CBIS 13th Biennial meeting 2022

Abstracts (alphabetic order - last name)

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Structural basis for the activation of insulin receptor

We determined the full-length structure of the IR in its insulin bound, active state. This structure unveiled a previously unknown secondary insulin binding site in IR (named site 2) beyond that observed in previous structures (site 1). Using designed site selective insulin mutants, we further showed that IR with insulin only bound at site 1 adopts an asymmetric conformation, which correlates with partial activation in cells. Insulin binding to site 2 destabilizes the asymmetric state, promoting formation of a more stable symmetric 'T'-shaped IR dimer and full activation. Together, our studies revealed how insulin binding to both sites 1 and 2 cooperatively promotes the full activity of IR, and explained how IR can respond differently to a wide range of insulin concentrations in different metabolic states.

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Structure and mechanism of type V CRISPR-Cas12 nucleases

CRISPR-Cas systems are adaptive immunity systems in bacteria and archaea against mobile genetic elements and have been developed as tools for genome editing. These systems use guide RNAs and effector proteins to specifically target foreign nucleic acids for degradation. Recently, functionally diverse Cas12 nucleases have been developed to expand genome editing applications. I will discuss our structural and mechanistic studies of Cas12 nucleases and how these nucleases are adapted to their respective functions.

Rui Chang **Yale University** rui.chang@yale.edu

The coding logic of interoception

Interoception, the ability to timely and precisely sense changes inside the body, is critical for survival. Vagal sensory neurons (VSNs) form an important body-to-brain connection, navigating visceral organs along the rostral–caudal axis of the body and crossing the surface–lumen axis of organs into appropriate tissue layers. The brain can discriminate numerous body signals through VSNs, but the underlying coding strategy remains poorly understood. Here we show that VSNs code visceral organ, tissue layer and stimulus modality—three key features of an interoceptive signal—in different dimensions. Large-scale single-cell profiling of VSNs from seven major organs in mice using multiplexed projection barcodes reveals a 'visceral organ' dimension composed of differentially expressed gene modules that code organs along the body's rostral–caudal axis. We discover another 'tissue layer' dimension with gene modules that code the locations of VSN endings along the surface–lumen axis of organs. Using calcium-imaging-guided spatial transcriptomics, we show that VSNs are organized into functional units to sense similar stimuli across organs and tissue layers; this constitutes a third 'stimulus modality' dimension. The three independent feature-coding dimensions together specify many parallel VSN pathways in a combinatorial manner and facilitate the complex projection of VSNs in the brainstem. Our study

highlights a multidimensional coding architecture of the mammalian vagal interoceptive system for effective signal communication.

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Reversing lysosome-mTORC1-ribosome axis dysregulation mitigates C9FTD/ALS neurodegeneration and behaviors

G4C2 repeat expansion in C9orf72 causes the most common familial frontotemporal dementia and amyotrophic lateral sclerosis (C9FTD/ALS). The pathogenesis includes haploinsufficiency of C9orf72, which forms a protein complex with Smcr8, as well as G4C2 repeat-induced gain-of-function including toxic dipeptide repeats (DPRs). The key in vivo disease-driving mechanisms and how loss- and gain-of-function interplay remain poorly understood. Here we identified a lysosome-mTORC1-ribosome biogenesis dysregulation as an early and key disease mechanism using a physiologically relevant mouse model with combined loss- and gain-of-function across the ageing process. C9orf72 deficiency exacerbates FTD/ALS-like pathologies and behaviors in C9ORF72 bacterial artificial chromosome (C9-BAC) mice with G4C2 repeats. Single nucleus RNA sequencing (snRNA-seq) and bulk RNA-seq revealed that C9orf72 depletion disrupts lysosomes in neurons and leads to mTORC1 signaling overactivation coupled with transcriptional dysregulation of ribosomal protein (RP) genes. Importantly, ectopic expression of C9orf72 or its partner Smcr8 in C9FTD/ALS mutant mice promotes lysosomal functions and restores mTORC1 activity and ribosome biogenesis gene transcription, resulting in the mitigation of DPR accumulation, neurodegeneration, as well as FTD/ALS-like motor and cognitive behaviors. Therefore, we conclude that loss- and gain-of-function crosstalk in C9FTD/ALS converge to cause neuronal dysregulation of a lysosome-mTORC1-ribosome biogenesis axis leading to the proteotoxicity, neurodegeneration, and behavioral defects.

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Circadian Regulator CLOCK Drives Immunosuppression in Glioblastoma

The symbiotic interactions between cancer stem cells and the tumor microenvironment (TME) are critical for tumor progression. Here, we show that CLOCK and its heterodimeric partner BMAL1 in glioma stem cells (GSC) drive immunosuppression in GBM. Mechanistically, CLOCK-directed olfactomedin-like 3 (OLFML3) upregulates legumain (LGMN) in GSCs via HIF1α signaling. As a result, LGMN promotes microglial infiltration into the GBM TME via upregulating CD162 and polarizes infiltrating microglia toward an immune-suppressive phenotype. In GBM mouse models, inhibition of the CLOCK-OLFML3-HIF1α-LGMN-CD162 axis reduces intratumoral immune-suppressive microglia, increases CD8+ T-cell infiltration, activation, and cytotoxicity, and synergizes with anti-PD-1 therapy. In human GBM, the CLOCK-regulated LGMN signaling correlates positively with microglial abundance and poor prognosis. Together, these findings uncover the CLOCK-OLFML3-HIF1α-LGMN axis as a molecular switch that controls microglial biology and immunosuppression, thus revealing potential new therapeutic targets for patients with GBM.

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Tagging endogenous proteins for structural studies by single particle cryo-EM

INO80 is an ATP dependent chromatin remodeling complex, and plays essential roles in regulating transcription, DAN replication and DNA repair. Structural studies of INO80 in complexes with its substrates by single particle cryo-EM provide new mechanistic insights into how INO80 slides nucleosome.

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Small-molecule inhibitor screen reveals novel biology of SARS-CoV-2

Using a recombinant SARS-CoV-2 reporter virus-based compound screening approach, we identified several small-molecule inhibitors that potently block viral replication. Among them, JIB-04 showed potent in vitro and in vivo antiviral activities against SARS-CoV-2. In addition, several calpain inhibitors suppressed the replication of SARS-CoV-2, a chimeric vesicular stomatitis virus (VSV) encoding the SARS-CoV-2 spike protein (VSV-SARS-CoV-2), but not wild-type VSV. This came as a surprise because many protease inhibitors target the SARS-CoV-2 main protease (Mpro) and VSV-SARS-CoV-2 does not encode Mpro. Genetic knockout of calpain-2 by CRISPR/Cas9 rendered the host cells resistant to VSV-SARS-CoV-2 and a clinical isolate of SARS-CoV-2. Reduced viral RNA and spike protein levels were observed early in infection in calpain-2 knockout cells, suggesting a potential role of calpain-2 in viral entry. Our results highlight an Mpro-independent antiviral mechanism of action by calpain inhibitors and shed light on a novel function of calpain-2 in mediating SARS-CoV-2 entry.

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New tricks for old proteins: Numb regulates Hedgehog signaling in the cilium

Hedgehog (Hh) signaling is widely involved in embryonic development and adult stem cells homeostasis. The transduction of Hedgehog (Hh) signaling relies on the primary cilium, a cell surface organelle acting as a signaling hub for the cell. Using proximity labeling and mass spectrometry, we studied the ciliary proteome and identified Numb as a new ciliary protein that positively regulates the Hh pathway. Numb localizes to the ciliary pocket and acts as an endocytic adaptor to incorporate Ptch1 into clathrin-coated vesicles, thereby promoting Ptch1 exit from the cilium, a key step in Hh signaling activation. Numb loss impaired Sonic Hedgehog-induced Ptch1 departure from the cilium, resulting in severe attenuation of Hh signaling. Genetic ablation of Numb and its homolog Numblike in the developing cerebellum impaired the proliferation of granule cell precursors, a Hh-dependent process, resulting in reduced cerebellar size. This study demonstrates a key function of Numb in controlling protein levels in the cilium, and highlights Numb's critical role in the regulation of Hh signaling and Hh-dependent developmental events.

