Annual Research Day 2024 Platform Presentations - April 17, 2024, PHAB 2B

Time	Name	Sponsor	Department	Classification	Title
1:00 PM	Sailee Chavan	Chongmin Huan	Cell Biology	Molecular & Cellular Biology	Effect of hydroxychloroquine on B cell tolerance in the germinal center
1:15 PM	Shreya Desikan	Chongmin Huan	Department of Cell Biology	Molecular & Cellular Biology	The Potential Role of Insufficient X Chromosome Inactivation in Impairing SMS2-Regulated Germinal Center B Cell Tolerance in Systemic Lupus Erythematosus
1:30 PM	Pargol Mashati	Prem Premsrirut	Cell Biology	Molecular & Cellular Biology	Evaluating the therapeutic potential of a novel ADAM10 modulator in Colorectal Cancer
1:45 PM	Joao Moreira	Salvador Dura-Bernal	Physiology and Pharmacology	Neural & Behavioral Science	Computational Model of the Mouse Whisker Thalamocortical Pathway
2:00 PM	Riley Morrone	Peter Bergold	Physiology and Pharmacology	Neural & Behavioral Science	3R Tau Mediates Acute Neurodegeneration Following Closed Head Injury
2:15 PM	Andrew Patera	Prem Premsrirut	Molecular and Cellular Biology	Biomedical Engineering	Engineering Molecular Probes for Diagnostic Applications
2:30 PM	Victoria Tung	Oleg Evgrafov	Cell Biology	Molecular & Cellular Biology	Elucidating the Neurodevelopmental Mechanisms of Schizophrenia through Cultured Mesenchymal Cells Derived from Olfactory Neuroepithelium
2:45 PM	Andrew Wang	Jin Montclare	Cell Biology	Biomedical Engineering	Targeted Multidomain Protein Nanomicelles for the Treatment of HER2+ Breast Cancer

Sailee Chavan Advisor(s): Chongmin Huan

Effect of hydroxychloroquine on B cell tolerance in the germinal center

Rationale: Systemic lupus erythematous is an autoimmune disease mediated by antinuclear antibodies. Hydroxychloroquine (HCQ), an antimalarial agent, has been used for nearly 60 years as a first-line lupus treatment. HCQ prevents lupus flares by suppressing lupus autoimmunity but sparing normal immune functions. However, the underlying mechanism of HCQ remains unknown. According to our hypothesis, HCQ may enhance the protective B cell tolerance mediated by SMS2 in the germinal center. We reported that SMS2 is required for preventing lupus pathogenesis in mice by activating the pro-apoptotic activity of PKCδ is autoreactive GC B cells. As HCQ has been reported to increase SM synthesis, we hypothesize that SMS2-regulated GC B cell tolerance is mediated by HCQ. Methods: In vivo analysis involved treating NZBWF1 mice with 16mg/kg/day HCQ for 4 weeks. Disease indicators such as serum autoantibody levels (ELISA), proteinuria (Bradford Assay), GC B cell proportion (Flow cytometry) were analyzed. For mechanistic studies, In vitro analysis was conducted by isolating B cells from wild-type and SMS2KO mice using MACS protocol. HCQ's effects on apoptosis and SMS2 expression were analyzed using Flow cytometry. The role of reactive oxygen species (ROS) in SMS2 expression and HCQ's impact on ROS-mediated SMS2 expression were also investigated in vitro. Results: After 4 weeks, 16mg/kg/day HCQ significantly reduced proteinuria and GC B cell proportion compared to controls. However, no significant decrease in serum autoantibody levels was observed, suggesting the need for treatment optimization. Mechanistically, HCQ increased apoptosis and SMS2 expression in cultured B cells. ROS inhibition decreased SMS2 expression, indicating ROS's role in SMS2 expression. Significance: 30-40% of lupus patients stop HCQ due to intolerance or toxicities, causing frequent flares. Understanding HCQ's mechanism can help develop therapies that can reduce disease burden and disparities in lupus treatment.

