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Mapping Footprints of Transcription Factors' Combination on the Human Genome: Regulation of Gene Modules
Xiaoliang Sunney Xie

An individual's somatic cells have the same genome but exhibit cell-type-specific transcriptome regulated by a combination of transcription factors (TFs) for each gene. Mapping of TF sites on the human genome is critically important for understanding functional genomics. We have developed a novel technique to measure human TFs' binding sites genome-wide with single-base resolution by footprinting with deaminase (FOODIE). Single-molecule sequencing reads from thousands of cells after in situ deamination yielded site-specific TF binding fractions and the cooperativity among adjacent TFs. In a human lymphoblastoid cell line, we found that genes in a correlated gene module (CGM) share TF(s) in their cis-regulatory elements to participate a particular biological function. Finally, single-cell resolved experiments (scFOODIE) allow cell-type-specific TF footprinting in heterogeneous brain tissues.

Enhancing Broad-Spectrum Disease Resistance in Plants Through Basic Research
Xinnian Dong
HHMI/Duke University, Durham, North Carolina, USA

In plants, a local infection can trigger long-lasting systemic acquired resistance (SAR) against a broad spectrum of pathogens. During my lecture, I will present recent advancements that we made in understanding the structure and function of NPR1, a key regulator of SAR, as well as our discoveries of highly conserved translation regulatory modules for reprogramming the defense proteome. I will demonstrate how this new knowledge may lead to new strategies for controlling crop diseases in agriculture.

Barrier-cell inflammasome activation and pyroptosis in antibacterial defense and sepsis
Feng Shao, PhD
National Institute of Biological Sciences, Beijing

The canonical (caspase-1) and noncanonical (caspase-4/5/11) inflammasomes both cleave gasdermin D (GSDMD) to induce pyroptosis. Whereas caspase-1 processes IL-1β and IL-18 for maturation, no cytokine target has been firmly established for LPS-activated caspase-4/5/11. Here we show that activated human caspase-4, but not mouse caspase-11, directly and efficiently processes IL-18 in vitro and during bacterial infections, which mainly occurs in epithelial cells. Crystal structure of the caspase-4/pro-IL-18 complex reveals a binary substrate-recognition mechanism, including a unique exosite that binds to a specific structure formed jointly by the propeptide and post-cleavage-site sequences in pro-IL-18. In caspase-11, a structural deviation around the exosite underlies its inability to target pro-IL-18, which can be restored by rationally designed mutations. The structure of pro-IL-18 features autoinhibitory interactions between the propeptide and the post-cleavage-site region, preventing recognition by the IL-18Rα receptor. Meanwhile, we also find that GSDMD activation by LPS-ligated caspase-4/11 specifically in brain endothelial cells, but not TLR4-induced cytokines, mediates BBB (blood brain barrier) breakdown in response to circulating LPS or during LPS-induced sepsis. Electron microscopy

records ultrastructural changes in the disrupted BBB, including pyroptotic endothelia, abnormal appearance of tight junctions, and vasculature detachment from basement membrane. Delivery of active GSDMD into brain endothelial cells bypasses LPS stimulation and opens the BBB. In *CASP4*-humanized mice, Gram-negative *Klebsiella pneumoniae* infection disrupts the BBB, which is blocked by a GSDMD-neutralizing nanobody expressed in brain endothelial cells. These findings together shift the paradigm in the understanding of noncanonical-inflammasome-mediated antibacterial defenses and sepsis.

Marker-guided effective therapy (Mget)

Mien-Chie Hung
President / Chair Professor, China Medical University, Taiwan

Cancer therapy has moved into a new era, including mechanism-driven marker-guided target therapy and immune therapy. Anti-PD-1/PD-L1 therapy is a promising immune therapy for multiple cancer types. Glycosylation of PD-L1 is required for its protein stability and interaction with PD-1 (***Nature Comm*** 2016). Impressive therapeutic effect of developed glycosylation-specific PD-L1 mAb was observed through antibody-drug-conjugate approach (***Cancer Cell*** 2018[a] & ***Cancer Res*** 2020). Through identifying potential targets, we developed marker-guided effective therapy **(Mget)** to enhance therapeutic efficacy and/or overcome drug resistance by combination therapy with immune checkpoint therapy, including metformin (***Mol Cell*** 2018), c-MET inhibitors (***Gastroenterology*** 2019); and targeting IL-6/JAK1 pathway (***J Clin Invest*** 2019), Galectin-9 (***Nature Comm*** 2021, ***IJBS*** 2023[a]), Tyro 3 (***J Clin Invest*** 2021). Several PARP inhibitors have been approved to treat cancer patients with BRCA mutation and/or homologous recombination defective tumors, we also investigate the mechanisms inducing resistance to PARP inhibitors and develop marker-guided combination therapy to overcome the resistance. The goal is to use identified markers to stratify patients for the right combination therapy. These include reports on c-Met, ALK and GSDMC (***Nature Medicine*** 2016; ***Nature Cancer*** 2022 & JCI 2024). This talk will also include our discoveries on novel therapy overcoming resistance to EGFR TKI in lung cancer (***Cancer Cell*** 2018[b]) and other caner types as well as a new methodology to retrieve antigen by protein de-glycosylaton that improves predictive ability of PD-L1 as a biomarker for immunotherapy. (***Cancer Cell*** 2018[b], ***Cancer Cell*** 2018[c], ***Cancer Cell*** 2019, ***AJCR*** 2022[a], ***Nature Reviews Clinical Oncology*** 2022, ***Nature***, 2020, ***Nat. Cell Biol*** 2020; ***Mol Cell*** 2021, ***IJBS*** 2023[a,b,c,] ***Nature Comm*** 2024, ***JCI*** *2024*). We will also share with our recent unpublished data for markers-guided effective therapy. All efforts are focused on mechanism-driven marker-guided effective therapy in a hope to benefit cancer patients.

Infection, inflammation and gastrointestinal cancers

Professor Xin Lu
Ludwig Institute for Cancer Research, Nuffield Department of Clinical Medicine, University of Oxford.

Over 2 million cancer cases are attributed to viruses, including EBV, HPV and HBV, and new ways to tackle these cancers would be highly beneficial. Many viruses are able to escape host immune responses by adopting a latent state, which enables them to achieve long-term infection. Each year ~210,000 cancer cases are attributed to

EBV: B-cell malignancies, nasopharyngeal cancer (NPC), gastric cancer (GC), and some rare T/NK cell lymphomas, leukaemias and leiomyosarcomas. The epithelial cancers NPC and GC are the major group of EBV-associated malignancies with high mortalities, and EBV+GC is the largest category of EBV-cancer with >80,000 cases per year. Harnessing host immunity to selectively eliminate tumour virus infected cancers could benefit millions of patients. Additionally, the bacteria *H.Pylori* is one of the most known cancer causing pathogens of gastric cancers and the oncoprotein of CagA is a potent inducer of cell plasticity changes such as epithelial mesenchymal transition (EMT).

One of the main research focuses of my group is the molecular switches of cell plasticity – the ability of cells to change their characteristics and fate. Cell plasticity is a key feature of development, regeneration and cancer and we recently revisited the role of cancer-causing pathogens, such as *H. pylori* and Epstein Barr virus on their ability to control the cell plasticity of host cells and vice versa. I will discuss our recent advances in achieving an effective 'kick and kill' strategy to purge EBV-infected epithelial cancer cells through a combination of high throughput drug screening and state of the art single cell sequencing technologies.

Immunochemotherapy is the fourth pillar of cancer therapy but the key challenge is to understand why some patients achieve clinical benefit here whereas others do not. I will also discuss the details of a unique clinical trial of immunochemotherapy-treated inoperable oesophageal cancer, one of the cancers with the highest rising incidence in the last few decades. Our uniquely designed window-of-opportunity trial (LUD2015-005), in which 35 inoperable oesophageal adenocarcinoma patients received first-line immune checkpoint inhibitor (ICI) for four weeks (ICI-4W), followed by immunochemotherapy (ICI+CTX). Comprehensive biomarker profiling was conducted, including generation of a 65,000-cell single-cell transcriptomic atlas of esophageal cancer, as well as multi-timepoint transcriptomic profiling of EAC during ICI-4W, revealing a novel T-cell inflammation signature (INCITE) whose upregulation correlated with ICI-induced tumor shrinkage. Deconvolution of pre-treatment gastro-esophageal transcriptomes using our single-cell atlas identified high tumor monocyte content (TMC) as an unexpected ICI+CTX-specific predictor of greater overall survival (OS) in LUD2015-005 patients, and of ICI response in an independent cohort of selected gastric cancer subtypes. Tumor mutational burden was an additional independent and additive predictor of LUD2015-005 OS. TMC can improve patient selection for emerging ICI+CTX therapies in gastro-esophageal cancer.

The Piwi-piRNA Pathway: a New Paradigm of Genomic Regulation

Haifan Lin, Ph.D.
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For more than a century, research on genetic regulation has predominantly focused on genes. In this presentation, I will introduce a whole-genome paradigm of genetic regulation that extends beyond genes to encompass all major genetic constituents within the genome. This novel regulatory framework is mediated by tens of thousands of small non-coding RNAs known as Piwi-interacting RNAs (piRNAs) and their associated Piwi proteins, a subfamily of the small-RNA binding Argonaute (Ago) protein family discovered by my lab in 1998. PiRNAs typically range from 24 to 32 nucleotides in length and correspond to diverse genomic sequences. Our recent

investigations indicate that the Piwi-piRNA pathway orchestrates the expression of protein-coding genes, transposons, pseudogenes, long noncoding RNAs (lncRNAs), and governs the functions of centromeres and telomeres at epigenetic and post-transcriptional levels. This holistic regulation across the entire genome is crucial for determining germline fate and sustaining stem cell self-renewal across the animal and plant kingdoms.

Receptor-Interacting Protein Kinase 1 (RIPK1) and Human Diseases
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Receptor-Interacting Serine/Threonine-Protein Kinase 1 (RIPK1) is a master regulator of the cellular decision between pro-survival NF-κB signaling and death in response to a broad set of inflammatory and pro-death stimuli in human diseases. Activation of RIPK1 kinase promotes both cell death and inflammation. Inhibition of RIPK1 has shown efficacy in a wide range of animal models of human diseases. Activation of RIPK1 kinase has been demonstrated in human pathological samples of neurodegenerative and inflammatory conditions. An unique hydrophobic pocket in the allosteric regulatory domain of RIPK1 has enabled the development of highly selective small molecule inhibitors of its kinase activity, which have demonstrated safety in pre-clinical models and human clinical trials. Potential applications of these RIPK1 inhibitors for the treatment of monogenic and polygenic autoimmune, inflammatory, neurodegenerative conditions are emerging. I will discuss RIPK1 biology and disease-associated mutations in RIPK1 signaling pathways, highlighting how basic research may be translated into advancement for the treatment of human diseases.

RNA methylation in gene expression regulation
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Over 150 types of post-transcriptional RNA modifications have been identified in all kingdoms of life. We have shown that reversible RNA modification could impact a wide range of biological processes. We have also characterized proteins that selectively recognize m^6A-modified mRNA and affect the translation status and lifetime of the target RNA. I will present our recent discoveries on chromatin state regulation by chromatin-associated regulatory RNA (carRNA) methylation. We found that carRNAs contain different chemical marks which facilitate recruitment of chromatin factors to shape local and global chromatin state. Some of these carRNA methylation-dependent pathways explain oncogenic roles of well-known oncogenes, which provides potential new targets for future anti-cancer therapies.