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A karyopherin inhibits nuclear condensation of MOS4-associated complex to prevent aberrant immune activation in plants

The nucleocytoplasmic exchange of macromolecules is fundamentally important for eukaryotic life. This essential process is mediated by karyopherins, a superfamily of nuclear transport receptors. Despite their functional importance, the substrate identities of different karyopherins and mechanisms by which

karyopherins regulate their substrate activities (dependent or independent of nuclear transport) are poorly understood, particularly in plants. Here, we identified an Arabidopsis karyopherin KA120 that is required for the suppression of immunity activated by the nucleotide-binding leucine-rich repeats receptors (NLRs), including SNC1. We leveraged the newly developed proximity labeling proteomics to profile in vivo substrates of KA120 and identified a conserved protein complex, namely the Prp19 complex, which has been reported to play a role in a series of critical cellular processes in humans and yeast, including RNA splicing and DNA damage response. In plants, the Prp19 complex is known as the MOS4-associated complex (MAC), which is essential for immune activation. Remarkably, we found that the MAC complex forms MAC-defined nuclear condensates (MDNCs) through phase separation, and their formation is induced by various immune elicitors and leads to defense gene expression. We demonstrated that the formation of MDNC is inhibited by KA120, revealing an unexpected chaperoning function of KA120 in a transport-independent manner.

Guoye Guan **Center for Quantitative Biology, Peking University** **guanguoye@gmail.com**

Comparison between phase-field model and coarse-grained model for characterizing cell-resolved morphological and mechanical properties in a multicellular system

Embryonic development is a precise and complex process involving the cell morphology and mechanics interacting in space and time. The difficulty in quantitatively acquiring cellular morphological and mechanical information in vivo makes mathematical modeling a challenging problem and impedes model validation. Recently, the three-dimensional time-lapse live imaging and delineated developmental programs in the roundworm Caenorhabditis elegans provide an excellent platform for establishing the quantitative models. In this paper, we study two popular computational models for multicellular systems, i.e., the phase-field model and coarse-grained model, and compare their performance in characterizing the cell morphologies, cell adhesion, and cell stiffness in a real C. elegans embryo. We show that both models can capture cell-cell contact areas and heterogeneous cell adhesion, but only the phase-field model succeeds in inferring the heterogeneous cell stiffness by fitting cell shapes or cell-cell interface curvatures. Moreover, we demonstrate that the phase-field model converges to the coarse-grained model when increasing cell surface tension to dominance, obtaining a distance-dependent isotropic intercellular force. The paper is under review in Communications in Nonlinear Science and Numerical Simulation.

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Using Drosophila to study SARS-CoV-2 entry, pathogenesis, and therapeutics

How to use the powerful genetic tools of Drosophila to fight COVID-19 is a question that fascinated me during the pandemic. Inspired by the work using flies to identify the Zika virus NS4A protein to be the cause of microcephaly from Dr. Hugo Bellen's lab, my lab developed fly models to screen the tissue-specific pathogenicity of SARS-CoV-2 proteins and identified Orf6 & Nsp6 as two major pathogenic proteins. We then identified host interactions of Orf6 & Nsp6, as well as the hijacked host pathways underlying their pathomechanisms. We also identified mechanism-based drugs (Selinexor for Orf6 and 2G for Nsp6), and demonstrated that these drugs could block the pathogenesis of these SARS-CoV-2 proteins in both flies and human cells. To study SARS-CoV-2 entry, we developed the ACE2 Fly Model with VSV-Spike infection system. This system allows quick testing of new SARS-CoV-2 Spike variants, as well as drugs that could block the entry mediated by Spike and ACE2 binding. This system can also be

used to test and validate human ACE2 variants associated with SARS-CoV-2 infection susceptibility. Our work here established a powerful new animal model to aid our fight against COVID-19 and new coronavirus in the future.

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TMEM106B regulates microglial proliferation and survival in response to demyelination

TMEM106B, a type II transmembrane protein located within the late endosome/lysosome compartments, is intimately linked to brain aging and brain disorders. An interesting link between TMEM106B and brain inflammation has been shown in recent studies, but how TMEM106B regulates inflammation is unknown. Here we report that TMEM106B deficiency in mice leads to reduced microglia proliferation and activation and increased microglial apoptosis in response to demyelination. Lysosomal pathway is significantly altered in TMEM106B deficient microglia. In addition, TMEM106B loss results in a significant decrease in the protein levels of the triggering receptor expressed on myeloid cells 2 (TREM2), an innate immune receptor essential for microglia survival and activation. Specific ablation of TMEM106B in microglia results in similar microglial phenotypes and myelination defects in mice, supporting that microglial TMEM106B is critical for proper microglial activities and myelination process. Furthermore, TMEM106B risk allele is associated with myelin loss and decreased microglial numbers in the white matter in humans. Collectively, our study revealed a novel function of TMEM106B to promote microglial survival and myelination.

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What do Whales tell us about Fractal, Endothermy and Organ Regeneration?

Why do mammalian organs including the heart lose regenerative potential during the perinatal window remains enigmatic. Through phylogenetic analysis of vertebrate cardiomyocyte ploidy as a proxy for cardiac regenerative potential, we uncover that certain monotreme, edentate, cetacean, chiropteran species have unusually high percentages of diploid cardiomyocytes in the adult heart. Cardiomyocyte abundance across 41 vertebrate species conforms to Kleiber's law, the 3/4-power law scaling of metabolism with body weight, and decreases when the standard metabolic rate and body temperature increase during the ectotherm-to-endotherm transition. Recently, following the fractal feature of the cardiovascular system, we further strengthen the link between endothermy and heart physiology. Thermogenesis increases by more than 10-fold during the ectotherm-to-endotherm transition requiring similar increases in blood flow and cardiac function that may impede heart regenerative potential. Moreover, we report sympathetic nerve-adrenergic receptor and thyroid hormone signaling as two major pathways promoting both thermogenesis and cardiomyocyte cell cycle arrest. Combined inhibition of postnatal adrenergic and thyroid hormone signaling results in juvenile mice with strikingly low body temperatures (~25°C), significantly elevated abundances of diploid cardiomyocytes (~60%), dramatically increased cardiomyocyte proliferation, and enhanced cardiac regenerative abilities when analyzed at postnatal day 14. Our findings support critical roles of two major thermogenic pathways in suppressing heart regenerative capacity, and implicate that the limited regenerative capacity in various adult mammalian tissues may be a tradeoff for the acquisition of endothermy in ontogeny and phylogeny.

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Targeting Neuroinflammation in Epilepsy and Glioma

The research in our laboratory has long been dedicated to a better understanding of the molecular mechanisms whereby a normal brain is transformed to one generating seizures, a process called epileptogenesis. Uncovering the biology of epileptogenic processes is critical to developing a cure for acquired forms of epilepsy, as current FDA-approved antiseizure drugs (ASDs) merely provide symptomatic relief, not to mention their wide-ranging and often unbearable adverse effects. Our early work revealed that the prostaglandin E2 (PGE2) via its receptor subtype EP2 contributes to brain cytokine storm and injury, blood-brain barrier breakdown, reactive gliosis, and behavioral abnormality in rodents following de novo status epilepticus, resulting in lifelong epilepsy. Our most recent study suggested that this Gαs-coupled receptor is also involved in the development and progression of glioma, the most common and lethal brain tumor that constitutes a major cause of epilepsy, particularly in the elderly. In collaboration with medicinal chemistry laboratories, we have developed the first-in-class brain-permeable bioavailable antagonists that are highly selective and potent to EP2 receptor (US Patent: 10758516). These novel small-molecule compounds hold translational potential to interrupt both glioma genesis and electrogenesis, two highly interactive pathogenic processes within glioma-bearing brains.