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Shreya Desikan Advisor(s): Chongmin Huan

The Potential Role of Insufficient X Chromosome Inactivation in Impairing SMS2-Regulated Germinal Center B Cell Tolerance in Systemic Lupus Erythematosus

Background: Systemic Lupus Erythematosus (SLE) is a female-biased autoimmune disease that is normally prevented by the self-protective mechanism of germinal center (GC) B cell tolerance in healthy individuals. Current evidence points to overexpression of X-linked genes, caused by dysregulated X chromosome inactivation, as a key mechanism underlying the female-bias of SLE. However, how overexpression of X-linked genes may contribute to the loss of GC B cell tolerance remains unknown. We have reported that GC B cell tolerance is regulated by sphingomyelin synthetase 2 (SMS2), whose expression is drastically reduced in SLE patients' B cells through unknown mechanisms. Here, we study whether loss of SMS2-regulated GC B cell tolerance is a female biased phenomenon, and if this process is driven by an overexpressed X-linked gene.
Methods: Published genetic studies were analyzed to identify a candidate X-linked gene that inhibits SMS2 expression. Genetic mouse models were studied to confirm this activity in GC B cells. RNAseq data was used to analyze the candidate gene in SLE patients' B cells. Candidate gene expression in GC B cells the NZBWF1 SLE mouse model was analyzed by RT-PCR.
Results: 1) Loss of SMS2-regulated GC B cell tolerance in SLE is female-specific. We found that impaired SMS2-regulated GC B cell tolerance in female NZBWF1 mice was associated with reduced GC B cell Sgms2 mRNA compared to their male counterparts. 2) Lysine demethylase 6A (KDM6A), an XCI escapee and transcriptional regulator, is overexpressed in XX splenocytes, SLE patient B cells, and in GC B cells of female NZBWF1 mice. 3) KMD6A inhibits SMS2 expression in GC B cells. We found that KDM6A deficient GC B cells had increased SMS2, consistent with the published data in other cell types.
Conclusions: Loss of SMS2-regulated germinal center B cell tolerance is female-specific, likely due to overexpression of KDM6A. Additional work is needed to elucidate this mechanism further.

Pargol Mashati Advisor(s): Prem Premsrirut

Evaluating the therapeutic potential of a novel ADAM10 modulator in Colorectal Cancer

Colorectal cancer, the world's second leading cause of cancer death, necessitates new therapeutic strategies due to current treatment side effects. ADAM10 metalloproteinase plays a crucial role in colorectal cancer tumorigenesis by activating oncogenic pathways like Notch and EGFR, making it a potential therapeutic target. Interestingly, ADAM10 is known to adopt two states: an open active conformation and a closed inhibited conformation. The active conformation of ADAM10, recognized for its increased proteolytic activity, appears more frequently in colorectal tumor cells. Our study focuses on the therapeutic potential of a novel human monoclonal antibody (hmAb) that specifically targets the active conformation of ADAM10 in colorectal cancer. This hmAb binds ADAM10's Disintegrin and Cysteine-rich domains, which are known to facilitate the transition between ADAM10's open and closed states.

In vitro, we evaluated this antibody anti-tumoral effects via cell viability assays and analysis of the impacts on major signaling pathways. Our preliminary findings show that the antibody effectively reduces cell viability in colorectal cancer cell lines COLO205, SW620, and DLD-1. Notably, cellular treatment with this anti-ADAM10 antibody inhibits Notch activation in COLO205 and SW620 cells, respectively. In the DLD-1 cell line, characterized by activation of EGFR and lacking active Notch signaling, the antibody successfully inhibits the phosphorylation of EGFR. This suggests the breadth of the antibody's impact across different signaling pathways, emphasizing its therapeutic potential. Our future in vivo studies, used in conjunction with chemotherapy regimens, will help elucidate the full therapeutic potential of this antibody in colorectal cancer management.

In conclusion, our findings underscore the therapeutic potential of this novel hmAb. Its demonstrated ability to diminish cellular viability in colorectal cancer cell lines and impede activation of key oncogenic pathways sig

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Joao Moreira Advisor(s): Salvador Dura-Bernal