From Epigenome Atlases to the Gene Regulatory Code
Bing Ren

Department of Cellular and Molecular Medicine
Center for Epigenomics
University of California, San Diego

The human genome, with its 3-billion base pairs of DNA, encodes the blueprint for human development and individual phenotypic traits. Despite rapid advances in genome sequencing technologies, understanding the genome has been challenging because less than 2% of it is functionally annotated as protein-coding, while human disease heritability lies predominantly in the non-coding regions. To address this challenge, my lab has carried out systematic studies to provide functional annotation to the non-coding genome, especially those involved in gene regulation. I will focus on our recent advances in epigenomics technologies that enabled the profiling transcription factor binding and chromatin modifications genome-wide in complex tissues and at single cell resolution. Applications of these tools to diverse human tissues have led to the identification of millions of potential regulatory elements in the human genome and annotation of their usage across different cell types and the lifespan. These results have been integrated with genome-wide association studies and deep-learning approaches to decode the functional consequences of genetic variants on gene regulation, paving the way for personalized medicine and targeted therapeutics.

Mechanisms of neuronal injury and maintenance

Yishi Jin,
Department of Neurobiology, University of California, San Diego, USA

Using single axon injury assay in C. elegans, we systematically screened the function of >1500 C. elegans genes, based on their orthology to human genes and potential neuronal function or known biochemical role. We identified numerous axon regeneration factors and their signaling pathways. My talk will focus on axonal cellular dynamics in response to axon injury and mechanistic conservation of the DLK kinases.

Molecular pathogenesis of brain injury caused by endemic fluorosis and clinical treatments

Zhi-Zhong Guan[1,2]
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Endemic fluorosis is characterized by dental and bone fluorosis, as well as multi-system damages including brain, liver, kidney, endocrine and others. Endemic fluorosis is divided into three types: drinking water, coal-burning pollution and tea drinking. Among them, coal-burning type of endemic fluorosis was firstly discovered by the research team of Guizhou Medical University in the 1970s. The main reason of the disease is that the residents in the area use indoor open stoves to dry food during the autumn harvest and heating in winter, and therefor the smoke emitted when burning coal with high fluoride content in the stoves causes fluoride pollution in food and indoor air. In China, about 35 million people live in the areas with endemic fluorosis caused by burning coal, in which18 million people had dental fluorosis and

1.5 million suffered from skeletal fluorosis. At present, China has adopted the improved stoves and health education to eliminate and control the disease. However, since the systemic damage mechanism of fluorosis is very complex, the effective development of drugs is affected. We first proposed that the elevated level of oxidative stress induced by fluoride may be the main pathogenesis of multi-organ damages of the disease, which has been recognized by peer scholars. In recent years, we have obtained significant results in the pathogenesis of fluoride-induced brain injury. Fluoride exposure could aggravate the learning and memory deficits and neuropathological changes. The cognitive level of diabetic patients in fluorosis areas was much worse than that in non-fluorosis areas, which may be involved in the overactivation of poly (ADP-ribose) polymerase-1/P53 pathway. In addition, the clinic researchers in our research team have achieved progress in the clinical treatments for the patients with dental or skeletal fluorosis and obtained valuable results in the drug treatment to chronic fluorosis. The investigations of molecular mechanism in-depth and clinical comprehensive treatments with long-term should have important practical significance to effectively eliminate the hazards of coal-burning type of endemic fluorosis.

Growth Control by a Novel Tumor Suppressor Pathway Impinging on Regulated Protein Degradation
Duojia Pan

Tumor suppressors play critical roles in normal tissue homeostasis and their dysregulation underlies human diseases including cancer. Besides human genetics, model organisms such as Drosophila have been instrumental in discovering tumor suppressor pathways, such as the Hippo pathway, that were subsequently shown to be highly relevant in human cancer. I will present recent studies from my laboratory showing that Hyperplastic disc (Hyd), one of the first tumor suppressors isolated genetically in Drosophila and encoding an E3 ubiquitin ligase with hitherto unknown substrates, and Lines (Lin), best known for its role in embryonic segmentation, define an obligatory E3-adaptor complex (Hyd-Lin) that targets the zinc-finger-containing oncoprotein Bowl for ubiquitin-mediated degradation. Interestingly, the activity of the Hyd-Lin complex is directly inhibited by a micropeptide encoded by another zinc-finger protein drumstick (drm), which functions as a pseudosubstrate by displacing Bowl from the Hyd-Lin complex and thus stabilizing Bowl. We further identify the epigenetic regulator polycomb repressive complex 1 (PRC1) as a critical upstream regulator of the Hyd-Lin-Bowl pathway by directly repressing the transcription of the micropeptide drm. Consistent with these molecular studies, genetic inactivation of Hyd, Lin or PRC1 resulted in Bowl-dependent hyperplastic tissue overgrowth in vivo. We also provide evidence that the mammalian homologues of Hyd (UBR5, known to be recurrently dysregulated in various human cancers), Lin (LINS1) and Bowl (OSR1/2) constitute an analogous protein degradation pathway in human cells, and that OSR2 promotes prostate cancer tumorigenesis. Altogether, these findings define a previously unrecognized tumor suppressor pathway that links epigenetic program to regulated protein degradation in tissue growth control and tumorigenesis.

The Interaction with Bacteria Confers Competitive Edge of Insects

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The intricate evolutionary dynamics of symbiotic relationships confer a competitive edge to insects by enhancing their adaptability and survival capabilities. Here, we reported separately on the physiological effects of two symbiotic bacteria on their host insects. For endosymbiont, we selected the cotton mealybug (an invasive pest) and its symbiont *Tremblaya phenacola* PSOL as our study model. A comprehensive analysis of essential amino acid metabolic pathways demonstrated complementarity between the host and endosymbiont metabolism. Elimination of *T. phenacola* PSOL significantly decreased the cotton mealybug's fecundity. Further co-expression network analysis demonstrated a correlation between genes associated with essential amino acid synthesis and those associated with mTOR signaling pathway, oocyte meiosis and oocyte maturation. These findings demonstrate a mechanism by which the endosymbiont *T. phenacola* PSOL contributed to high fecundity in the cotton mealybug.
For exosymbiont, we found the symbiont (*Serratia harmoniae*) of harlequin ladybird is highly lethal to other ladybird species, which endows the harlequin ladybird with strong survival capabilities, helping it avoid predation by other close species. Moreover, this symbiotic relationship is regulated by harmonine, a defense compound synthesized in the harlequin ladybird. Taken together, the symbiotic relationship between insects and bacteria highlights the importance of microorganisms in animal evolution and ecosystem dynamics. This relationship not only helps insects gain a competitive advantage in their environments but also underscores the critical role of microorganisms in maintaining ecological balance and biodiversity.

SAMD9 is a novel sensor of cytosolic dsDNA and dsRNA molecules
Siyuan Ding

Rotavirus (RV) is a non-enveloped virus with a triple-layered particle and a segmented double-stranded RNA genome. While RVs infect a wide range of mammalian species, the replication and pathogenesis occur in a host range restricted manner. In suckling mice, only murine RVs cause high levels of fecal virus shedding and transmission of virus to uninfected littermates. Our previous studies highlight an indispensable role of non-structural protein 1 (NSP1) in murine RV infection *in vivo*. Interestingly, the replication defect of an NSP1-deletion virus was not rescued in *Irf3*$^{-/-}$ mice, suggesting additional functions of NSP1. Utilizing an optimized reverse genetics system, we successfully generated isogenic recombinant murine RVs with its native NSP1 replaced with that derived from human, monkey, and bovine RV strains. Intriguingly, all monoreassortant RVs carrying non-murine RV NSP1s were highly attenuated *in vivo*. Using recombinant RVs expressing chimeric NSP1s, we mapped the key domain to the last 17 amino acids of murine NSP1 C-terminus. Successful viral replication *in vivo* did not correlate with degradation of IRF3 but with another antiviral host factor, sterile alpha motif domain containing protein (SAMD9). The

replication defect of chimeric NSP1-expressing viruses was restored in mice lacking *Samd9l*, the murine homolog of human SAMD9. Mechanistically, RV NSP1s bind to SAMD9/9L and cause its degradation via the neddylation pathway and in a host species specific way. Our investigations not only elucidate that murine Samd9l serves as a host range determinant for RV infections but also lay the groundwork for designing novel RV vaccine candidates by disrupting NSP1 function to antagonize SAMD9 activity.

Supramolecular assemblies in immunity
Tianmin Fu
Department of Biological Chemistry and Pharmacology, The Ohio State University, Columbus, OH, USA

Immune signaling plays a crucial role in protecting the host from pathogen infections. Recently, higher-order assemblies have emerged as a central principle in governing immune signaling. In my talk, I will use a few examples from my lab to highlight the assembly process of these supramolecular complexes in immunity and their functional significance in immune defense.

Preparation for next pandemic: lessons from Hong Kong
By Kwok Yung Yuen

Animal surveillance in Hong Kong between 2003 to 2019 has identified many animal viruses with over 30 novel coronaviruses (CoV). Some of these viruses or their close relatives have subsequently jump into humans several years later. This is best exemplified by the bat SARS related CoV found in 2005 which turns out to be the ancestral virus of 2003 SARS-CoV-1 and 2019 SARS-CoV-2. The bat CoV -HKU4 and -HKU5 are closely related to the human and camel MERS-CoV of the 2012 outbreak in Middle East. The relative of porcine DeltaCoV-HKU15 found in 2012 was reported to infect Haitian children in 2021. However, often only the full genome sequences of these 30 novel animal CoVs are available because the majority of them cannot be cultured in vitro. Since most of these animal CoVs are found in enteric specimens while human CoVs are more often found in respiratory specimens, we use adult stem cells harvested from bat intestinal tissue and human lung tissue for setting up organoids which could be useful for the isolation of these viruses and the assessment of their potential to jump into human. These candidate viruses should be considered in the development of multiplexed rapid diagnostic test, targeted next generation genome sequencing, broad spectrum antiviral, and mucosal vaccine platform for the preparation against the next pandemic.

Advancing Precision Cancer Care through Innovative Therapeutics and Technologies
Tianhong Li, MD PhD
University of California Davis Comprehensive Cancer Center

The landscape for the diagnosis and treatment of cancer has significantly evolved over the past decade. These advances have improved survivals and quality of life of cancer patients. Taking lung cancer as an example, advances in basic and translational research has rapidly expanded the armamentarium of targeted therapeutics and cancer immunotherapy for patients with non-small cell lung cancer (NSCLC). Increasingly,

cancer patients are being treated as a chronic disease and cancer survivors are being followed in a cancer-free status. All the translation of innovative treatment strategies and technology advances into direct patient care starts with first-in-human, early-phase clinical trials. Several challenges and opportunities have emerged with the exponential growth in the number of available novel cancer drugs. This presentation will highlight recent translation of a few novel cancer immunotherapeutics, including next generation CTLA-4 inhibitor ONC-392 (gotistobart), peptide vaccine for a pan-adenocarcinoma neoantigen Labyrinthin (LabVax), and novel strategies for treating immune-mediated adverse events (irAEs). The correlative sciences associated with these early phase clinical trials have become complexed and require a close collaboration of multidisciplinary team for validating drug targets, identifying predictive biomarkers, and developing biomarker assays for patient selection. It is essential to develop an interdisciplinary, early phase clinical research program to support the evaluation of cancer immunotherapy and precision therapeutics across different tumor types.