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Repeat-RNA-mediated toxicity in C9orf72 ALS/FTD

GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene is the most common genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). The repeat is bidirectionally transcribed and confers gain of toxicity by RNA-mediated toxicity (one proposed mechanism is via sequestering essential RNA binding proteins into RNA foci) and/or by producing toxic dipeptide repeat proteins via non-canonical repeat-associated non-AUG-dependent translation. However, the underlying toxic species is debated. This talk will focus on repeat-RNA-mediated toxicity by C9orf72 repeat expansions.

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A unified framework for realistic in silico data generation and statistical model inference in single-cell and spatial omics

In the single-cell and spatial omics field, computational challenges include method benchmarking, data interpretation, and in silico data generation. To address these challenges, we propose an all-in-one statistical simulator, scDesign3, to generate realistic single-cell and spatial omics data, including various cell states, experimental designs, and feature modalities, by learning interpretable parameters from real datasets. Furthermore, using a unified probabilistic model for single-cell and spatial omics data, scDesgin3 can infer biologically meaningful parameters, assess the quality of cell clusters and trajectories, and generate in silico negative and positive controls for benchmarking computational tools.

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Machine learning assisted elucidation of circulating tumor stem cell clusters in cancer metastasis
Tumor-initiating cells with reprogramming plasticity or stem-progenitor cell properties (stemness) are thought to be essential for cancer development and metastatic regeneration in many cancers; however, elucidation of the underlying molecular network and pathways remains demanding. Combining machine learning and experimental investigation, here we report CD81, a tetraspanin transmembrane protein known to be enriched in extracellular vesicles (EVs), as a newly identified driver of breast cancer stemness and metastasis. Membrane CD81 interacts with CD44 through their extracellular regions in promoting tumor cell cluster formation and lung metastasis of triple negative breast cancer (TNBC). CD81 and CD44 regulate endocytosis pathways, enhance EV-associated stemness-stimulation of recipient cells, and promote circulating tumor cell (CTC) cluster formation in cancer metastasis. Our study highlights machine learning as a powerful tool in facilitating the molecular understanding of new molecular targets in regulating stemness and metastasis of TNBC.

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Immune responses to SARS-CoV-2 infection and mRNA vaccination
As an RNA virus, SARS-CoV-2 continues to evolve and evade the host immunity. In this talk, I will discuss the host antibody response to SARS-CoV-2 natural infection and mRNA vaccination. I will highlight the correlations between the titters of neutralizing antibody and disease severity, as well as differences between COVID infection and vaccination, especially with different doses of mRNA vaccines. I will also describe the extent and durability of antibody in neutralizing major SARS-CoV-2 variants, including Omicron subvariants. Our results underscore the importance of booster administration in control of the virus spread and global COVID pandemic.

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The kinase PLK1 promotes the development of Kras/Tp53-mutant lung adenocarcinoma through transcriptional activation of the receptor RET
Increased abundance of polo-like kinase 1 (PLK1) is observed in various tumor types, particularly in lung adenocarcinoma (LUAD). Here, we found that PLK1 accelerated the progression of LUAD through a mechanism that was independent of its role in mediating mitotic cell division. Analysis of human tumor databases revealed that increased PLK1 abundance in LUAD correlated with mutations in KRAS and p53, with tumor stage, and with reduced survival in patients. In a mouse model of KRASG12D-driven, p53-deficient LUAD, PLK1 overexpression increased tumor burden, decreased tumor cell differentiation, and reduced animal survival. PLK1 overexpression in cultured cells and mice indirectly increased the expression of the gene encoding the receptor tyrosine kinase RET by phosphorylating the transcription factor TTF-1. Signaling by RET and mutant KRAS in these tumors converged to activate the mitogen-activated protein kinase (MAPK) pathway. Pharmacological inhibition of the MAPK-pathway kinase MEK combined with inhibition of either RET or PLK1 markedly suppressed tumor growth. Our findings show that PLK1 can amplify MAPK signaling and reveal a potential target for stemming progression in lung cancers with high PLK1 abundance.

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Infer 1D and 3D epigenomes by the fragmentation patterns in circulating cell-free DNA

Circulating cell-free DNA (cfDNA) in the plasma is not randomly fragmented into small pieces and is recently associated with the cellular epigenomes, suggesting the possibility of computationally inferring the cellular epigenomes from cfDNA fragmentation. However, the computational tools are still not available to comprehensively infer the cellular epigenomes from cfDNA whole-genome sequencing (WGS). Here, we developed a set of computational tools to facilitate the study of cellular epigenomes non-invasively by fragmentation patterns measured from cfDNA WGS. First of all, current research on the development of computational methods for cfDNA fragmentation patterns is significantly limited by the controlled access of the cfDNA WGS. Thus, we built and maintained a comprehensive database and browser to host >3,000 uniformly processed and curated de-identified cfDNA WGS for the liquid biopsy community. Second, given that local nucleosome structure reduces the fragmentation process, we developed a computational method to de novo characterize the genome-wide fragmentation hotspots at cfDNA WGS. In healthy, hotspots are enriched in gene-regulatory elements, including open chromatin regions, promoters, hematopoietic-specific enhancers, and, interestingly, 3'end of transposons. The aberration of hotspots detected in early-stage cancers allows us to understand the gene-regulatory mechanism in early-stage diseases and diagnose early-stage cancers and their tissues of origin with high performance. Finally, the fragment sizes of cfDNA from active and repressive compartments are known to be different. Therefore, we developed a computational method to reconstruct the three-dimensional epigenome using the cfDNA co-fragmentation patterns between pairs of genomic bins. The computational methods developed for cfDNA fragmentation in our lab will eventually pave the roads for our understanding of the variation of cis-regulatory elements non-invasively across different physiological and pathological conditions.

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Deciphering functional links between genome organization and gene regulation

Deconstructing the mechanism by which the 3D genome encodes genetic information to generate diverse cell types during animal development remains a major challenge in biology. The contrast between the elimination of chromatin loops and domains upon Cohesin loss and the lack of downstream gene expression changes at the cell population level instigates intense debates regarding the structure-function relationship between genome organization and gene regulation. Here, by analyzing single cells after acute Cohesin removal with sequencing and spatial genome imaging techniques, we discover that, instead of dictating population-wide gene expression levels, 3D genome topology mediated by Cohesin coordinates cross-domain gene co-regulation at the single cell level. Notably, Cohesin loss increases gene expression correlation between active domains in cis, limiting the efficiency and ability of a cell population to acquire proper cell fates during lineage specification. In addition, Cohesin separates Mediator hubs, prevents distant genes from localizing into shared hubs and blocks intersegment transfer of diverse transcriptional regulators. Together, these results support that spatial organization of the 3D genome orchestrates dynamic gene co-expression and related regulatory activities in single living cells.

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Heterogeneity and coordination of individual hematopoietic stem cells

Tissue homeostasis and regeneration are sustained by many individual stem cells. To understand the roles of individual stem cells in these processes, we have developed an integrated in vivo clonal tracking and single cell RNA sequencing system to perform high-throughput and quantitative analyses of the hematopoietic stem cell network at a genome-wide scale. Using mouse hematopoietic stem cells as a primary model, we will present our findings on the quantitative differences in blood production between individual stem cells and how these differences are coordinated to maintain an overall balanced blood supply. We will also discuss cellular heterogeneity during hematopoietic aging and leukemia progression using primary mouse and human cells. Our findings provide new insights into the cellular network underlying tissue regeneration during both healthy and diseased conditions from a single cell perspective.

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Targeting antigen presentation to potentiate cancer immunotherapy

While most of the immunotherapies are deemed to increase the activity of cytotoxic T lymphocytes (CTLs), cancer cells can be eradicated only if their tumor antigens are presented and recognized by CTLs. Not surprisingly, cancer cells have developed a number of mechanisms to evade from immune attacks by molecular evolution during tumor progression. For a majority of human tumors with functional MHC-I genes, they may carry those so-called “soft lesions” to suppress tumor antigen processing and presentation. To better understand immune evasion of human cancer, we developed a computational biology tool to identify target genes in human cancer that are significantly associated with immune cell infiltration and cytotoxicity. Furthermore, we have been establishing a patient-derived tumor organoid model to facilitate the discovery of new drugs as adjuvant in immune checkpoint blockade therapy.