Computational Model of the Mouse Whisker Thalamocortical Pathway

Recent studies in the whisker pathway of rodents awake rodents highlighted the existence of monosynaptic projections from thalamocortical (TC) to layer 6 corticothalamic (L6 CT) neurons. These projections enable a short-latency feedback pathway that bypasses the full loop in the cortical column, but its function remains poorly understood [1]. This feedback could work as a mechanism to selectively increase the responsiveness of specific thalamic neurons to incoming streams of information while silencing others, contributing to the emergence of direction-selective angular tuning in the somatosensory network. In this work, we developed a detailed computational model of the mouse whisker pathway in NetPyNE to study the effect of this direct L6 CT feedback in regulating network excitability [1]. We characterized the network based on the angular tuning response of thalamic neurons to different whisker deflection angles and evaluated the contribution of direct activation of L6 CT neurons by the thalamus in this process. We also developed a novel realistic model of whisker deflection responses in the brainstem based on different deflection angles, providing topological feedforward inputs to the thalamus. Our current results show that the architecture of thalamic projections is crucial for preserving the angular tuning across the network and that CT feedback is essential to keep the balance of thalamic excitation. Next, we will test the influence of the timing of this CT feedback, which we believe is key to sharpening the angular tuning in the thalamic network to brainstem inputs. Ultimately, our model will provide insights into the mechanisms that regulate thalamocortical excitability and how interactions between L6 CT neurons and the thalamus can shape the information arriving at the cortex.

1. Hirai D, Nakamura KC, Shibata K-I, et al. Shaping somatosensory responses in awake rats: cortical modulation of thalamic neurons. Brain Struct Funct. 2018;223: 851–872.

Riley Morrone Advisor(s): Peter Bergold

3R Tau Mediates Acute Neurodegeneration Following Closed Head Injury

White matter is particularly vulnerable to traumatic brain injury (TBI). Acceleration-deceleration of the head during TBI injures and demyelinates axons both proximal and distal to the injury site. This produces cytoskeleton damage that impairs axonal transport. Axonal microtubules are crosslinked and stabilized by tau protein. Alternative splicing of the tau gene results in tau protein isoforms containing either three (3R) or four (4R) microtubule binding sites. Adult mouse axons express only 4R tau; human axons express both 3R and 4R tau. 3R tau containing-axons are less stable and more flexible than 4R tau containing-axons. TBI produces cognitive deficits that arise, in part, from white matter degradation. I tested if 3R tau expression alters disease course after an experimental TBI. Using a closed head injury TBI model, white matter damage and cognition was compared between C57/Bl6 wildtype mice (WT) with 4R tau containing-axons, and microtubule-associated protein tau knock-in (MAPTKI) mice with 3R and 4R tau containing-axons. Righting reflex, a measure of initial injury, does not differ between WT and MAPTKI mice. At 14 days post injury, spatial memory is evaluated using Active Place Avoidance in WT and MAPTKI mice. Injured MAPTKI mice acquire Active Place Avoidance, whereas injured WT mice are significantly impaired. Following behavioral testing, amyloid precursor protein assesses impaired fast axonal transport; the myelin probe Fluoromyelin-Red assesses myelin content; and NeuN assesses hippocampal neuronal density. The corpus callosum of injured WT mice has significantly greater demyelination and accumulates more amyloid precursor protein than injured MAPTKI mice. Injured WT and MAPTKI mice, however, have similar hippocampal neuronal loss. These data suggest that Active Place Avoidance deficits in injured WT mice arise from increased white matter damage. These data suggest that 3R tau expression mediates acute neurodegeneration following closed head injury.

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Andrew Patera Advisor(s): Prem Premsrirut

Engineering Molecular Probes for Diagnostic Applications

The SARS-CoV-2 pandemic highlighted the need for highly sensitive and rapid pathogen testing as the use of lateral flow and PCR-based screening assays failed to adequately limit the spread of disease. High affinity molecular probes coupled to electronic transducers can be adapted to develop sensitive and portable diagnostics. We analyzed an array of biomolecular probes, including antibodies, computationally designed peptides and aptamers and tested their integration into a graphene field effect transistor (GFET). By studying the performance of each biomolecular probe in a variety of media, we were able to develop a GFET-based system that could rapidly detect 10s of SARS-CoV-2 viral particles per reading. To fabricate this system, we used thermochemical scanning probe lithography (tSPL) to selectively expose reactive sites on the GFET channel to conjugate probes at the nanometer scale, allowing for multiplexing and miniaturization. The compatibility, affinity, and detection conditions for each probe was explored to develop an optimized GFET that was able to detect spike proteins and live viral particles. Beyond traditional biosensors, this work also resulted in the development of a new type of molecular probe that detects pathogens by exploiting the presence of pathogen-specific restriction endonucleases as a biomarker. DNA duplexes and hairpins were engineered to produce sequence-specific restriction endonuclease probes (REProbes) which are resistant to exonucleases, but sensitive to the desired restriction enzyme(s) of specific species of pathogens. This REprobe was validated with cultured E. Coli lysates at detection limits as low as 10^4 CFU/mL utilizing techniques such as spectrophotometry, surface plasmon resonance (SPR) and pulse voltammetry. Importantly, we demonstrate a clinical application of this technology with cultured N. gonorrhoeae validated by spectrophotometry, with the potential to implement this technology on the described GFET platform.