Identification of New Therapeutic Targets in Cancer Oxygen Sensing Signaling
Qing Zhang
Department of Pathology, Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX

Hypoxia contributes to the malignancy and metastasis of most solid cancers. Our lab is interested on studying how hypoxia and oxygen sensing pathway contribute to cancer progression, especially in breast and kidney cancers. We use genomic and proteomic approaches to identify some new signaling molecules that may play important roles in breast and kidney cancers. We have identified some new therapeutic targets in oxygen sensing enzymes in breast cancer, especially for triple negative breast cancer. By performing genome-wide screenings, we also identified some new therapeutic targets in kidney cancer, by either acting as Von Hippel Lindau (VHL) substrates or VHL synthetic lethality targets in kidney cancer. Recently, we have implemented CRISPR-Cas9 in vivo screening approaches and identified new therapeutic vulnerabilities in primary cancer growth, metastasis and therapeutic resistances. In this presentation, we will discuss these latest findings and explore the potential rationale combination in targeting oxygen sensing signaling in cancer.

Decoding the transcription circuitry when the life begins
Wei Xie
School of Life Sciences, Tsinghua University, Beijing, China, 100084

Zygotic genome activation (ZGA) marks the first transcription event in development. Deciphering key regulators of ZGA is crucial for understanding how life begins and how a totipotent embryo arises from terminally differentiated gametes. Probing these questions in mammals was long hindered by the scarce experimental materials that are available from early embryos. By developing a set of ultra-sensitive chromatin analysis technologies, we previously investigated chromatin accessibility, epigenetic modifications, 3D chromatin architecture, and RNA Pol II engagement during mammalian ZGA. These studies unveiled highly dynamic and non-canonical transcription and chromatin regulation during the maternal-to-zygotic transition. However, how ZGA is kickstarted and how the early development program is progressively driven by transcription factors (TFs) remain enigmatic. Recently, we

sought to identify key TFs that act at the onset of ZGA, and those that connect ZGA to the first cell fate commitment. In this talk, I will discuss how these findings help illuminate the core transcription circuitry underlying the beginning of the life.

Structural Pharmacology of Na_v and Ca_v channels

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

Voltage-gated sodium (Na_v) and calcium (Ca_v) channels are responsible for the initiation of electrical signaling. Being associated with a variety of disorders, Na_v and Ca_v channels are targeted by multiple pharmaceutical drugs and natural toxins. Taking advantage of the resolution revolution of single particle cryo-EM, we have determined the structures of different Na_v and Ca_v subtypes from human and other eukaryotes. These structures, alone or in complex with distinct auxiliary subunits, toxins, and drugs, not only afford unprecedented insights into the working and disease mechanism of these channels, but also reveal novel pharmacological sites. In light of these structural advances, we proposed a structure-based nomenclature for ligand binding sites on Na_v and Ca_v channels, which may facilitate rational drug design and optimization.

Regulation of Cholesterol Metabolism

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Cholesterol from low-density lipoprotein (LDL) can be transported to many organelle membranes by non-vesicular mechanisms involving sterol transfer proteins (STPs). Fatty acid-binding protein (FABP) 7 was identified in our previous study searching for new regulators of intracellular cholesterol trafficking. Whether FABP7 is a bona fide STP remains unknown. Here, we found FABP7 deficiency resulted in the accumulation of LDL-derived cholesterol in lysosomes and reduced cholesterol levels on the plasma membrane. A crystal structure of human FABP7 protein in complex with cholesterol was resolved. In vitro, FABP7 efficiently transported the cholesterol analog dehydroergosterol between the liposomes. Further, silencing of FABP3 and 8 that belong to the same family as FABP7 caused robust cholesterol accumulation in lysosomes. These two FABP proteins could transport dehydroergosterol in vitro as well. Collectively, our results suggest that FABP3, 7 and 8 are a new class of STP mediating cholesterol egress from lysosomes. I will discuss our latest advances on cholesterol transport in cells.

Estimation of Disease Burden and Clinical Outcomes of Acute Leukemia in Mainland China

[1]**Chen Sai-Juan**, [1]Yin Wei, [2]Cai Jiao-Yang, [1]Zhang Jia-Nan, [2]Shen Shu-Hong，[1]Yan Xiao-Yu
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Acute leukemia (AL) is a life-threatening hematological malignancy with a poor prognosis. It is the most common cancer in children, posing a significant disease burden. In adults, an increased incidence and poorer prognosis with aging are the hallmarks of AL. Given China's vast population and aging demographic, it is crucial to elucidate the current state of AL diagnosis and treatment nationwide and provide real-world data to inform health policy decisions. In this comprehensive nationwide cross-sectional study, we harnessed data from the Chinese Childhood Leukemia Registry (CCLR) and the National Adult Acute Leukemia Registry of China (NALRC), encompassing 596 hospitals and 515 hospitals respectively. Most participating hospitals are tertiary facilities, spanning 31 provinces/autonomous regions/municipalities across mainland China. We meticulously characterized the diagnostic landscape, therapeutic regimens, and survival outcomes of AL subgroups, including non-acute promyelocytic leukemia (APL) acute myeloid leukemia (non-APL AML), APL, B-cell precursor acute lymphoblastic leukemia (BCP-ALL), and T-lymphoid progenitor ALL (T-ALL), among pediatric (0-14 years) and adult (≥15 years) patients from 2016 to 2020. Diagnostic patterns were analyzed using the French-American-British (FAB) classification, immunophenotyping, and the World Health Organization Classification of Haematolymphoid Tumours (WHO-HAEM). Treatment modalities were delineated across age groups and AL subgroups. Crucially, the CCLR and NALRC databases were linked with the China Cause of Death Reporting System (CDRS), enabling regular incorporation of patient survival data, with all follow-up records updated to December 31, 2021. Survival rates were stratified and compared based on sex, age groups, treatment approaches, and molecular subgroups. A total of 104,952 newly diagnosed AL cases were extracted from 2016 to 2020, with 33,530 cases in children and 71,422 cases in adults, with a range of male to female ratio of 1.18-1.33. For children, the median age for AL was 5 years, with the predominant subtype being ALL, comprising 25,106 cases (74.9%). Conversely, for adults, the median age for AL was 53 years, and the primary subgroup was AML, accounting for 56,150 cases (78.6%). To facilitate accurate diagnoses, a comprehensive panel encompassing Morphologic, Immunophenotypic, Cytogenetic, and Molecular biological (MICMb) tests has been widely implemented. According to WHO-HAEM classification, molecular genetic abnormalities characterized both AML and BCP-ALL. The distinct age-related molecular pattern was revealed, with the proportion of fusion genes decreasing while the proportion of AML, myelodysplasia-related (AML-MR), and NPM1 mutations gradually increasing with age. In APL and Ph+ B-ALL, respectively, the synergistic regimen of ATRA and ATO and the TKI-based regimen constitute a significant advancement that drastically improves the prognosis of the disease. By incorporating outcome data from the CDRS, the study presented a childhood cohort with a median follow-up of 36.8 months and an adult cohort with a median follow-up of 38.8 months. The standardized treatment and hematopoietic stem cell transplantations have significantly improved the prognosis of AL patients, translating into the estimated 5-year survival rates for non-APL AML, APL, BCP-ALL, and T-ALL of 66.3% (64.8-67.8), 90.9%

(89.1-92.6), 86.1% (85.6-86.7), and 72.5% (70.2-74.8) in children, and 34.3% (33.8-34.8), 84.7% (83.9-85.6), 40.6% (394-41.9), and 37.2% (34.7-39.9) in adults, respectively. Among all AL subgroups, age was a strong discriminating factor for survival. In conclusion, our study represents the first nationwide attempt to leverage the extensive CCLR and NALRC databases, providing a landscape view of AL in China, encompassing diagnostic patterns, treatment modalities, and prognostic outcomes across both pediatric and adult populations. Our data unveils a higher disease burden and poorer prognosis of AL among the elderly compared to children. With China's rapidly aging population, there is an urgent need to raise societal awareness about the importance of risk factor control, early detection, and prompt treatment for AL.

Day 1, July 26 (Friday):

Concurrent workshops 1-9,61 1:30-3:00pm

Workshop 1: DNA Repair and Cancer Therapy (Room 2A)

Chairs: Fen Xia, Zhenkun Lou

W1-1: CDK12 Deficiency Instigates Cgas-STING Activation and Innate Immunity via Inducing DNA2 Nuclease/Helicase Stabilization and Replication Stress

Rui Sun, Binyuan Yan, Zhijun Wang, Shouhai Zhu, Jianong Zhang, Donglin *Ding, Xiang Li, Liguo Wang, Zhenkun Lou, Jun Pang, Dan Xia and* ***Haojie Huang***

CDK12 is known as a transcription-regulatory cyclin-dependent kinase. Cyclic GMP-AMP (cGAMP) synthase (cGAS) is critical for activation of the innate immune response induced by cytosolic double-stranded DNA (dsDNA). DNA replication nuclease/helicase 2 (DNA2) is a structure-specific nuclease/helicase that plays an important role in regulating genomic stability. Here, we demonstrate that CDK12 promotes DNA2 phosphorylation and subsequent polyubiquitination and degradation. CDK12 inactivation induces genome-wide upregulation of interferon response and antigen processing and presentation machinery genes. Besides causing aberrant DNA2 stabilization, replication stress, genomic instability and cytosolic double-stranded DNA (dsDNA) accumulation, CDK12 loss also triggers cGAS-STING activation and innate immune response. Our findings identify DNA2 as a phosphorylation target of CDK12 and reveal aberrant stabilization of DNA2 as a critical mechanism mediating CDK12 deficiency-induced genomic instability, cGAS-STING signaling activation and innate immune response.

W1-2: Glycogen Synthase Kinase-3B Controls DNA Double-Strand Break Repair Choice through Phosphorylation and Regulation of 53BP1 Function

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Genotoxic therapy resistance is a major cause of tumor recurrence and treatment failure. The underlying mechanisms that allow cancer cells to escape genotoxic therapies by activating the DNA damage response (DDR) remain unclear. Here, we report a novel mechanism through which glycogen synthase kinase-3β (GSK3β), a multifunctional serine/threonine kinase involved in various cellular processes and signaling pathways, integrates with the DDR to regulate double-strand break (DSB) recognition and repair pathway choice. GSK3β phosphorylates 53BP1 at threonine 334 amino acid (T334), inhibiting 53BP1 recruitment to DNA DSB sites and its function in non-homologous end-joining. Conversely, T334 phosphorylation enhances single strand DNA resection and promotes homologous recombination repair in response to

DNA damage. Importantly, genetic and pharmacologic manipulation of the GSK3β-53BP1 axis dramatically enhances the cytotoxic response of both BRCA1-proficient and BRCA1-deficit cancer cells to PARP inhibitor (PARPi). Together, these data identify GSK3β-dependent regulation of 53BP1 and provide a strategy for targeting cancer cell resistance to PARPi.