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Epigenetic regulation of breast cancer initiation and progression

27-hydroxycholesterol (27-HC) is the most abundant oxysterol that increases the risk of breast cancer progression. However, little is known about epigenetic regulation of 27-HC metabolism and its role in breast tumor initiation. Using genetic mouse mammary tumor and human breast cancer models, we showed here that the histone reader ZMYND8 was selectively expressed in breast cancer stem cells (BCSCs) and promoted epithelial-mesenchymal transition (EMT), BCSC maintenance and self-renewal, and oncogenic transformation through its epigenetic functions, leading to breast tumor initiation. Mechanistically, ZMYND8 was a master transcriptional regulator of 27-HC metabolism. It increased cholesterol biosynthesis and oxidation but blocked cholesterol efflux and 27-HC catabolism leading to accumulation of 27-HC in BCSCs. Consequently, 27-HC promoted EMT, oncogenic transformation, and tumor initiation through activation of liver X receptor. These findings reveal that ZMYND8 is an epigenetic booster that drives breast tumor initiation through metabolic reprogramming.

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Dissecting Brain Circuitry Using Connectomic and Novel Imaging Approaches

The Mao laboratory is interested in elucidating brain circuit mechanisms underlying animal behaviors, such as sensori-motor interactions and motor control, and understanding how these circuits are changed and modulated by disease, brain state and behavioral context. We use cutting-edge technology including modern anatomy, imaging, computation, genetics and functional circuit mapping in the mouse model to examine the principles governing neuronal connectivity and their regulation. This talk will discuss using whole brain imaging approach combined with machine learning based algorithms to establish comprehensive connectomic maps at the mesoscopic scales (~300 μm) between brain regions that process external and internal information and the motor output based on such information. Novel structural principles governing the neuronal connectivity will be presented. I will next illustrate the examples of using the comprehensive structural connectomic maps to further our understanding of circuit function. Combined with a novel imaging modality that we recent developed, I will present single cell resolution live imaging of the changes of these circuits under the modulation of opioids and different brain states. This work highlights the necessity and importance of large scale, data-driven structure-function analyses, as well as the power of cutting-edge imaging technology to guide the understanding of circuit functions.

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The NF-κB-microRNA regulatory network tunes inflammatory responses in alcohol associated liver diseases

Background: Alcohol associated liver disease (ALD) is a syndrome of progressive inflammatory liver injury associated with long-term heavy intake of ethanol. Elevated miR-34a expression and hepatic NF-κB activity in alcoholic liver disease, and their correlation with the degree of inflammation and fibrosis have been reported. The current study aims to characterize the functional role of miR-34a-regulated steatohepatitis during ALD. Methods: Expression of inflammation related genes and miR-34a was assessed using a PCR Array and/or real-time PCR analysis in LPS-treated human hepatocytes (N-Heps), as well as in liver specimens from a mouse model of chronic and binge ethanol feeding for five weeks (the NIAAA model) relative to control liver tissue. The upstream modulators and downstream mediators of steatohepatitis were defined in LPS-treated N-Heps in vitro by Western blot and real-time PCR assay. The in vivo anti-inflammation effects were evaluated in TLR-4 knockout mice or the morpholino antisense oligomer against miR-34a (miR-34a Morpho/AS) treated mice with chronic and binge ethanol feeding. Results: We identified that 5 weeks of ethanol feeding significantly increased the total liver histopathology score and miR-34a expression, along with the liver inflammation by enhanced myeloperoxidase (MPO) staining. Treatment of N-Heps with LPS (20 ng/ml) for 24 hr significantly increased miR-34a expression, along with the enhanced NF-κB activity and reduced Sirt1 expression. Silencing of miR-34a decreased LPS-induced activation of NF-κB in N-Heps by upregulation of Sirt1. Interestingly, silencing of miR-34a in human N-Heps also stimulated oxidative energy production via the activation of AMPK, PPARα and PGC-1α, and deacetylated the p65 subunit of NF-κB complex, whereas silencing of the LPS receptor, TLR4, inhibited NF-κB activation through Sirt1 upregulation in the same group of N-Heps, suggesting anti-miR-34a reversed LPS-mediated NF-κB activation through Sirt1 related oxidative energy production mechanism. Furthermore, the expressions of miR-34a and its target Sirt1, and NF-κB subunit p65 were significantly altered in isolated hepatocytes from ethanol-fed mouse liver specimens compared to controls. TLR4 knockout mice and miR-34a Morpho/AS treated mice displayed less sensitivity to alcoholic injury, along with enhanced Sirt1, AMPK, PPARα and PGC-1α levels in isolated

hepatocytes, and reduced hepatic expressions of inflammation markers MPO, LY6G, CXCL1 and CXCL2. Summary and Conclusion: Our results show that miR-34a mediated Sirt1/NF-κB signaling is essential for the steatohepatitis during alcohol induced liver injury. These findings provide new insight into the function of microRNA-regulated liver inflammation, and implication for reversing steatohepatitis with potential therapeutic benefits in human alcoholic liver diseases.

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Post-translational Modifications of Soluble α-Synuclein Regulate the Amplification of Pathological α-Synuclein

Cell-to-cell transmission and the subsequent amplification of pathological proteins is a key process for the progression of various neurodegenerative diseases. Recent studies on the transmission process have focused on pathological seeds. What has generally been ignored is the function of the normal counterparts of pathological proteins in regulating the transmission process. Here, using α-Synuclein (α-Syn), the pathological protein for a group of neurodegenerative diseases including Parkinson's disease, as an example, we show that phosphorylation of soluble α-Syn dramatically affects the amplification of pathological α-Syn. Importantly, this effect is conformation and phosphorylation site-specific. To systematically explore the landscape of post-translational modifications (PTMs) on soluble α-Syn, LC-MS/MS analyses were performed on soluble α-Syn purified from PD and various α-Synucleinopathies, leading to the identification of a large number of novel α-Syn PTMs. Significantly, acetylation of soluble α-Syn also modified the transmission of pathological α-Syn in a site and conformation specific manner. Moreover, phosphorylation of soluble α-Syn could also modulate the seeding properties of pathological α-Syn. In summary, our study represents the first systematic analysis of the role of soluble α-Syn PTMs in the spreading of pathological α-Syn, which is a critical novel mechanism that modulates the amplification of pathological proteins and affects disease progression.

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Tissue Proteomics Reveals RNA Splicing Dysfunction in Alzheimer's Disease: From Discovery to Animal Models

Recent developments in proteomics and transcriptomics enable deep profiling of proteome and transcriptome in Alzheimer's disease (AD), holding the promise of discovering novel disease genes/proteins. These profiling studies of postmortem human brain tissues reveal RNA splicing dysfunction and U1 small nuclear ribonucleoprotein (snRNP) pathology containing U1-70K and its N-terminal 40-KDa fragment (N40K). Here we present a causative role of U1 snRNP dysfunction to neurodegeneration in primary neurons and transgenic mice (N40K-Tg), in which N40K expression exerts a dominant-negative effect to downregulate full-length U1-70K. N40K-Tg recapitulates N40K insolubility, erroneous splicing events, neuronal degeneration and cognitive impairment. Specifically, N40K-Tg shows the reduction of GABAergic synapse components (e.g., the GABA receptor subunit of GABRA2), and concomitant postsynaptic hyperexcitability that is rescued by a GABA receptor agonist. Crossing of N40K-Tg and the 5xFAD amyloidosis model indicates that the RNA splicing defect synergizes with the amyloid cascade to remodel the brain transcriptome and proteome, deregulate synaptic proteins, and accelerate cognitive decline. Thus, our results support the contribution of U1 snRNP-mediated splicing dysfunction to AD pathogenesis, providing a novel pathway for disease treatment. (The related manuscript is provisionally accepted by Nature Aging.)