Victoria Tung Advisor(s): Oleg Evgrafov

Elucidating the Neurodevelopmental Mechanisms of Schizophrenia through Cultured Mesenchymal Cells Derived from Olfactory Neuroepithelium

The development of schizophrenia (SCZ) is associated with disruption during brain development, but direct studies of these processes in affected individuals are not feasible due to the inability to observe live brain development in living individuals. We seek to overcome this challenge by examining neurodevelopment in the olfactory neuroepithelium, which continuously regenerates throughout human life and can serve as a cellular model for studying neurodevelopmental processes. We generated cell lines obtained from olfactory neuroepithelium (CNON) from more than 300 individuals. Using single-cell sequencing (scRNA-seq), we identified these cultured cells as cultured mesenchymal cells, based on their transcriptomic profile, which resembles that of mesenchymal stem cells. Furthermore, the gene expression profile of these cultured mesenchymal cells corresponded to a distinct cell type found in the early stages of brain development, notably around week 5 to 6. In culture mesenchymal cells obtained from an individual with SCZ (CNON-SCZ), we identified an upregulated gene expression of genes associated with WNT signaling pathway and extracellular matrix (ECM) organization. These pathways are important for the brain's structural development, indicating that alterations in these processes may contribute to etiology of schizophrenia. By characterizing these cultured mesenchymal cells, we aim to elucidate their role in neurodevelopment and establish their utility as a cellular model for investigating cellular mechanisms of schizophrenia and identifying potential therapeutic targets.

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Andrew Wang Advisor(s): Jin Montclare

Targeted Multidomain Protein Nanomicelles for the Treatment of HER2+ Breast Cancer

Approximately 15-20% of breast cancers overexpress the human epidermal growth factor receptor-2 (HER2/erbB2) and are traditionally known to be aggressive, metastatic, and associated with a poor prognosis. Pioneering work by Langer and others have demonstrated the appeal and feasibility of nanoparticle drug delivery systems for treating metastatic cancer. Nanoparticle formulations can be designed to possess favorable pharmacokinetic properties, including the sustained release of chemotherapeutics, or be targeted to alter their biodistribution, reducing off-target effects. Previously, the Montclare group has developed a multidomain fusion protein called Thermo-Responsive Assembled Protein (TRAP), which forms nanomicelles that encapsulate and release drug under hyperthermic conditions. This drug encapsulation was shown to be due in part to a hydrophobic pore in the coiled-coil ("C") domain. We hypothesize that 1) adding an additional C domain to our TRAP construct (TRAP2) will improve its drug loading capacity and 2) attaching a HER2+ binding peptide (P51) to the N-terminus will improve cellular targeting. Therefore, we designed and characterized the novel protein construct P51-TRAP2, and evaluated its ability to deliver cargo to HER2 overexpressing SKBR-3 cells. As expected, the addition of a C domain increased the helicity of the construct as measured by circular dichroism. P51-TRAP2 displays significantly improved hydrophobic area compared to TRAP, and encapsulates an increased amount of drug. Micellar size was not significantly increased, but the number of monomers per micelle (Nagg) was decreased. Flow cytometry and ELISA were used to assess P51-TRAP2 association with HER2. P51-TRAP2 bound to HER2 with high nanomolar affinity and demonstrated improved uptake in and cytotoxicity against SKBR-3 cells compared to untargeted TRAP2 and TRAP. As a result, P51-TRAP2 shows promise as a targeted drug delivery carrier, and future in vivo experiments are planned.