W1-3: PARP Inhibitors Trap PARP2 at Nicks, and Catalytically Inactive PARP2 Leads to Lethal Anemia In Mice By Selectively Interfering with DNA Replication

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Dual inhibitors of PARP1 and PARP2 (PARPi) are promising anti-cancer drugs. In addition to blocking PARP1 and PARP2 enzymatic activity, PARPi also prolong the presence of DNA damage-induced PARP1 and PARP2 foci, a phenomenon known as trapping. While PARP1 trapping is recognized for its therapeutic effects, the mechanism and biological significance of PARP2 trapping remain unclear. Using live-cell imaging, we discovered that PARP inhibitors cause persistent PARP2 foci by altering the mode of PARP2 recruitment from a PARP1- and PAR-dependent rapid exchange to a WGR domain-mediated stalling of PARP2 on DNA. Mechanistically, niraparib and talazoparib, but not olaparib, prevent PARP2 exchange and significantly enhance PARP2 foci intensity in PARP1-deficient cells. This PARP2 trapping is independent of auto-PARylation and is abolished by the R140A mutation in the WGR domain and the H415A mutation in the catalytic domain. Interestingly, the DNA-binding defective PARP2-R140A forms robust foci in PARP1-proficient cells. While PARPi completely abolish residual PARP2-R140A foci in PARP1-proficient cells, they only delay PARP2-WT foci. Our findings indicate that PARPi trap PARP2 by stalling it on DNA via WGR-DNA interactions while inhibiting the PARP1- and PAR-dependent rapid exchange of PARP2. Unlike PARP1, PARP2 selectively binds to 5' phosphorylated DNA ends. Structural analyses suggest that the WGR domain can embrace both ends of the nick. To explore the biological consequences of PARP2 trapping on DNA 5'p-nicks—ideal substrates for DNA ligase—we generated a mouse model with PARylation-defective Parp2 (Parp2-E534A). While mice with a complete loss of Parp2 (knockout) are viable and healthy, those expressing PARylation-deficient Parp2 (Parp2EA/EA) exhibited dose-dependent anemia and p53- and Chk2-dependent embryonic lethality, mimicking the phenotype of DNA Ligase I knockout mice. In conclusion, unlike PARP1 inhibition, which affects base excision repair and nick ligation during replication, PARP2 inhibition traps PARP2 and primarily disrupts nick ligation during replication by competing with DNA Ligase I. In proliferating erythroblasts with rapid fork progression, inactive PARP2 causes fork collapses and lethal anemia. Our findings elucidate a cellular mechanism underlying the severe anemia observed with clinical PARP inhibitors.

W1-4: Mitotic-Specific Function of DNA Polymerase Iota in Post-Replication DNA Damage Response

Can Yi and **Lei Li**
Life Sciences Institute, Zhejiang University

DNA polymerase iota is a member of the Y family lesion bypass DNA polymerases. Pol iota has the largest catalytic pocket and exhibits exceedingly low fidelity on

primer extension. Aside from its synthetic lethality with the Fanconi anemia pathway, Pol iota's biological function is unclear. Deletion of Pol iota in human cells and mice has not revealed any detectable phenotype. In this study, we find that Pol iota is involved in mitotic DNA synthesis (MiDAS) which occurs during mitosis. Cell deficient in Pol iota are specifically vulnerable to low-level stress. Expression of Pol iota is restricted to G2/M cells via ubiquitination-mediated proteasomal degradation, rendering the activity of Pol iota available only in mitotic cells. These results suggest that Pol iota plays a major role in post-replicative DNA damage response.

W1-5: Mono-Ubiquitination of Topbp1 By PHRF1 Enhances ATR Activation and Genomic Stability

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The TopBP1-ATR axis plays a critical role in maintaining genomic stability during DNA replication stress-induced checkpoint activation. However, the precise regulation of TopBP1 in replication stress responses remains poorly understood. In this study, we identified the PHD and Ring Finger Domains 1 (PHRF1) as an important ATR activator through its interaction with TopBP1. Our analysis revealed a strong correlation between PHRF1 and genomic stability in cancer patients. Mechanistically, PHRF1 is recruited to DNA damage sites in a PHD domain and H3K9me3-dependent manner. Subsequently, PHRF1 mono-ubiquitinates TopBP1 at lysine 73, leading to enhanced TopBP1-ATR interaction and ATR activation. Depletion of PHRF1 impedes ATR activation and hypersensitizes cells to replication stress-inducing agents. Additionally, conditional knockout of *Phrf1* in mice led to early lethality and diminished ATR-Chk1 axis signaling. Overall, our findings establish PHRF1 as a novel E3 ligase for TopBP1 in coordinating the replication stress response by enhancing TopBP1-ATR signaling.

Workshop 2: Identifying and Exploiting New Molecular Targets for Neurological Disorders (Room 2B)

Chairs: Zhong-Ping Feng, Xiangnan Zhang

W2-1: The Role of Autophagy in Psychiatric Disorders

Xiangnan Zhang, Zhejiang University

W2-2: Semiconducting Polymer Dots for Highly Specific NIR-II Fluorescence Imaging of Glioma

Meihua Bao, Changsha Medical University

W2-3: Pancreatic and Extra-Pancreatic Function of GLP-1 and GLP-1 Receptor Agonists

Tianru Jin, University of Toronto

W2-4: Sigma-1 Receptor Positive Allosteric Modulator Promotes the Neuronal Survival and Improves Cognitive Deficits of AD Mice by the Sigma-1 Receptor/ERK Pathway

Wenhua Zheng, University of Macau

W2-5: Calcium Binding Protein Regulates Neuronal Maturation

Zhong-Ping Feng, University of Toronto

Workshop 3: Structural Biology in the Era of Cryo-EM I (Room 2C)

Chairs: Peijun Zhang, Yifan Cheng

W3-1: Cryo-EM Structure of TMEM63C Suggests it Functions as a Monomer

Yuqi Qin[1,8], Daqi Yu[1,8], Dan Wu[2,8], Jiangqing Dong[1,8], William Thomas Li[1], Chang Ye[2], Kai Chit Cheung[1], Yingyi Zhang[3], YunXu[2], YongQiangWang[4], Yun Stone Shi[2,5] & **Shangyu Dang**[1,6,7]

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The TMEM63 family proteins (A, B, and C), calcium-permeable channels in animals that are preferentially activated by hypo-osmolality, have been implicated in various physiological functions. Deficiency of these channels would cause many diseases including hearing loss. However, their structures and physiological roles are not yet well understood. In this study, we determine the cryo-electron microscopy (cryo-EM) structure of the mouse TMEM63C at 3.56 Å, and revealed structural differences compared to TMEM63A, TMEM63B, and the plant orthologues OSCAs. Further structural guided mutagenesis and calcium imaging demonstrated the important roles of the coupling of TM0 and TM6 in channel activity. Additionally, we confirm that TMEM63C exists primarily as a monomer under physiological conditions, in contrast, TMEM63B is a mix of monomer and dimer in cells, suggesting that oligomerization is a regulatory mechanism for TMEM63 proteins.

W3-2: How Physiological Temperature Drives Ligand Recognition and Channel Gating

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Temperature profoundly affects macromolecular function, particularly in proteins with temperature sensitivity. However, its impact is often overlooked in biophysical studies typically performed at non-physiological temperatures, potentially leading to inaccurate mechanistic and pharmacological insights. This is demonstrated in our present study of TRPM4, a temperature-sensitive Ca^{2+}-activated ion channel. By studying TRPM4 prepared at physiological temperature using single-particle cryo-electron microscopy, we discovered a "warm" conformation, distinct from those observed at lower temperatures. This conformation is driven by a temperature-dependent Ca^{2+} binding site in the intracellular domain, and is essential for TRPM4 function in physiological contexts. We demonstrated that ligands, exemplified by decavanadate (DVT, a positive modulator) and ATP (an inhibitor), bind to different locations at physiological temperature than at lower temperatures; and that these sites have *bona fide* functional relevance. We elucidated the TRPM4 gating mechanism by capturing structural snapshots of its different functional states at physiological temperature, revealing the elusive channel opening not observed at lower temperatures. Our work represents the first example of temperature-dependent ligand recognition and modulation of an ion channel, underscoring the importance of studying macromolecules at physiological temperature. It also provides a potential molecular framework for deciphering how thermosensitive TRPM channels perceive temperature changes.

W3-3: Structure and Topography of the Synaptic V-ATPase

Qiang Guo[1], Chuchu Wang[2], Wenhong Jiang[1], Jeremy Leitz[2], Kailu Yang[2], Xing Wang[1], Michael Stowell[3], Wolfgang Baumeister[4], and Axel Brunger[2]
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Synaptic vesicles are organelles with a precisely defined protein and lipid composition, yet the molecular mechanisms for the biogenesis of synaptic vesicles are mainly unknown. Here, we discovered a well-defined interface between the synaptic vesicle V-ATPase and synaptophysin by *in situ* cryo-electron tomography and single particle cryo-electron microscopy of functional synaptic vesicles isolated from mouse brains. The synaptic vesicle V-ATPase is an ATP-dependent proton pump that establishes the protein gradient across the synaptic vesicle, which in turn drives the uptake of neurotransmitters. Synaptophysin and its paralogs synaptoporin and synaptogyrin belong to a family of abundant synaptic vesicle proteins whose function is still unclear. We performed structural and functional studies of synaptophysin knockout mice, confirming the identity of synaptophysin as an interaction partner with the V-ATPase. Although there is little change in the conformation of the V-ATPase upon interaction with synaptophysin, the presence of synaptophysin in synaptic vesicles profoundly affects the copy number of V-ATPases. This effect on the topography of synaptic vesicles suggests that synaptophysin assists in their

biogenesis. In support of this model, we observed that synaptophysin knockout mice exhibit severe seizure susceptibility, suggesting an imbalance of neurotransmitter release as a physiological consequence of the absence of synaptophysin.

W3-4: Ultrastructural Interplay between Host and Bacteria as Revealed by Correlative Cryo-Electron Tomography

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Pathogen infection, a cause of numerous diseases, poses a significant threat to human health. Understanding the intricate host-pathogen interaction underlying disease pathogenesis is crucial for effective disease prevention. Xenophagy, a typic autophagy, is an innate immune response to eliminate intracellular bacteria. The host and bacteria engage in a tug-of-war regarding the phagophore biogenesis, a critical interface between the host and pathogen that is not fully understood. In our study, we have embarked on a complex research journey, employing state-of-the-art cryo-electron tomography (cryo-ET) integrating cryo-fluorescent microscopy, high-pressure freezing, and cryo-FIB milling techniques, to dissect phagophore biogenesis at subnanometer or near-atomic levels in an unperturbed environment. This intricate process has allowed us to characterize the ultrastructures of variable-shaped phagophore intermediates engulfing *Salmonella* or *Staphylococcus aureus* at the molecular level, revealing two pathways for phagophore generation.

W3-5: High-resolution In-situ Structures of Mammalian Mitochondrial Respiratory Supercomplexes in Reaction within Native Mitochondria

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Mitochondria play a pivotal role in ATP energy production through oxidative phosphorylation, which occurs within the inner membrane via a series of respiratory complexes. Despite extensive *in-vitro* structural studies, revealing the atomic details of their molecular mechanisms in physiological states remains a major challenge, primarily because of the loss of the native environment during purification. Here, we directly image porcine mitochondria using an *in-situ* cryo-electron microscopy approach. This enables us to determine the structures of various high-order assemblies of respiratory supercomplexes in their native states, achieving up to 1.8-Å local resolution. We identify four major supercomplex organizations: $I_1III_2IV_1$, $I_1III_2IV_2$, $I_2III_2IV_2$, and $I_2III_4IV_2$, which can potentially expand into higher-order arrays on the inner membranes. The formation of these diverse supercomplexes is largely contributed by 'protein-lipids-protein' interactions, which in turn dramatically impact the local geometry of the surrounding membranes. Our *in-situ* structures also capture numerous reactive intermediates within these respiratory supercomplexes, shedding light on the dynamic processes of the ubiquinone/ubiquinol exchange mechanism in complex I and the Q-cycle in complex III. By comparing supercomplex structures from mitochondria treated under distinct conditions, we elucidate how conformational

changes and ligand binding states interplay between complexes I and III in response to environmental redox alterations. Our approach, by preserving the native membrane environment, enables structural studies of mitochondrial respiratory supercomplexes in reaction at high resolution across multiple scales, spanning from atomic-level details to the broader subcellular context.