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structural insights into dynein motor coordination

Thousands of outer-arm dyneins (OADs) are arrayed in the axoneme to drive a rhythmic ciliary beat. Coordination among multiple OADs is essential for generating mechanical forces to bend microtubule doublets (MTDs). Using electron microscopy, we determined high-resolution structures of Tetrahymena thermophila OAD arrays bound to MTDs in two different states. OAD preferentially binds to MTD protofilaments with a pattern resembling the native tracks for its distinct microtubule-binding domains. Upon MTD binding, free OADs are induced to adopt a stable parallel conformation, primed for array formation. Extensive tail-to-head (TTH) interactions between OADs are observed, which need to be broken for ATP turnover by the dynein motor. We propose that OADs in an array sequentially hydrolyze ATP to slide the MTDs. ATP hydrolysis in turn relaxes the TTH interfaces to effect free nucleotide cycles of downstream OADs. These findings lead to a model explaining how conformational changes in the axoneme produce coordinated action of dyneins.

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Stromal cell-immune cell interactions in the lung pre-metastatic niche

Primary tumors are drivers of pre-metastatic niche formation, but the coordination by the secondary organ toward metastatic dissemination is underappreciated. By single-cell RNA sequencing and immunofluorescence, we identified a population of cyclooxygenase 2 (COX-2)-expressing adventitial fibroblasts that remodeled the lung immune microenvironment. At steady state, fibroblasts in the lungs produced prostaglandin E2 (PGE2), which reprogramed different types of myeloid cells to be immunosuppressive or undergo metabolic changes. This lung-intrinsic stromal program was propagated by tumor-associated inflammation to foster a pre-metastatic niche formation. Genetic ablation of Ptgs2 (encoding COX-2) in fibroblasts was sufficient to reverse the immune-suppressive phenotypes of lung-resident myeloid cells, resulting in heightened immune activation and diminished lung metastasis in breast cancer models. Moreover, fibroblast-specific Ptgs2 deletion or dual inhibition of PGE2 receptors EP2 and EP4 effectively improved the therapeutic effects of immunotherapies. Collectively, lung-resident fibroblasts reshape the local immune landscape to facilitate breast cancer metastasis.

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Targeting MTDH/SND1 Protein Complex to Boost Immunotherapy Response in Metastatic Breast Cancer

Metastatic breast cancer remains a significant health threat to women worldwide. Curative treatment options for this disease are urgently needed. A growing body of evidence suggests that tumor microenvironment (TME) plays pivotal roles in cancer treatment responses. However, how TME contributes to breast cancer progression and metastasis, and the therapeutic potential by remodeling TME are still elusive. Recently, we found that the protein complex of Metadherin and Staphylococcal nuclease domain-containing 1 (MTDH/SND1) inhibits antigen presentation in tumors to result in an immune suppressive microenvironment, and thus, promotes breast cancer progression and metastasis. The small chemical inhibitor C26-A6 that we developed specifically and effectively disrupts MTDH/SND1 complex, and thus, remodels TME to enhance anti-PD-1 treatment response in metastatic breast cancer.

Our study provides novel insights into the mechanism of TME in promoting breast cancer progression and metastasis, and opens a new avenue for developing therapies against metastatic breast cancer.

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HUMAN IPSC-BASED DISEASE MODELING AND THERAPEUTIC DEVELOPMENT

The iPSC technology has provided great hope for serving as a platform for disease modeling, drug discovery, and cell therapy development. We have used the human iPSC (hiPSC) platform to model neurological disorders and develop cell therapies for these debilitating diseases. For example, using neurons and astrocytes derived from hiPSCs, we demonstrated ApoE isoform-dependent SARS-CoV-2 neurotropism and cellular response. Moreover, our findings suggest that ApoE4 may play a causal role in COVID-19 severity. In addition, we have established stem cell therapy candidates for Canavan disease (CD), a devastating neurological disease that has neither a cure nor a standard treatment. We tested the hiPSC-derived cellular products in a Canavan disease mouse model and demonstrated robust efficacy and preliminary safety of the cellular products. This study could provide an effective therapeutic approach for Canavan disease, and other related neurological diseases.

Huizhong Tao **University of Southern California** htao@usc.edu

A bottom-up sensory pathway for reward-related behavior

Valence detection and processing are essential for the survival of animals and their life quality in complex environments. Neural circuits underlying the transformation of external sensory signals into positive valence coding to generate appropriate behavioral responses remain not well-studied. Here, we report that somatostatin (SOM) subtype of GABAergic neurons in the mouse medial septum complex (MS), but not parvalbumin subtype or glutamatergic neurons, specifically encode reward signals and positive valence. Through an ascending pathway from the nucleus of solitary tract and then parabrachial nucleus, the MS SOM neurons receive rewarding taste signals and suppress the lateral habenula. They contribute essentially to appetitive associative learning via their projections to the lateral habenula: learning enhances their responses to reward-predictive sensory cues, and suppressing their responses to either conditioned or unconditioned stimulus impairs acquisition of reward learning. Our results suggest that MS serves as a critical hub for transforming bottom-up sensory signals to mediate appetitive behaviors.

Dong Wang **University of California San Diego** dongwang@ucsd.edu

A Scenic Byway: Molecular Basis of Transcription Elongation, Blockage, and Repair

During transcription elongation, RNA polymerase II (Pol II) moves along DNA template, recognizes the template base, and synthesizes RNA with a high fidelity. Transcription elongation process is subject to pausing and arrest by various obstacles such as pause-inducing DNA sequences or secondary structures, DNA modifications, DNA lesions, DNA-binding proteins and small molecules. Here we will present our recent progress in understanding the structural basis of lesion recognition, pausing, arrest, and repair. In particular, we will focus on recent results related to transcription blockage, recognition of DNA lesions and unnatural base pairs, as well as transcription-coupled repair.

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SARS-CoV-2 main protease inhibitor design and a comprehensive study of nirmatrelvir drug resistance
SARS-CoV-2 main protease is a validated antiviral drug target of Pfizer's oral COVID drug nirmatrelvir. As Mpro is a cysteine protease, the target specificity of Mpro inhibitors is a major hurdle in drug design. This talk will focus on two aspects, one is our efforts in developing selective Mpro inhibitors with novel reactive warheads or non-covalent binding mode, and the other is the comprehensive study of nirmatrelvir drug resistance. With the increasing prescription of Paxlovid, resistance is going to emerge sometime soon. Our study has identified multiple nirmatrelvir resistant mutants that could be clinically relevant. The results will also help gudie the design of second generation of Mpro inhibitors with a high genetic barrier to drug resistance.

Shizhen Emily Wang **UC San Diego** emilywang@ucsd.edu

The Systemic Effects of Cancer-derived Extracellular miRNA
Recent studies find that adaptation of a distant niche (the “soil” for disseminated cancer cells) during pre-/metastatic stages is critical for the development of metastases. In addition, cancer cells cause a wide range of systemic effects to interfere with normal physiological functions. These distant effects can be mediated by extracellular vesicles (EVs, including exosomes) secreted by cancer cells. Our group has been interested in understanding cancer-directed systemic effects with a focus on the miRNA cargo of cancer cell-secreted EVs, which can be transferred to neighboring or distant cells to modulate cell functions. Circulating miRNA has emerged as potential biomarkers for cancer diagnosis and prognosis. Overall, cancer-secreted miRNAs encapsulated in EVs could mediate tumor-directed adaptations of non-cancer tissues at the systemic level and may serve as novel therapeutic targets for patients with high blood levels of those miRNAs.

