accumulation of T cells and complete clearance of metastasis. We also found that microglia displayed a robust antigen presentation phenotype in Treg-depleted mice. Localized Treg depletion in the brain resulted in preferential expansion of conventional CD4 T cells (Tconv) 24h after Treg depletion and enrichment of antigen presentation in microglia at 48h. Single-cell RNA sequencing following localized Treg depletion identified microglia as the dominant transcriptional responders among all immune populations. These shifts were defined by upregulation of antigen presentation and interferon response programs, positioning microglia as the central cellular target of Treg-mediated suppression. In situ analysis showed that Tregs and microglia co-localize at the tumor-stroma interface, indicating crosstalk between these two populations. Using uLIPSTIC, a proximity-based contact labeling system, we further demonstrate that Tregs physically engage activated microglia in vivo. Strikingly, microglia depletion reversed tumor clearance after Treg loss and

significantly reduced Tconv infiltration. Integrated analysis of human brain metastasis and mouse BCBM scRNAseq data identifies ICOS-ICOSL signaling as the candidate axis mediating Treg-microglia suppression. Our data demonstrates that Tregs are central to BCBM progression through suppression of microglial antigen presentation and effector T cell responses, identifying Treg-microglia crosstalk as a promising immunotherapeutic target.

Unveiling organ crosstalk via circulating metabolite exchange at the whole-body level

Hosung Bae, Postdoc Jang Lab

Mammalian organs continuously produce and consume circulating metabolites for organismal health and survival. However, the landscape of this fundamental process and its perturbation by diet and disease is unknown. To address this gap, I applied advanced analytical platforms, including liquid chromatography–mass spectrometry (LC-MS)-based metabolomics, lipidomics, and stable isotope tracing, to map the regulation of metabolites across organs in vivo. I generated a cross-organ metabolic atlas encompassing eleven key organs under fasting/feeding states, Western diet exposure, and LDL receptor (LDLR) deficiency-induced cardiovascular disease. This work revealed both feeding-dependent and -independent patterns of metabolite production and consumption, as well as mechanisms by which Western diet perturbs these fluxes through altered metabolite gradients and hormone signals. Notably, I found that both Western diet and LDLR deficiency promote the release of bile acids from extrahepatic organs, contributing to elevated circulating bile acid levels and vascular inflammation. Together, these findings uncover complex inter-organ metabolic crosstalk and offer new biochemical insights into the systemic effects of diet and the pathogenesis of metabolic diseases.

Supervised contrastive learning and experimental validation identify QPCT-expressing monocytes implicated in Alzheimer's disease

Fujing Ge, Postdoc Yu Lab

The peripheral immune system is recognized as an important contributor to the pathogenesis of Alzheimer's disease (AD). However, the peripheral cell subpopulations linked to central nervous system pathology remain poorly defined. Here, we integrated multi-omics, biology-guided deep learning, and experimental validation to characterize the systemic immune landscape associated with AD. We identified a binary pathological signature in peripheral myeloid cells of AD patients, characterized by a hyperinflammatory state coupled with defective phagocytic clearance. Leveraging this biological discovery, we developed a supervised contrastive learning (SupCon) framework. The framework prioritized a QPCT-centered signature, from which a compact 7-gene panel achieved 95.5% accuracy in an external qPCR cohort. Subpopulation-level analyses together with β-amyloid (Aβ) uptake and degradation assays revealed that S100A12+CD14+ monocytes, characterized by high QPCT expression, were aberrantly increased in the peripheral blood of AD patients. Multiplex immunofluorescence (mIF) of hippocampus from patients with AD showed that S100A12+CD14+ myeloid cells were detected to be localized near damaged neurons. Collectively, our study identifies S100A12+CD14+ monocytes as a dysregulated peripheral myeloid state associated with neuronal injury, providing evidence for a periphery-to-brain immune axis in AD and suggesting a potential blood-based biomarker for AD diagnosis and monitoring.

Germinal Center Response Quality is Shaped by Immune-History Dependent Differences in the Memory B Cell Compartment

Erika Joloya, Grad Student Wagar Lab

Germinal center-derived responses form the foundation of protective immunological memory against subsequent exposure to viral antigens. As such, established immune memory in adults is usually correlated with robust antibody responses to recall antigens and characterized by increased somatic hypermutation (SHM). However, it remains unclear how different memory cell compartments derived from limited or more experienced immune histories influence the development of antigen-specific B cell responses. We used influenza, a common seasonal virus, as a model antigen to compare memory B