Workshop 4: Genome Regulation in Development and Disease (Room 2D)

Chairs: Zhao Zhang, Chao Lu

W4-1: DNA Replication Shapes Chromatin Architecture and Regulates Cell Reprogramming

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Stem cells display asymmetric histone inheritance while non-stem progenitor cells exhibit symmetric patterns in the *Drosophila* male germline lineage. Here, we report that several DNA replication components have significantly reduced levels in stem cells compared to progenitor cells. Compromising these factors genetically induces the replication-coupled histone incorporation pattern in progenitor cells to be indistinguishable from that in stem cells, which can be recapitulated using a specific inhibitor in a concentration-dependent manner. Furthermore, under these conditions the preexisting (old) *versus* newly synthesized (new) histone asymmetry is comparable between stem cells and progenitor cells at both S-phase and M-phase. These results indicate that developmentally programmed expression of key DNA replication components is important to shape stem cell chromatin. Furthermore, reducing the level or activity of a key component facilitates cell reprogramming in diverse stem cell systems across species, including *Drosophila* male and female germline stem cell lineages, *Drosophila* intestinal stem cell lineage, and induced pluripotent stem cells (iPSCs) derived from human embryonic fibroblasts. These findings unveil a novel role of the DNA replication component in regulating cellular reprogramming potential. Together, these discoveries hold promise for promoting tissue health, facilitating post-injury rehabilitation, and enhancing healthspan.

W4-2: A Unified Model for the Surveillance of Translation in Diverse Noncoding Sequences

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Translation is pervasive outside of canonical coding regions, occurring in long noncoding RNAs (lncRNAs), canonical untranslated regions (UTRs) and introns, especially in cancer, aging, and neurodegeneration. Notably, the majority of tumor-specific antigens are results of noncoding translation. Although the resulting polypeptides are often nonfunctional, translation of noncoding regions is nonetheless necessary for the birth of new coding sequences. Intriguingly, functional polypeptides

derived from annotated noncoding sequences often localize to membranes. The mechanisms underlying the surveillance of translation in diverse noncoding regions, how most noncoding-derived polypeptides are processed into antigens, and how escaped polypeptides evolve new functions remain unclear. Combining massively parallel analyses of more than 10,000 human genomic sequences and millions of random sequences with genome-wide CRISPR screens and extensive validations, we have uncovered an unexpected mechanism for noncoding translation mitigation in human cells with important implications in understanding tumor immunogenicity, aging and neurodegeneration, as well as the evolution of new genes (Kesner & Chen et al., Noncoding translation mitigation, *Nature*, 2023).

W4-3: ecDNA Biogenesis

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Although originally reported six decades ago, extrachromosomal circular DNA (henceforth, abbreviated as “ecDNA” for all circles regardless of size) have recently emerged as a common DNA form broadly produced in eukaryotes. With the size ranging from hundreds to millions of bases, ecDNA often harbor genes or genetic regulatory elements. Together with the feature of unrestricted copy numbers—often up to hundreds of copies per cell, ecDNA bring one important layer of genome dynamics that allows cells to fully exploit their genetic information for adaptation and evolution. Reflecting this fundamental function, ecDNA have been documented to play pivotal roles in diverse processes: magnifying histone genes in yeast to achieve dose compensation, amplifying rDNA to drive frog egg development, and re-writing the cattle genome for coat color sidedness patterning. Notably, forming ecDNA is a common mechanism for the amplification of oncogenes and genes that render cancer cells drug resistant. Despite their essential functions, our understanding of the ecDNA formation process is limited. I will present our recent endevors on understanding how ecDNA can be produced from both repetitive and non-repetetive genomic regions.

W4-4: Chromatin Crosstalk: Mechanistic Insights and Therapeutic Opportunities

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Chemical modifications of DNA and histones are important carriers of chromatin regulatory information. While the modifiers and the biological functions for many histone and DNA marks have been defined individually, less is understood about how chromatin modifications can “communicate” with each other. Enzymatic activities of chromatin modifiers are known to be sensitive to local chromatin environment, fostering various trans-regulatory interactions among epigenetic marks. This interplay, often termed as chromatin "crosstalk," is believed to contribute to the stable and sometimes heritable partitioning of the epigenome. I will present our recent efforts using genetic approaches to dissect the complex mechanisms underlying the molecular crosstalk between DNA and histone methylation and discuss its implication for the development and treatment of human diseases such as cancer.

W4-5: Dynamics of Extreme Long Polycomb Loops in Cancer

Xiaotian Zhang[1]
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Chromosomal loops are CTCF-cohesion mediated 3D genomic structure in mammalian nucleus. Besides the CTCF-cohesion loops, other epigenomic marks could also form long-range interactions in the nucleus. Polycomb targeted loci, which is regulated through Polycomb repressive complex and trithorax complex during development, form long-range chromatin interactions independent of CTCF-cohesion and demarcated by low DNA methylation. We found such extremely long Polycomb loops (XL-Polycomb loops) could occur between loci separated by up to 60 Mbs, and exclusively in cells of self-renewal status like human hematopoietic stem cells and mouse embryonic stem cells. Due to the interactions' association with self-renewal status of cells, we tested if XL-Polycomb loops could been observed and played a role in cancer. We therefore profiled 7 primary acute myeloid leukemia (AML) blast and 3 AML cell lines. To our surprise, we found in most of primary AMLs a significant loss of XL-Polycomb loops, and such loss is exacerbated in AML cell lines. Accompanied with loss of XL-Polycomb loops, DNA hypermethylation and loss of Polycomb binding is observed at the anchor loci of XL-Polycomb loops in primary AML samples and further exacerbated in cell line. Interestingly, these silenced loci (most of them are mesoderm transcriptional factors) switched to activated chromatin status in the AML samples and forms *de novo* 3D genomic interaction anchor sites in AMLs.
Surprisingly, in one specific AML which is of more stem cell subtype, AML-specific XL-Polycomb loops are particularly strong despite the DNA hypermethylation and loss of Polycomb mark. We found that AML is sensitive to EZH2 inhibition. EZH2 inhibition also caused differentiation of this AML and activated neutrophil-associated differentiation transcriptional program. This case suggests certain cancer may display strong XL-Polycomb loops interactions, which plays a role in the cancer development and renders the sensitivity to Polycomb disruption.

W4-6: RNA Polymerase II Stalling Facilitates Termination and Pirna-Guided Heterochromatin Formation

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The Piwi-interacting RNA (piRNA) pathway plays a crucial role in protecting animal germ cells by repressing transposons. However, the mechanism of piRNA-guided heterochromatin formation and its relationship to transcriptional termination remains elusive. Through RNA interference screening, we discovered Pcf11 and PNUTS as essential for piRNA-guided silencing. Enforced tethering of Pcf11 leads to co-transcriptional repression and RNA polymerase II stalling, both are dependent on an α-helical region of Pcf11 capable of forming condensates. An intrinsically disordered region can substitute for the α-helical region of Pcf11 in its silencing capacity and support animal development, demonstrating a causal relationship between phase separation and Pcf11's function. Pcf11 stalls RNA polymerase II by preferentially forming condensates with the unphosphorylated Spt5, promoted by the PP1/PNUTS phosphatase during termination. We propose that Pcf11/Spt5

condensates control termination by decelerating polymerase elongation, a property exploited by piRNAs to silence transposons and initiate RNA-mediated heterochromatin formation.

Workshop 5: Cancer Metabolism: Oncogenesis and Therapeutics (Room 2E)

Chairs: Wei-Xing Zong, Weiping Han

W5-1: Deciphering the Warburg Effect: Metabolic Reprogramming, Epigenetic Remodeling, and Cell Dedifferentiation

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A century ago, Otto H. Warburg discovered the Warburg effect, the metabolic hallmark of cancer. Our laboratory has focused on exploring how metabolic reprogramming influences the epigenetic landscape, and developing therapeutic approaches to revert cancer cells to a normal state.

Hypoxia, which induces the Warburg effect, is one common microenvironmental stress in solid tumor. We found that hypoxia inhibits cell differentiation by reducing acetyl-CoA levels, leading to decreased global histone acetylation and chromatin accessibility, demonstrating a direct connection between the Warburg effect and epigenetic remodeling (Li et al., Cell Death & Dis. 2020). Contrary to the hypoxia, serine deprivation seems to induce an 'anti-Warburg effect' by reducing lactate production. However, despite this contrasting metabolic feature, serine deprivation also led to acetyl-CoA reduction and histone hypoacetylations as observed under hypoxia. In ER+ breast cancer cells, serine deprivation repressed estrogen receptor alpha (ERα) expression, diminished sensitivity to antiestrogens, and prompted a transition to an ER-/PR- state, suggesting that these acute metabolic stresses divert pyruvate flux away from acetyl-CoA generation to initiate dedifferentiation (Li, et al., PNAS 2023).

Contrary to the transient and dynamic nature of histone modifications, DNA methylation offers stability and exerts a long-lasting effect on gene expression and cell fate control. Inhibition of the mitochondrial ETC led to a reduced NAD+/NADH ratio. This disruption not only drives the Warburg effect, but also promoted conversion of α-ketoglutarate (αKG) to 2-hydroxyglutarate (2-HG), inhibiting DNA demethylase TET. We discovered that mitochondrial uncoupling can effectively increase the NAD+/NADH and α-KG/2-HG ratios. This metabolic reprogramming promotes CpG island DNA demethylation and neuroblastoma differentiation (Jiang, et al., Cancer Res, 2023). Additionally, mitochondrial uncoupling also inhibits reductive carboxylation (RC), often considered the Warburg effect's sibling(Jiang et al., Mol Cancer Res. 2023).

In conclusion, these discoveries unveiled the missing link between the Warburg effect and dedifferentiation, establishing a new theoretical framework to interpret the origin of tumorigenesis. Additionally, they highlight promising novel metabolic therapies that could potentially lead to a paradigm shift in the standard of care for cancer management.

W5-2: Metabolic-Epigenetic Regulation of Lineage Plasticity in AR-/Low Prostate Cancer Development

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Prostate cancer (PCa) is one of the most frequent types of cancer and a leading cause of cancer related death among males worldwide. Androgen deprivation therapy (ADT) has generated impressive effects in treating advanced PCa. However, most PCa patients suffer from disease relapse inevitably. New treatment approaches or novel combination therapies in addition to ADT are urgently needed for advanced PCa. Lineage plasticity is often exploited by cancer cells to acquire therapeutic resistance. Lineage transition from adenocarcinoma (AD) to aggressive neuroendocrine (NE) derivatives is a common type of cancer cell plasticity in ADTtreated prostate adenocarcinoma (ADPC). Treatment-induced neuroendocrine prostate cancer (NEPC) are highly aggressive and lack effective clinical interventions. We show that neuroendocrine prostate cancer cells display a prominent change in mitochondrial status and rely on glycolysis for energy metabolism compared to corresponding adenocarcinoma subtype. On the other hand, we find that proline metabolism is upregulated by an activation of the ADORA2A/ERK/MYC/PYCR cascade. The metabolic change, characterized by a significant increase in the production of metabolites including lactate acid, subsequently leads to a reprogramming of the histone modification landscape and biases the global transcriptional output towards a neuroendocrine lineage profile. Ablation of Adora2a in genetically engineered mouse models or pharmacological blockade of ADORA2A suppresses the development of neuroendocrine prostate and lung cancers. Other than NEPC, double negative prostate cancer (DNPC) with a low or negative expression of AR and NE markers, is an emerging CRPC subtype following application of potent AR antagonists. We delineated the molecular mechanism by which cancer cells acquire enhanced cell lineage plasticity and identified actionable drug target Gremlin1. Blocking antibody for Gremlin1 can be used as a potential novel therapeutic agent for DNPC.