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High-content image-based CRISPR screening reveals regulators of 3D genome folding architectures
Three-dimensional (3D) genome organization is significantly altered in development, aging, and diseases. Recent technological advances have enabled systematic characterization of the 3D genome across multiple length scales. However, mechanistic and functional investigations of the 3D genome are limited by a lack of methods to screen for molecular regulators of the multi-scale 3D genome folding architectures. To enable high-throughput and efficient discovery of regulators of the 3D genome, here we developed an image-based high-content CRISPR screen method. We designed a pooled loss-of-function screen targeting 137 genes, identified individual gene perturbations in single cells in situ with an image-based cellular barcoding technique, and visualized the effects of the perturbations on chromatin organization by tracing the folding conformations of human chromosomes with megabase resolution in the same single cells. Using 1.4 million imaged 3D positions along chromosome traces, we identified candidate regulators controlling one or more chromatin folding and nuclear features at different length scales, including previously known 3D genome regulators NIPBL and CTCF and hits unknown to function in higher-order 3D genome organization. Particularly, CHD7 promotes chromatin compaction at multiple length scales. The high-content image-based screen method allows for investigating the effects of diverse gene mutations on complex 3D genome phenotypes.

Tina Wang **University of Texas Medical Branch** ti1wang@UTMB.EDU

Investigating acute and long-term impacts of SARS-CoV-2 infection on the central nervous system
SARS-CoV-2 and its emerging variants of concern (VOCs) have posed a significant threat to the global healthcare system. Acute neurological complications, ranging from mild symptoms to life-threatening encephalopathy and central nervous system (CNS)-mediated respirator stress have been reported in COVID19 patients. Up to 50% survivals suffer a post-viral syndrome known as long-COVID. Currently, the underlying mechanism of SARS-CoV-2 -induced neurological complications are not well understood. We recently characterized acute and chronic infections of SARS-CoV-2 VOCs and the virus- induced immune responses in the CNS in K18-hACE2 mice. We also found that mice surviving acute infection of VOCs displayed abnormalities in neuropsychiatric state, motor behavior, autonomic function, and sensory function for several months, regardless of the severity of virus-induced acute diseases, which recapitulated long-COVID symptoms in humans. Identifying viral and host factors associated with long-COVID will provide valuable information for development of strategies to prevent and treat patients with long-COVID.

Yingfei Wang **UT Southwestern Medical Center** yingfei.wang@utsouthwestern.edu

AIF3 splicing, mitochondrial dysfunction and neurodegeneration
Apoptosis-inducing factor (AIF) is a mitochondrial flavoprotein controlling both cell life and death. Recently, we identified a novel disease-inducible AIF isoform defined as AIF3 distinct to two other known isoforms. AIF3 was undetectable under physiological conditions, but its expression was induced in human diseases including ischemic-hypoxic brain injury and Alzheimer's disease. We established conditional inducible AIF3 splicing mouse model as well as AIF3 transgenic mouse model. Induction of AIF3 splicing in the mouse brain caused mitochondrial dysfunction and neurodegeneration. AIF3 splicing mice died 3-4 months after birth with neuron loss in the cortex and hippocampus and enlarged ventricles. Mechanistically, AIF3 inhibited NADH oxidase activity, ATP production, oxygen consumption, and mitochondrial biogenesis. Expression of AIF3 also increased chromatin condensation and nuclear shrinkage prior to the neuronal cell death observed in mouse brain. The synergistic effect of loss-of-AIF and gain-of-AIF3 is likely to contribute to AIF3 splicing-induced mitochondrial dysfunction and neurodegeneration. Together, our study provides valuable tools to understand the role of AIF3 splicing in brain and a potential therapeutic target to prevent/delay the progress of neurodegenerative diseases.

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Modeling Fragile X syndrome with human iPSC models
Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and a leading genetic cause of autism. FXS is caused by the loss of functional fragile X mental retardation protein (FMRP), an RNA-binding protein that can regulate the translation of specific mRNAs. Despite major progress to characterize underlying disease mechanisms in animal models that has led to several clinical trials, improvements of behavioral and cognitive outcomes in patients have unfortunately been unsuccessful, a strong need for human-specific models of FXS to understand the unique factors that underlie human disease and to test the efficacy of candidate compounds. Here we have developed human induced pluripotent stem cell (iPSC) models, including 3-D cortical organoids and microglia, to molecular and cellular mechanisms underlying pathogenesis of FXS, and revealed molecular, cellular,

and electrophysiological abnormalities associated with the loss of FMRP during human brain development.

Stephen Wong **Houston Methodist Academic Institute** STWong@houstonmethodist.org

Systems Biology and omics-driven approaches for target identification and drug discovery

During the past decade, bioinformatics-driven approaches have been emerged to be a powerful means for delineating mechanisms of dynamic, complex cell-cell interactions within tissue microenvironment and for discovering novel targets for drug repositioning and development. This short talk will present an overview of a multicellular systems biology strategy to understand the underlying cell-cell communication within tumor microenvironment using cell-specific, single cell, and spatial transcriptomics, multiplex imaging, and multi-cellular crosstalk modeling in a comprehensive, unbiased manner. We will provide an overview applications and findings of tumor microenvironment of different organs, such as the bones, brain, lungs, and ovaries to illustrate the power of systems biology modeling for hypotheses generation, tumor heterogeneity delineation, and target and drug discovery

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Decoding RNA dynamics and cell state specific regulatory network with Time-resolved Single-cell RNA Sequencing

Single-cell RNA sequencing offers snapshots of whole transcriptomes but obscures the temporal RNA dynamics. Here we present an improved version of single-cell metabolically labeled new RNA tagging sequencing (scNT-Seq), a method for massively parallel analysis of newly-transcribed ("new") and pre-existing ("old") mRNAs from the same cell.. Using scNT-Seq, we jointly profiled new and old transcriptomes in tens of thousands of mouse primary cortical cells. These data revealed distinct patterns of newly synthesized mRNAs at single-cell level in response to brief or sustained neuronal activation. We further showed that measuring new RNA levels of target genes linked to a neuronal activity regulated transcription factor (TF) can temporally resolve TF regulatory network activity in single neurons. Using a computational approach that explicitly incorporates metabolic RNA labeling-based single-cell measurements, we performed labeling based RNA velocity analysis to infer cell state trajectories during the highly dynamic neuronal activation process (minutes to hours). Finally, with pulse chase experiments, scNT-Seq can more accurately estimate RNA synthesis and degradation rates, revealing RNA regulatory strategies in rare stem cell populations. High-throughput time-resolved single-cell transcriptomics thus provides a broadly applicable strategy to investigate cell-type-specific RNA regulatory mechanisms in dynamic biological processes such as neuronal activation and stem cell plasticity.

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Delineating the Heterogeneity and Regulation of Astrocytes in Spinal Cord Injury

We previously generated an RNA-Seq transcriptome and splicing database of neurons, glia and vascular cells of the cerebral cortex, including not only coding but also long non-coding RNAs (lncRNAs) which are regulatory RNAs playing important roles in the CNS. Recently, we have investigated the molecular changes in the injury environment and the astrocyte-specific responses by isolating astrocyte lineage cells from injured spinal cords. We identified a lncRNA Zeb2os playing an essential role in astrogliosis through the Zeb2os/Zeb2/Stat3 axis. Currently, we are dissecting the heterogeneity of astrocyte

lineage cells in SCI by scRNA-seq. We found subpopulations with distinct functional enrichment and their identities defined by subpopulation-specific transcription factors and regulons. Immunohistochemistry, RNAscope and stereology experiments verified the molecular signature, location and morphologies of potential resident neural progenitors in the adult spinal cord, and uncovered the previously undescribed populations. This study reveals new insights in the heterogeneity and cell state transition of glial progenitors before and after injury.

Longjun Wu **Mayo Clinic** wu.longjun@mayo.edu

Neuroimmune interaction: how microglia sense neuronal activity

This talk will discuss using whole brain imaging approach combined with machine learning based algorithms to establish comprehensive connectomic maps at the mesoscopic scales (~300 μm) between brain regions that process external and internal information and the motor output based on such information. Novel structural principles governing the neuronal connectivity will be presented. I will next illustrate the examples of using the comprehensive structural connectomic maps to further our understanding of circuit function. Combined with a novel imaging modality that we recent developed, I will present single cell resolution live imaging of the changes of these circuits under the modulation of opioids and different brain states. This work highlights the necessity and importance of large scale, data-driven structure-function analyses, as well as the power of cutting-edge imaging technology to guide the understanding of circuit functions.