cell contributions to germinal center dynamics in pediatric and adult tissues. Using a tonsil immune organoid platform, we stimulated tonsillar tissues with influenza vaccine and deeply characterized hemagglutinin (HA)-specific B cell expansion using flow cytometry and single-cell RNA-sequencing (scRNAseq). We also assessed influenza-specific antibody production and neutralization. Although most pediatric donors responded poorly to influenza antigens, a subset of children mounted B cell and antibody responses comparable to that of vaccine-responding adults, suggesting underlying differences in memory B cell and germinal center behaviors in pediatric and adult tissue. To examine the relative contributions of different memory cell compartments derived from limited to more experienced immune reservoirs, we depleted memory cells and harvested influenza vaccine-stimulated organoids for subsequent scRNAseq analyses of HA-specific B cells, including transcriptional profiles and B cell receptor dynamics. Preliminary results suggest that memory B cell depletion attenuated robust HA-specific B cell responses to in vitro vaccine stimulation in both pediatric and adult donors. SHM analyses will further elucidate how memory depletions impact BCR repertoire diversification essential for the development of robust antigen-specific responses. Altogether, this study aims to elucidate how memory compartments shaped by disparate immune exposure histories in young children and adults may influence antigen-specific germinal center dynamics. These results provide important context for improving influenza vaccine strategies and on the establishment of immune memory.

Modeling ASXL1-driven Clonal Hematopoiesis in Human Stem Cells

Daniella Lu, Grad Student Byun Lab

Age is the dominant risk factor for many chronic diseases, yet the mechanisms linking aging to disease susceptibility are poorly understood. One key process implicated in age-related disease is the accumulation of somatic mutations in hematopoietic stem and progenitor cells (HSPCs), leading to clonal expansion of these advantageous mutations. This phenomenon, known as clonal hematopoiesis of indeterminate potential (CHIP), has been associated with an increased risk of adverse outcomes, including cardiovascular disease, myeloid malignancies, and all-cause mortality. Among recurrent CHIP-associated genes, ASXL1 is one of the most clinically significant yet mechanistically unresolved drivers.

ASXL1 encodes a chromatin regulator that modulates gene expression through epigenetic mechanisms, including histone modifications and chromatin remodeling. In both CHIP and myeloid malignancies, ASXL1 mutations are predominantly heterozygous frameshift or nonsense mutations clustered within the last two exons, resulting in escape from nonsense-mediated decay and production of stable C-terminally truncated proteins. Despite this, most functional ASXL1 studies rely on complete gene knockout or overexpression of mutant alleles, primarily in murine models, which do not recapitulate the physiologic consequences of heterozygous truncations in human cells. These limitations hinder our ability to understand whether ASXL1 mutations act through loss-of-function, dominant-negative, or gain-of-function mechanisms, and how these alterations reshape epigenetic landscapes during human hematopoietic differentiation.

To address these gaps, my work aims to model ASXL1 truncation in a precise and physiologic manner by generating isogenic human stem cells harboring a heterozygous, CHIP-relevant nonsense mutation. This approach enables direct comparison of wild-type and mutant ASXL1 function within a controlled human genomic background while maintaining the ability to differentiate these cells into hematopoietic progenitors and other relevant lineages. Using this system, I will characterize how ASXL1 truncation alters histone modification profiles, chromatin accessibility, and transcriptional programs across early hematopoietic development. Together, these studies will establish a human stem cell-based model for dissecting ASXL1 mutation biology and will provide mechanistic insight into how ASXL1-driven epigenetic dysregulation promotes clonal expansion and predisposition to early myeloid transformation. Elucidating these mechanisms is critical for understanding the adverse clinical impact of ASXL1 mutations and for identifying therapeutic vulnerabilities in ASXL1-mutant disease.

School of Pharmacy

Zebrafish as an efficient low-cost model for expression studies of mRNA-LNP developed for Vaccine or Therapeutic applications

Jichen Yang, Kim Lab

"Lipid nanoparticle (LNP) is a cheap, safe, and efficient method to deliver carriers to the host through the uptake by the target cells. For vaccine or therapeutic purposes, LNP can carry mRNA molecules that encode pathogenic proteins or metabolic enzymes, which will stimulate the immune system or compensate for the cell deficiency, according to their respective goals. Either way, as quality control, a flexible and efficient system for mRNA expression is usually required, and zebrafish embryos, as an in vivo fish model, fulfill all these technical requirements.

In this study, we designed a new mRNA structure adding the UTRs from the Salmon Albumin gene, flanking the gene of the m-NEON Green fluorescent protein (mNG), which was codon optimized for zebrafish expression. This mRNA was encapsulated in LNP composed of the cationic lipid ALC3015, a similar formulation to the Pfizer-BioNTech COVID-19 vaccine. The mRNA-LNP was soluble in a buffer 10% sucrose at 400 ug/ml, and the following doses, 10 ng/uL and 400 ng/uL were injected into yolk or early embryonic cells. The expression of the mNG was recorded on a fluorescence microscope, and the fluorescence quantification was performed from the recorded image using the ImageJ software.

The time-lapse experiment shows that in both yolk and embryonic cell injection, the green fluorescence started to express in the injected area. On top of that, higher dosage injection showed a higher fluorescence level while maintaining the same survival rate.

Zebrafish injected with our LNP-mRNA can successfully express mNG in both yolk and cells with a higher dosage. Taken together, this work supports zebrafish embryos as a rapid and accessible in vivo platform for testing LNP-mRNA formulation for future vaccine or therapeutic development."