W5-3: Bilateral Effects of Nitrogen Metabolism in Oncogenesis

Wei-Xing Zong

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Glutamine is the most abundant free amino acid in human blood. Although glutamine can be obtained from dietary sources, the majority of glutamine in mammals is produced via de novo synthesis that is catalyzed by glutamine synthetase (GS), also termed glutamate ammonium ligase (GLUL). In addition to fueling the TCA cycle via glutaminolysis, glutamine serves as the obligatory nitrogen donor via its side-chain terminal amine group for the biosynthesis of nitrogen-containing metabolites such as nucleotides, asparagine, NAD, and hexosamines. Hence GS-mediated glutamine synthesis is particularly important for cancer cell growth and proliferation in poorly vascularized tumors where circulatory glutamine supply is limited. Genetic ablation of GS in the pancreas impedes the development of pancreatic ductal carcinoma (PDAC) and prolongs animal survival in a KrasG12D/p53-driven (KPC) mouse models. On the other hand, another important

function of GS is to remove ammonia in the liver. Oncogenic activation of -catenin leads to defective urea cycle and elevated ammonia waste burden in the liver. GS expression is paradoxically induced, presumably to help alleviate the hyperammonemia condition. Genetic ablation of hepatic GS accelerates the onset of liver tumors in several mouse models that involve b-catenin activation. In vivo transcriptome and metabolomics analyses show that GS ablation exacerbates hyperammonemia and facilitates the production of glutamate-derived alanine, which subsequently stimulates mTORC1. Pharmacological and genetic inhibition of mTORC1 and glutamic-pyruvic transaminase (alanine transaminase) suppresses tumorigenesis facilitated by GS ablation. Therefore, while GS plays a pro-tumorigenic role in many cancers such as PDAC, GS-mediated ammonia clearance serves as a tumor-suppressing mechanism in livers that harbor b-catenin activation mutations.

W5-4: Tyrosine Degradation: a Boost for Cancer Chemotherapy

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Amino acid metabolism has been studied as a way to treat cancer, but it is not clear how it affects the response to chemotherapy. We found that removing an enzyme called fumarylacetoacetate hydrolase (FAH), an enzyme that catalyzes the final step of tyrosine degradation, made ovarian cancer cells less sensitive to chemotherapy. Our findings indicate that the expression level of FAH correlates significantly with chemotherapy efficacy in patients with ovarian cancer. In terms of the underlying mechanism, under genotoxic chemotherapy, FAH is oxidised at Met308 and translocates to the nucleus, where FAH-mediated tyrosine catabolism predominantly supplies fumarate. FAH-produced fumarate binds directly to REV1, resulting in the suppression of translesion DNA synthesis (TLS) and improved chemosensitivity. Furthermore, in vivo tyrosine supplementation improves sensitivity to genotoxic chemotherapeutics and reduces the occurrence of therapy resistance. Our findings demonstrate a novel function for tyrosine-derived fumarate in regulating TLS, offering a potential avenue for enhancing genotoxic chemotherapy through dietary tyrosine supplementation.

W5-5: Pentose Metabolism in Cancer: Regulation and Therapeutic Opportunities

Xuemei Tong

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Transketolase (TKT) catalyzes two reversible reactions in the non-oxidative pentose phosphate pathway (non-oxidative PPP), bridging PPP and glycolytic metabolites. The physiological and pathological significance of TKT remains to be elucidated. We found that hyperinsulinemia promoted TKT expression and TKT was critical for the ribose moiety of nucleosides to enter glycolysis. By studying the function of TKT in cancer cells, regulatory T cells and $CD8^+$ T cells, we revealed intriguing roles of

pentose metabolism in regulating genome instability, mitochondrial function, DNA methylation and nutrient uptake, providing new therapeutic opportunities for prevention and treatment of cancer.

W5-6: Protein Metabolism in Health And Disease
Weiping Han
Duke-NUS Medical School

Although epidemiological studies indicate that increased consumption of proteins, especially those with high BCAA content, is associated with 2-3 fold higher all cause cancer mortality, and replacement of proteins with carbohydrates or lipids as fuel source leads to dramatically reduced risk of such death, it continues to dominate scientific views and public beliefs that protein is a better and healthier macronutrient. For example, it is believed that increased de novo lipogenesis (DNL), a hallmark of nonalcoholic fatty liver disease (NAFLD) in obesity, relies on carbohydrates as the macronutrient source to support hepatic fatty acid synthesis. However, ex vivo isotope tracing studies support amino acids as a primary carbon supplier for tricarboxylic acid (TCA) cycle and lipogenesis in hepatocytes. Here I will provide our latest studies in determining the source and relative contribution of macronutrients towards DNL.

Workshop 6: Hematopoiesis and Immune Responses (Room 2F)

Chairs: Wei Du, Qing Li

W6-1: Persistent DNA Damage-Induced Immune Response in Aging Hematopoiesis
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Systemic immune effects of DNA damage in aging and disease remain elusive. It is generally believed that immune responses triggered by transient DNA damage are beneficial while those associated with persistent DNA damage are detrimental. We recently reported that NLRP12 (NOD-like receptor 12), a member of the NLR inflammasome family that plays a central role in innate immunity, is induced by persistent DNA damage, and that persistent DNA damage-induced NLRP12 improves the function of hematopoietic stem and progenitor cells (HSPCs) in both mouse and human models of DNA repair deficiency and aging. These results argue a protective role of NLRP12 in the context of immune response to persistent DNA damage under conditions of DNA repair deficiency and aging.
However, the mechanism underpinning the link between persistent DNA damage and NLRP12 expression has not been defined. More recently, we show that persistent DNA damage induces abundant cytosolic dsDNA and consequently activation of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway in HSPCs from DNA repair-deficient (*Fanca*$^{-/-}$) and aged mice treated with ionizing

radiation (IR) or the DNA cross-linker mitomycin C (MMC). Concomitantly, a major transcription factor for *Nlrp12*, PU.1, is also elevated. Inhibition of cGAS-STING pathway PU.1 abolishes persistent DNA damage-induced NLRP12 expression in these damaged HSPCs. By using a novel hematopoietic specific *Nlrp12*-KO mouse model, we observed exacerbated aging phenotypes in *Nlrp12*-deficient *Fanca*$^{-/-}$ and aged mice, accompanied with the senescence associated secretory phenotype (SASP) in the bone marrow (BM) and a significant increase in pyroptosis in BM HSPCs. Furthermore, deletion of *Nlrp12* significantly increases cytoplasmic localization of NLRP3, a hallmark of NLRP3 inflammasome activation in the *Nlrp12*-deficient *Fanca*$^{-/-}$ and aged HSPCs. Together, our findings suggest a novel interplay between persistent DNA damage and immune response as a systemic pro-homeostatic effector of immune response to otherwise detrimental persistent DNA damage.

W6-2: Developing New Cancer Cellular Immunotherapies for Solid Tumors

Li Peng
Guangzhou Institute of Biomedicineand Health

Adoptive cell therapy (ACT) is highly personalized and represents a promising cancer therapy. ACT therapy uses both T cells and NK cells. Engineered T and NK cells that express chimeric antigen receptors (CARs) have been successfully used to treat hematopoietic malignancies but exhibit limited clinical benefits for solid tumor patients. Given the cellular and molecular heterogeneity in solid tumors, new sources of immune cells are demanded to use multiple machineries to recognize and eliminate tumor cells. In addition, TGFβ is known to play a crucial role in tumor progression and T cell dysfunction within the TME. I am leading a team to study tumor immunology in two aspects: 1. Reprogram T cells to NK-like cells as new sources of ACTs; 2. Develop new CAR-T cells to disrupt the TME. In the presentation, I will show recent progress in developing and characterizing anti-TGFβ CAR T cells (T28z T cells). CAR-transduced $CD4^+$ but not $CD8^+$ T cell compartment that is enriched with $TCF\text{-}1^+IL7R^+$ memory T cells persists in PBMCs and tumors and downregulates exhausted T cell markers, including PD-1 and LAG3. In addition, the expansion and persistence of untransduced $CD8^+$ T cells is improved in the presence of T28z T cells *in vivo*. Notably, a combination of $CD4^+$ T28z T cells and $CD8^+$ anti-GPC3 or anti-MSLN CAR T cells exhibit augmented antitumor effects in xenograft. To evaluate the safety and preliminary efficacy of the combination TbM or TbG (TbM/G), we initiated a phase I clinical trial and have enrolled 17 patients with solid tumors, including 6 with NSCLC, 4 with HCC, 4 with PC and 3 with other types of tumors. Preliminary clinical trial results will be presented.

W6-3: Fatty Acid Metabolism in Hematopoietic Stem Cells

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Hematopoietic homeostasis requires that a rare pool of hematopoietic stem cells (HSCs) balance the processes of self-renewal and differentiation throughout the life of an organism. HSCs are maintained in a quiescent state and enter the cell cycle for homeostatic proliferation and under times of stress. The distinct metabolic states of HSCs under these conditions are largely unknown and essential *in vivo* studies into

this fundamental aspect of HSC biology are lacking. Though fatty acid oxidation has been shown to be essential for hematopoietic stem cell maintenance, the importance of fatty acid uptake from the bone marrow microenvironment vs. de novo lipogenesis has not been elucidated. The process of de novo lipogenesis begins with the generation of malonyl-CoA from acetyl-CoA and is catalyzed by the enzyme acetyl-CoA carboxylase 1 (ACC1), encoded by Acaca. Malonyl-CoA is then subsequently converted to palmitate by fatty acid synthase (FASN). To characterize the role of de novo lipogenesis, we deleted *Acetyl-CoA carboxylase 1* (*Acaca*), the gene encoding the DNL rate-limiting enzyme ACC1, in hematopoietic tissue using Mx1-cre mediated knockout. ACC1 loss results in aberrant myelopoiesis, splenomegaly, and the accumulation of HSCs and primitive progenitors in the bone marrow and spleen. Functional testing of HSCs revealed that ACC1-deficient HSCs lost the ability to facilitate trilineage hematopoiesis and self-renew following bone marrow transplantation. These findings are linked to an increased proliferation rate and altered metabolic profile. ACC1-deficient HSCs demonstrated increased fatty acid uptake however high fat diet feeding failed to completely rescue the phenotype of ACC1 knockout, suggesting fatty acid update and de novo synthesis play different roles in HSC regulation. Together, these data suggest that ACC1 is essential for normal hematopoiesis and HSC long term self-renewal. Collectively, these data are the first report of a critical role for ACC1 in hematopoiesis and LT-HSC function.