Zhuhao Wu **Icahn School of Medicine at Mount Sinai** zwu@rockefeller.edu

Holistic imaging approach to appreciate brain structural and functional complexity

The rapid advancements in tissue clearing and whole mount imaging approaches have greatly expedited systematic investigation of organ-wide cellular compositions and interactions. Our group has developed several iterations of iDISCO-family tissue clearing protocols (iDISCO, iDISCO+, and AdipoClear) to enable whole mount immunolabeling, high-throughput imaging, and volumetric analysis of large intact organs including adult mouse brain. We have improved upon 1) tissue delipidation and tissue refractive index matching to achieve homogeneous optical clearing for optimal volumetric imaging; 2) tissue morphology preservation for reliable histo-anatomical analysis; 3) reliable and quantitative molecular labeling with compatible monoclonal antibodies to ensure reproducibility and scalability. These improvements enable precise spatial molecular profiling, cell type distribution with molecular and genetic labeling, and complete neural projectome tracing in large intact brain samples. It will facilitate brain-wide anatomical and patho-histological profiling across scales.

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Structure, Function and Antigenicity of Henipavirus Surface Glycoproteins

Henipavirus (HNV), a viral genus named after the first two identified members, Hendra virus (HeV) and Nipah virus (NiV), is a group of expanding zoonotic viruses that have caused repeated outbreaks with case fatality rate reaching 75%. The exceptional broad species tropism and various transmission routes make HNV a risk of potential future pandemics. HNV glycoproteins F and G, coordinating the viral entry process, are the principal targets for vaccine and therapeutic development. The glycoproteins of recently emerged thirteen HNVs, which are genetically and antigenically distinct from the two prototypic HNVs, have not been well investigated. We used rational designs to create HNV glycoprotein

ectodomain constructs to facilitate structural and antigenicity characterization. Our structure-based functional analyses further delineated the steps of HNV glycoprotein-mediated entry. The results of our study provide insights to HNV entry mechanism, as well as inform future work on designing envelope glycoprotein-based vaccine and immunotherapeutic against HNVs.

Nieng Yan **Shenzhen Medical Academy of Research and Translation (SMART)**
School of Life Sciences, Tsinghua University

Targeting Nav channels for pain relief

Voltage-gated sodium (Nav) channels are responsible for the initiation and propagation of action potentials. Associated with a variety of disorders, Nav channels are targeted by multiple pharmaceutical drugs and natural toxins. Employing the modern methods of cryo-EM, we determined high resolution structures of a number of eukaryotic and eventually human Nav channels in complex with auxiliary subunits, toxins, and drugs, which reveal the mode of action of representative Nav modulators. Based on the structural discovery, we suggest a “door-wedge” allosteric blocking mechanism for fast inactivation of Nav channels. Structural comparison of the conformationally distinct Nav channels provides important insights into the electromechanical coupling mechanism of Nav channels, offers the 3D template to map hundredes of disease mutations, and will aid rational design of next-generation pain killers.

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Integrated proteomics reveals the autophagy landscape in human neurons and receptors in regulating neuronal activity

Autophagy is the major cellular pathway to degrade dysfunctional organelles and protein aggregates. Both neurodevelopment and the long-term maintenance of neuronal homeostasis require autophagy. Defects in autophagy are implicated in neurodevelopmental disorders and neurodegenerative diseases. However, the exact role and targets of autophagy in neurons remain elusive. Our recent systemic investigation of autophagy cargo in human stem cell-induced neurons (iN) and mouse brains through integrated proteomics and functional analysis reveals a role for autophagy in targeting a broad range of cellular pathways and organelles, including endoplasmic reticulum (ER), mitochondria, Golgi, and synaptic vesicle (SV) proteins, for degradation. We identified an LC3-binding protein calumenin as a novel ERphagy receptor and showed that autophagy degrades PRKAR1A through the AKAP11 receptor to regulate PKA and neuronal activity. Our study provides a global view of autophagy degradation in neurons and insight into mechanisms of neurological disorders linked to autophagy deficiency.

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Brain Gene Coexpression Map and Application to Decipher Perturbation and Disease Gene Signatures

Brain tissue transcriptomes may be organized into gene coexpression networks, but their underlying biological drivers remain incompletely understood. Here we undertook a large-scale transcriptomic study to define highly reproducible gene coexpression modules in two adult mouse brain tissues, the striatum and cortex. We found a subset of the modules are enriched in cell-type and/or molecular complex markers. Interestingly, one of latter modules is highly enriched in genes related to Parkinson’s

disease, mitophagy, and mitochondrial oxidative phosphorylation. Surprisingly, a majority of the coexpression modules in the two brain regions are highly enriched in daily rhythmically expressed genes that peak or trough throughout the 24-hour diurnal window, despite our study used only daytime transcriptomes. These modules provide an unbiased framework to define diurnal gene expression networks and uncover their underlying biology. Finally, we employed the reference striatal coexpression modules and a searchable online gene coexpression map (CoExMap) to decipher gene signatures from genetic perturbations and brain diseases.

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SF3B1 mutation leads to diverse changes in CLL-related pathways

SF3B1 is recurrently mutated in chronic lymphocytic leukemia (CLL), but its role in the pathogenesis of CLL remains elusive. Here, we show that conditional expression of Sf3b1-K700E mutation in mouse B cells disrupts pre-mRNA splicing, alters cell development, and induces a state of cellular senescence. Combination with Atm deletion leads to the overcoming of cellular senescence and the development of CLL-like disease in elderly mice. These CLL-like cells show genome instability and dysregulation of multiple CLL associated cellular processes, including deregulated B cell receptor signaling, which we also identified in human CLL cases. Notably, human CLLs harboring SF3B1 mutations exhibit similar RNA mis-splicing and altered response to BTK inhibition. Our murine model of CLL thus provides insights into human CLL disease mechanisms and treatment.

Kai (Jack) Zhang **Yale University** **Jack.zhang@yale.edu**

Toward dynein-driven ciliary motility and its regulation in atomic detail

Ciliary beating is driven by axonemal dyneins, of which the outer-arm dynein (OAD) generates the majority of mechanical forces required via ATP hydrolysis. OAD activity is regulated by numerous ciliary components, factors and extracellular signals, including the gigantic central apparatus (CA). By developing cryo-EM, we were able to obtain OAD arrays bound to microtubule doublets (MTDs) and an almost complete atomic model of the CA. Our work captures the OAD motors in arrays actively engaged on MTDs, provides a model for how OADs form arrays in two different steps in their motile cycle at near-atomic resolution, and explains how a rhythmic ciliary beat is generated through phased propagation of OAD nucleotide states and microtubule-binding states. Moreover, we were able to capture the CA kinesin arrays in different stepping states, providing direct evidence that the CA is also an active motor system in cilia driven by kinesin, which complements and remotely coordinates with the dynein motor systems.

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Immune Network Dysregulation of the Central Nervous System in Neurodegenerative Disease

Parkinson's disease is a prevalent neurodegenerative disorder where recent evidence suggests pathogenesis may be mediated by inflammatory processes. The molecular architecture of the disease remains to be fully elucidated. We hypothesize that Parkinson's disease is initiated by an autoimmune process involving alpha-synuclein-specific T cell activation, followed by neuro-immune interactions that establish the disease in the brain. We performed single-nucleus transcriptomics and unbiased proteomics using postmortem tissue obtained from the prefrontal cortex of late-stage Parkinson's disease and age-matched control brains. We analyzed ~80,000 brain nuclei and identified eight major

cell types, including brain-resident T cells, each with distinct transcriptional changes in line with the known genetics of Parkinson's disease. By analyzing Lewy body pathology in the same postmortem tissue, we found that α-synuclein pathology is inversely correlated with chaperone expression in excitatory neurons. Examining cell-cell interactions, we found a selective abatement of neuron-astrocyte interactions and enhanced neuroinflammation. Proteomic analyses of the same brains identified synaptic proteins in prefrontal cortex that were preferentially downregulated in Parkinson's disease. We next examined whether T cell-mediated autoimmunity initiates the neurodegeneration process in Parkinson's disease, and if these early immunological processes converge on classic archetypes of neurodegeneration. Using single-cell RNA sequencing, we characterized the immune cells in the cerebrospinal fluid (CSF) and paired blood samples from rapid eye movement (REM) sleep behavior disorder (RBD) patients with a high risk of Parkinson's disease and identified pleocytosis of CSF in RBD, as well as altered immune profiles in the central nervous system compared to healthy controls. This work will produce an unprecedented map of the neuro-immune interactions that are perturbed in Parkinson's disease, identifying rationale targets for clinical trials paving the way for the development of new treatments.