W6-4: Dynamic Changes in Lysine Succinylation as Important Regulators of Erythropoiesis

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Lysine succinylation has emerged as a recently discovered protein modification that significantly impacts the chemical environment and exhibits diverse functions in various biological processes. However, the specific role of lysine succinylation in erythropoiesis has not been fully elucidated. In this study, we investigated the levels of six common acylations (acetylation, crotonylation, succinylation, propionylation, butyrylation, and malonylation) in human erythroid cells. Interestingly, we observed a prominent accumulation of lysine succinylation during human erythroid differentiation, suggesting its potential importance in this process. To explore the functional significance of succinylation, we inhibited succinylation in human erythroid progenitor cell line by disrupting the expression of the key succinyltransferases and desuccinylases. The results revealed that succinylation inhibition led to suppressed cell proliferation, increased apoptosis, and disrupted differentiation, indicating the essential role of succinylation in erythropoiesis. Furthermore, integrative proteome and succinylome analysis identifies 939 quantifiable proteins with 2,871 Ksu sites. Notably, we observed inconsistencies between alterations in protein levels and succinylation levels, suggesting that the role of succinylation in proteins' function regulation. These succinylated proteins are widely distributed in various cellular compartments and involved in multiple cell processes, indicating that succinylation is a prevalent modification in erythropoiesis. Mechanically, we identified CYCS as a key target of succinylation during

erythropoiesis, emphasizing its essential role in this process. Specially, we implicated KAT2A-mediated histone succinylation in chromatin remodeling, further highlighting the regulatory significance of lysine succinylation in erythropoiesis at the epigenetic level. Collectively, our comprehensive investigation of the succinylation landscape during erythropoiesis provides valuable insights into its regulatory role and offer potential implications for erythroid-related diseases and therapeutic strategies.

W6-5: Delineating Metabolic Gatekeepers of B-cell Malignancy and CAR T-cell Functionality

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PIK3CA activating mutations and *PTEN* deletions are frequent in nearly all types of cancer but hardly detected in B cell acute lymphoblastic leukemia (B-ALL). Here we report unique crosstalk of PI3K and mTORC1 activities which is mediated by MYC to ensure balanced cellular energy supply and anabolism in B-ALL cells. We revealed the essentiality of *PTEN* in ALL cells was through keeping energy production by glutaminolysis. Based on the transcriptomic profiling of Pten deficient ALL cells, we found that PI3K hyperactivation decreased multiple cellular biosynthetic processes, while Myc overexpression significantly restored biosynthesis. Untargeted metabolomic analysis indicated depletion of several key amino-acid levels in Pten KO murine B-ALL cells, while Myc overexpression partially restored them and further initiated reprogram of multiple biosynthetic pathways. Our findings decipher a unique synthetic vulnerability of PI3K and MYC in malignant precursor B cells and highlight the balance between anabolism and energy supply for cell survival as a promising therapeutic strategy for B-ALL.

CAR T therapy has shown great success in treating B-ALL and other B-cell malignancies. However, during rapid *ex vivo* expansion or *in vivo* tumor eradication, metabolic shifts and inhibitory immune signals lead to terminal differentiation and exhaustion of CAR T cells, impairing their antitumor potency. We show that the FDA-approved isocitrate dehydrogenase 2 (IDH2) inhibitor enasidenib enhances memory CAR T-cell formation, and exhibits sustainedsustains anti-leukemic cytotoxicity *in vivo*. Mechanistically, IDH2 impedes metabolic fitness of CAR T cells by restraining glucose carbon utilization via the pentose phosphate pathway, which alleviates oxidative stress, particularly in nutrient-restricted conditions. In addition, IDH2 limits cytosolic Acetyl-CoA level in CAR T cells to prevent histone acetylation that promotes memory cell formation.

Workshop 7: Type I in Interferons in Infection, Autoimmune and Cancer (Room 2G)

Chairs: Genhong Cheng, Lishan Su

W7-1: Systems Medicine Approaches to Define the Type I interferon Gene Program

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We take systems medicine approaches to define the type I interferon (IFN-I) signal transduction pathway and gene expression network, which we called gene program. This IFN-I gene program upregulates of several hundred interferon stimulated genes (ISGs) and downregulates numerous metabolic genes including those involved in the synthesis of fatty acids, amino acids and nucleotides. Interestingly, both upregulation of ISGs and downregulation of metabolic genes play important roles in IFN-I-mediated antiviral activities. In addition, we have elucidated both physiological and pathological impacts of the IFN-I gene program on host immune responses against acute and chronic bacterial infections, metabolic diseases and inflammatory diseases. Our recent studies have also identified multiple pathways involved in regulating IFN-I-mediated anti-tumor immunity in the tumor microenvironment.

W7-2: Signal-Induced NLRP3 Phase Separation Initiates Inflammasome Activation

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NLRP3 inflammasome is activated by diversified stimuli including infections, intracellular and environmental irritators. Many inflammation-related autoimmune diseases involve NLRP3 overactivation. How NLRP3 senses these unrelated stimuli and what activate NLRP3 remains unknown. Here we reported that signal-dependent NLRP3 phase separation initiated its activation, in which the palmitoyltransferase ZDHHC7-mediated tonic NLRP3 palmitoylation and an IDR region in the FISNA domain of NLRP3 were important. Known NLRP3-activating stimuli including K+ efflux, imiquimod, intracellular metabolites like palmitate, and the mitochondrial lipid cardiolipin induced NLRP3 phase separation and activation in cells and in vitro. Surprisingly, amphiphilic molecules like di-alcohols usually used to inhibit biomolecular phase separation and chemotherapeutic drugs doxorubicin and paclitaxel also activate NLRP3 by inducing phase separation, suggesting a unique feature of protein palmitoylation-mediated phase separation. Our results demonstrated that signal-induced NLRP3 phase separation provides probably the simplest and most direct mechanistic basis for both K+-dependent and K+-independent NLRP3 activation.

W7-3: A Novel A-IFNAR1 Antibody Selectively Preserves IFN-B But Blocks All the Other Type I Interferons

Liguo Zhang[1], Jingyun Li[1], Yong Wang[1], Xinlu Wang[1], Liwei Zhang[1], Haojie Xu[2], Xiaohua Nie[1], Jiafeng Huang[1], Junxiao Ma[1], Xuyuan Zhang[1], Lidan Zhao[2], Guangxia Gao[1], Pu Gao[1]

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Type I interferons (IFN-Is) play pivotal roles in antiviral immune responses. However, the uncontrolled expression of IFN-Is is a crucial pathogenic factor in multiple autoimmune diseases. Among the various IFN-Is, IFN-β stands out both by its unique mechanisms of induction and distinct functions. Here we reported a unique monoclonal antibody 7G4, which binds IFN-I receptor IFNAR1 and blocks IFN-αs, IFN-ω, IFN-ε, and IFN-κ but selectively perseveres IFN-β. Additionally, we demonstrate that 7G4 efficiently blocks IFN-I activity in serum samples of systemic lupus erythematosus patients. By solving the structure of the 7G4-IFNAR1 complex, we reveal that the inhibitory mechanism of 7G4 is non-competitive. The binding of 7G4 induces conformational changes to IFNAR1 and disrupts the binding of IFN-αs but selectively preserves IFN-β due to its higher affinity for IFNAR1. Thus, 7G4 represents a promising candidate for the next generation of IFN-I blockade in the treatment of autoimmune diseases associated with IFN-αs but not IFN-β.

W7-4: The IFN-Is Paradox in Anti-Tumor Immunity

Guang Sheng Ling[1,2], Weixin Chen[1], Jia Ming Nickolas Teo[1], Siu Wah Yau[1], Yee-Man Melody Wong[3], Chun-Nam Lok[4], Chi-Ming Che[4], Asif Javed[1], Yuanhua Huang[1], Stephanie Ma[1,4,5,6]
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Identifying signals that govern the differentiation of tumor-infiltrating $CD8^+$T cells ($CD8^+$TILs) towards exhaustion can improve current therapeutic approaches for cancer. We found that type I interferons (IFN-Is) act as environmental cues enhancing terminal $CD8^+$T cell exhaustion in tumors. We observed the enrichment of IFN-Is-stimulated genes (ISGs) within exhausted $CD8^+$T cells (Tex cells) in patients across various cancer types, with heightened ISG levels correlating with poor response to immune checkpoint blockade (ICB) therapy. In preclinical models, $CD8^+$TILs devoid of IFN-Is signaling developed less exhaustion features, provided better tumor control and showed greater response to ICB-mediated rejuvenation. Mechanistically, chronic IFN-Is stimulation perturbed lipid metabolism and redox balance in Tex cells, leading to aberrant lipid accumulation and elevated oxidative stress. Collectively, these defects promoted lipid peroxidation, which potentiated metabolic and functional exhaustion of Tex cells. Thus, cell-intrinsic IFN-Is signaling regulates the extent of $CD8^+$TIL exhaustion, and has important implications for immunotherapy.

W7-5: Critical Roles of Type III Interferon Pathway In Protecting Host Against Viral Infection Beyond Mucosal Tissues

Meifang Pan, Jun Zhang, Jingrong Shi, Jianping Cui, Yujuan Xu, Xiaoping Tang, Fengyu Hu*, **Feng Li***

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Understanding the antiviral role of the type III interferon pathway in non-mucosal tissues, a relatively less explored domain compared to its widely recognized mucosal immune responses, is crucial for developing novel therapeutics. Our study investigates this aspect using a uniquely developed IFN**A**R, IFN**G**R, and IFN**L**R triple defective AGL mouse model created by deleting a large fragment containing four receptor subunits. First, we tested the infection permissiveness of this AGL mouse against Flavivirus such as Dengue virus (DENV) and Zika virus. Compared to the IFNAR and IFNGR defective mice (AGB6), AGL mice supported robust viral replications in both the mucosal and non-mucosal tissues but also exhibited obvious morbidity and mortality. Second, we found that Monkeypox (mpox) infected AGL mice had obvious weight loss and replicated robustly in multiple organs after intranasal infection. Our findings reveal that, in the absence of type I and II interferons, type III interferons provide essential defence against multiple viral infections in non-mucosal tissues. This discovery expands the conventional understanding of type III interferon-mediated immune responses beyond mucosal tissues.

W7-6: Type I Interferons and Pdc In Inflammatory Diseases: Lessons From HIV

Lishan Su, Degui Geng, James Ahodantin, Yaoxian Lou, Michael Wu and Guangming Li

Institute of Human Virology, Departments of Pharmacology and Microbiology and Immunology, University of Maryland Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA

Plasmacytoid dendritic cells (pDC) are the major type I interferon (IFN-I) producing cells and play important roles in antiviral immune responses during acute virus infection. However, sustained pDC activation and IFN-I signaling has been correlated with disease progression in chronic virus infection. We have functionally revealed the role of pDC/IFN-I in HIV-induced inflammatory diseases. The pDC-mediated inflammation (Inflammation-pDC) pathway can be targeted with novel therapeutics to i) resolve inflammation-associated diseases in HIV-infected hosts under ART, ii) recover anti-HIV immunity and iii) reduce or control HIV-1 reservoirs. Mechanistically, inflammatory pDC/IFN-I impair anti-HIV T cells via multiple pathways including metabolic repgramming and functional maturation. Interestingly, the inflammation-pDC pathway is also involved in other human inflammatory diseases including cancer. We have demonstrated that similar immune suppression is induced in tumor-immune-microenvironment (TIME) as HIV-infected lymphoid tissues. We have also discovered that tumor-associated pDC (TApDC) impairs anti-tumor T cells via similar mechanisms. Modulation of TApDC repgrams TIME, rescues anti-tumor T cells and reduces tumor growth alone or cooperatively with immune check point inhibition. The inflammation-pDC pathway will provide novel

therapeutic targets for treating inflammatory diseases including cancer and chroinic virus infection.