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Regulation of miRNA production in plants.

miRNAs are a group of small noncoding RNAs that are widely present in eukaryotes. miRNAs are loaded into Argonaute proteins to form RNA-induced silencing complexes (RISCs) to repress gene expression through target cleavage and/or translational repression. In metazoans miRNAs target more than 60% transcripts. Similarly in plants, they regulate essentially every aspect of growth and development as well as biotic and abiotic stress responses. Therefore, precise control of miRNA production and fine-tuning of homeostasis of miRNA accumulation warrants the functional accuracy and targeting efficacy of miRNAs. miRNAs originate from primary transcripts (pri-miRNAs), which feature stem-loop structures. The pri-miRNAs are sequentially cleaved by Microprocessor that minimally consists of Dicer-like 1 (DCL1), a double-stranded (ds)RNA-binding protein, Hyponastic leaves 1(HYL1), and Serrate (SE) to eventually produce miRNA. In the past years, our lab has extensively studied functions and mechanisms of SE protein in miRNA biogenesis. To our surprise, we have discovered that SE serves as a regulatory hub at the interface between microprocessor and epigenetic machinery that includes SWI2/SNF2 chromatin remodel factors, histone methyltransferases, and some yet characterized proteins. Moreover, the accumulation of SE protein itself is also tightly controlled through posttranslational modification and ubiquitin-independent degradation. In my talk, we will learn about how SE-centered regulatory network controls homeostasis of miRNA production among other RNA metabolism in plants.

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Oligodendrocyte-lineage cell exocytosis and L-type prostaglandin D synthase promote oligodendrocyte development and myelination

In the developing central nervous system, oligodendrocyte precursor cells (OPCs) differentiate into oligodendrocytes, which form myelin around axons. Oligodendrocytes and myelin are essential for the function of the central nervous system, as evidenced by the severe neurological symptoms that arise in demyelinating diseases such as multiple sclerosis and leukodystrophy. Although many cell-intrinsic mechanisms that regulate oligodendrocyte development and myelination have been reported, it remains unclear whether interactions among oligodendrocyte-lineage cells (OPCs and oligodendrocytes)

affect oligodendrocyte development and myelination. Here, we show that blocking vesicle-associated membrane protein (VAMP) 1/2/3-dependent exocytosis from oligodendrocyte-lineage cells impairs oligodendrocyte development, myelination, and motor behavior in mice. Adding oligodendrocyte-lineage cell-secreted molecules to secretion-deficient OPC cultures partially restores the morphological maturation of oligodendrocytes. Moreover, we identified L-type prostaglandin D synthase as an oligodendrocyte-lineage cell-secreted protein that promotes oligodendrocyte development and myelination in vivo. These findings reveal a novel autocrine/paracrine loop model for the regulation of oligodendrocyte and myelin development.

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Thalamic function in health and disease

Cortical functions critically depend on the integration of internal states (emotion) and external information (sensory inputs). The thalamus, being a crucial region for sensory processing and multi-sensory integration with both bottom-up and top-down connectivity, is in a powerful anatomical and functional position to guide cortical processes. While past thalamic research has focused mostly on pure sensory processing (i.e., their relay role), the importance of non-relay functions has recently been emphasized, but much less understood. In this talk, I will share our recent studies in which we found that parafascicular thalamic (PF) projections to ventral striatum play a major role in reward processing and depression-like behaviors, while distinct anterior thalamic nuclei (ATN) are necessary for contextual encoding, generalization, and working memory maintenance through their interaction with specific cortical regions. Further, we showed that targeting thalamic circuitry, specifically PF in neurodegenerative disease models and ATN in psychiatric disease models, using circuit- and molecular target-based approaches rescues a subset of the disease phenotypes. Together, these findings reveal non-relay thalamic contributions to various behaviors both in health and disease states and supports the idea that thalamic nuclei may serve as novel therapeutic targets.

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Novel Strategies Targeting Immune Checkpoint B7-H3 in Advanced Prostate Cancer

Prostate cancer is the most diagnosed cancer in men worldwide and the leading cause of cancer death in men worldwide. Genetic inactivation of PTEN and TP53 are common in advanced prostate cancers. Checkpoint immunotherapy has yielded meaningful responses across many cancers but shown modest activity in advanced prostate cancer.

Prior studies showed that overexpression of immune checkpoint B7-H3 (CD276) correlates with the increased risks of clinical recurrence, disease spread, and poor outcomes in various cancer types, including prostate cancer. However, the roles of B7-H3 in prostate cancer development and its tumor microenvironment remain unclear, partially due to the lack of tissue-specific deletion mouse models. This gap in knowledge hinders the application of immunotherapy targeting B7-H3 in prostate cancers. To identify PTEN- and p53-associated immune checkpoints, we performed multi-omics analyses of expression patterns of 51 checkpoint molecules in human prostate cancer samples and found that B7-H3 is one of the most significantly overexpressed immune checkpoints in prostate tumors containing PTEN and TP53 genetic inactivation. Mechanistically, we found that the PTEN-AKT pathway co-operates with p53 pathway in modulating B7-H3 expression in cancer cells. In Pten/Trp53 genetically engineered mouse (GEM) models, prostate-specific deletion of Cd276 resulted in markedly delayed tumor

progression and reversed immunosuppression in the tumor microenvironment. Our studies provide insights into biomarker-driven immunotherapy targeting B7-H3 in advanced prostate cancer.

Hong Zhao **Houston Methodist Academic Institute** hzhao@houstonmethodist.org

Tumor-interacting astrocytic signaling restrains the pathogenesis of Alzheimer's disease

Convincing epidemiological data indicates a considerable inverse relationship between Alzheimer's disease (AD) and cancer - elderly persons with cancer have significantly reduced risk of AD and vice versa. We examined a significant reduction of amyloid burden in the AD mice bearing brain tumors. Considering the statistically importance of astrocytes in the pathogenesis of AD and brain tumor, we have been exploring the spatiotemporal transcriptional changes of astrocytes induced by tumor cells seeded in the brain of AD mice. Our preliminary studies identified a list of astrocytic signaling in restraining the pathogenesis of AD, including inhibiting astrocytic Aβ production; facilitating astrocytic clearance of Aβ; and activating astrocytic interactions with other cell types in regulating the Aβ/tau homeostasis. The novel astrocytic signaling may open a new vista for novel mechanisms of action therapeutic strategies for AD.

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Immunization in NHP with Diverse HIV-1 Envelope Trimers Elicits Broadly Neutralizing CD4-Binding Site-targeting Antibodies

Vaccine elicitation of broadly neutralizing antibodies remains a goal yet to achieve for the development of an effective HIV-1 vaccine. Here we report that sequential boosts with glycans-restored and natively glycosylated HIV-1 Env could elicit cross-reactive responses in non-human primates (NHP). Neutralization assays showed that antibodies isolated from these NHPs could neutralize 54-56% of viruses in our 208-virus panel. Cryo-EM structures showed that two of the antibodies, named A13V144-91 and A11V093-10, targeted the CD4BS but with different orientations compared to that of VRC01. A13V144-91 achieved broad neutralization by mimicking key CD4 and VRC01 interactions to HIV-1 gp120. The other antibody, A11V093-10, bound to gp120 with an epitope that shifted away from glycan 276 to reduce interaction to this glycan and achieve broad neutralization. In summary, immunization using Env with glycans modulated around the CD4BS can elicit broad CD4BS antibodies in NHP with diverse modes of Env recognition.