Workshop 8: RNA Structure and Assembly (Room 3A)

Chair: Chuan He

W8-1: *De Novo* Assembly of Nuclear Stress Bodies Rearranges and Activates NFIL3 to Restrain Acute Inflammatory Responses

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Mammalian cells activate diverse defense mechanisms in response to stresses. Nuclear stress bodies (nSBs) are transient membrane-less organelles in primates that only present in sensing severe stresses. Little is known on how their formation contributes to cellular homeostasis or whether nSBs have any physiopathological roles. We report the widespread expression of Satellite III transcripts (*SatIII* RNAs), the hall marker of nSBs, across chromosomes. nSB components, including *SatIII* DNAs, RNAs and 30 proteins examined, are assembled into a well-organized structure within 3 hours, as shown by high-resolution imaging in living cells. Remarkably, the activated *SatIII* heterochromatin loci rapidly expand, resulting in adjacent gene activation, including the transcription suppressor *NFIL3* known to dampen proinflammatory cytokine production. Upon stresses, *NFIL3* loci were found within or close to nSB territory due to *SatIII* locus expansion, which enhances *NFIL3* chromatin accessibility and makes *NFIL3* promoters spatially more accessible to transcription factors HSF1 and BRD4 newly recruited to nSBs. Human peripheral blood mononuclear cell (PBMC)-derived macrophages under heat shock and LPS stresses exhibited increased expression of *SatIII* and NFIL3, the latter of which prevents the excessive expression of key inflammatory cytokines. Importantly, *NFIL3* expression positively correlates with *SatIII* activation in PBMCs from septic patients, which appears to be beneficial for patient survival. These findings unveil an unexpected role of the highly organized nSBs in shaping local gene organization upon stresses, and highlight a crucial role of nSBs in restraining acute inflammatory responses.

W8-2: Studying RNA Structures to Understand RNA Function

Yue Wan
*Genome Institute of Singapore, A*STAR, Singapore*

RNA structures play important roles in every stage of an RNA's lifecycle. Recent developments in coupling RNA structure probing with high throughput sequencing have greatly accelerated our ability to gain structural and biological insights into how an RNA can fold to achieve its function. In this talk, I will describe some of the new technologies that we have developed to study RNA structures and how they inform new understanding of cellular compartmentalization and development.

W8-3: A Deep Neural Network-Based Method for Predicting Small Molecule and RNA Target Interactions

Qiangfeng Zhang

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RNA-targeting small molecules have emerged as potential therapeutics for human diseases by specifically binding to RNA and regulating its function. However, progress in this field has been impeded by limited resolved interaction structures and ineffective virtual screening methods. In this study, we introduce SmrtNet, a deep neural network-based method for predicting **s**mall **m**olecule and **R**NA **t**arget interactions using deep neural **net**work. SmrtNet utilizes an RNA language model and a coarse-grained representation of RNA structures to characterize binding pockets on RNA molecules. Our results highlight the substantial superiority of SmrtNet over existing tools in terms of both accuracy and efficiency. Notably, SmrtNet successfully predicts 132 interactions that have already been experimentally validated in published studies. Moreover, we employ SmrtNet to screen a vast compound library for targeting mRNAs of undruggable proteins, viral RNAs, and onco-miRNAs. The predicted interactions and functional effects are subsequently validated through in vitro and in vivo experiments, underscoring the potential of SmrtNet to offer new solutions and insights in the development of RNA-targeting drugs.

W8-4: A New RNA Base Editor Repairs Nonsense Mutations

Chengqi Yi

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Nonsense mutations, which lead to premature termination codons (PTCs) in mRNA coding region, account for ~20% of mutations associated with human diseases. Current treatments for diseases related to nonsense mutations often lack specificity or may cause severe off-target effects and immunogenicity. Therefore, we developed a CRISPR-independent, programmable targeted pseudouridylation method RESTART, which can efficiently and specifically edit PTC sites on mammalian cell mRNA, thereby achieving effective readthrough and functional rescue in disease cell models related to nonsense mutations. Moreover, the restricted off-target edits induced by RESTART are generally “benign” as they do not change the coding information or the global gene expression. Collectively, RESTART is a promising RNA-editing tool for research and therapeutics.

W8-5: Detection and Processing of Transcription Activity by Ribozyme-Assisted RNA Editing

Yangming Wang[1,2], Jing Wang[1,2], Lu-Feng Hu[1,2], Jia-Zhen Wang[1,2]

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We introduce a method termed ribozyme-processed adenosine deaminase acting on RNA (ADAR)-engaging RNA-directed editing (REDDIT), which allows sensitive and quantitative detection with high dynamic range for the transcription of

protein-coding genes, long noncoding RNAs (lncRNAs), and primary-microRNAs (pri-miRNAs). Importantly, REDDIT faithfully reflects the transcription events associated with enhancer elements, marking an accomplishment not achieved before. Furthermore, we demonstrate the adaptability of REDDIT for detecting multiplexed transcription events and monitoring changes in cell states of human embryonic stem cells (hESCs). Finally, REDDIT can be adapted to record transient transcription events triggered by different signals combinatorially, and allows uncovering molecular pathways regulating specific stages of the biogenesis of a miRNA in a high-throughput screen. In conclusion, REDDIT is a robust platform for monitoring and processing transcription activity across diverse genome units, which holds potential to control cell states in response to specific transcription events and dissect molecular mechanisms underlying various biologic processes.

Workshop 9: Epigenetics, Chromatin and Transcription (Room 3B)

Chairs: Ting Wang, Guohong Li

W9-1: High-Resolution Profiling of Total RNA in Single Cells with STORM-Seq

Benjamin K. Johnson[1]*, Mary Rhodes[1,2]*, Rebecca A. Siwicki[2], Marc Wegener[2], Ayush Semwal[1], H. Josh Jang[1], Jacob Morrison[1], Pamela Himadewi[1], Kelly Foy[1], Joshua L. Schipper[4], Larissa L. Rossell[3], Emily J. Siegwald[3], Dave W. Chesla[3], Jose M. Teixeira[5], Rachael T. C. Sheridan[4], Ting Wang[6], Timothy J. Triche Jr.[1], **Hui Shen**[1‡]

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Recent advances in single-cell RNA sequencing (scRNA-seq) have provided techniques that capture both mRNA and total RNA – exemplified by Smart-seq3/xpress, VASA-seq, and Smart-seq-total. However, a primary limitation to broad adoption is the accessibility of these methods both at the bench and computationally. Here, we present STORM-seq, a fully kitted solution for single cell total RNA sequencing (sc-total-RNAseq) that makes use of random hexamer priming, incorporation of unique molecular identifiers (UMI), and requires no specialized equipment, generating the most complex scRNA-seq libraries to date. Further, data analysis is straightforward by integration of STORM-seq's library fragment structure into commonly used bioinformatics tools. STORM-seq is the only scRNA-seq method to reliably profile transposable elements (TEs) in single cells, and have unique sensitivity for splicing isoforms, enhancer RNAs, fusion transcripts and expressed variants. We applied STORM-seq to primary human benign distal fallopian tube epithelium (FTE), thought to harbor the cell of origin (COO) for high-grade serous ovarian carcinoma (HGSOC), and reveal a progenitor cell population that could serve as the COO for HGSOC.

W9-2: 2'-O-Methylation at Internal Sites on mRNA Promotes mRNA Stability

Yanqiang Li[1,2,10], Yang Yi[3,4,10], Xinlei Gao[1,2], Xin Wang[1,2], Dongyu Zhao[1,2], Rui Wang[3,4], Li-Sheng Zhang[5,6,7], Boyang Gao[6,7], Yadong Zhang[1,2], Lili Zhang[1,2], Qi Cao[3,4,*], **Kaifu Chen**[1,2,8,9,11,*]

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2′-O-methylation (Nm) is a prominent RNA modification well known in noncoding RNAs and more recently also found at many mRNA internal sites. However, their function and base-resolution stoichiometry remain under-explored. Here, we investigated the transcriptome-wide effect of internal site Nm on mRNA stability. Combining Nanopore sequencing with our developed machine-learning method, NanoNm, we identified thousands of Nm sites on mRNAs with a single-base resolution. We observed a positive effect of FBL-mediated Nm modification on mRNA stability and expression level. Elevated FBL expression in cancer cells is associated with increased expression levels for 2′-O-methylated mRNAs of cancer pathways, implying the role of FBL in post-transcriptional regulation. At last, we found FBL-mediated 2′-O-methylation connected to widespread 3′ UTR shortening, a mechanism that globally increases RNA stability. Collectively, we demonstrated that FBL-mediated Nm modifications at mRNA internal sites regulate gene expression by enhancing mRNA stability.

W9-3: Altered Chromatin Occupancy of Patient-Associated H4 Mutants Misregulate Neuronal Differentiation

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Chromatin is a crucial regulator of gene expression and tightly controls development across species. Mutations in only one copy of multiple histone genes were identified in children with developmental disorders characterized by microcephaly, but their mechanistic roles in development remain unclear. Here we focus on dominant mutations affecting histone H4 lysine 91. These H4K91 mutants form aberrant nuclear puncta at specific heterochromatin regions. Mechanistically, H4K91 mutants demonstrate enhanced binding to the histone variant H3.3, and ablation of H3.3 or the H3.3-specific chaperone DAXX diminishes the mutant localization to chromatin. Our functional studies demonstrate that H4K91 mutant expression increases chromatin accessibility, alters developmental gene expression through accelerating pro-neural differentiation, and causes reduced mouse brain size *in vivo*, reminiscent of the microcephaly phenotypes of patients. Together, our studies unveil a distinct molecular pathogenic mechanism from other known histone mutants, where H4K91 mutants misregulate cell fate during development through abnormal genomic localization.

W9-4: Single-Cell Total-RNA Profiling Unveils Regulatory Hubs of Transcription Factors

Chenghang Zong[1,2,3,4,5,6], Yichi Niu[1,2,] and Jiayi Luo[1,3]
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Recent development of RNA velocity uses master equations to establish the kinetics of the life cycle of RNAs from unspliced RNA to spliced RNA (i.e., mature RNA) to degradation. To feed this kinetic analysis, simultaneous measurement of unspliced RNA and spliced RNA in single cells is greatly desired. However, the majority of single-cell RNA-seq chemistry only captures mature RNA species to measure gene expressions. Here, we develop a one-step total-RNA chemistry-based scRNA-seq method: snapTotal-seq. We benchmarked this method with multiple single-cell RNA-seq assays in their performance in kinetic analysis of cell cycle by RNA velocity. Next, with LASSO regression between transcription factors, we identified the critical regulatory hubs mediating the cell cycle dynamics. We also applied snapTotal-seq to profile the oncogene-induced senescence and identified the key regulatory hubs governing the entry of senescence. Furthermore, through the comparative analysis of unspliced RNA and spliced RNA, we also identified a significant portion of genes whose expression changes occurred in spliced RNA but not to the same degree in unspliced RNA, indicating these gene expression changes are mainly controlled by post-transcriptional regulation. Overall, we demonstrate that single-cell total-RNA-seq data generated by snapTotal-seq can provide enriched information about gene regulation, especially during the transition between cell states.

W9-5: Pathogenic Germline Variants in Asian PAAD

Jing Li

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Putting pancreatic cancer screening into perspective for high-risk individuals could significantly reduce cancer morbidity and mortality. Previous studies have clarified the somatic mutations in PAAD. In contrast, the prevalence of mutations in PAAD predisposition genes has not been well investigated, especially in the Asian population. Here we revealed a comprehensive germline mutation landscape in 1,123 cancer patients compared to 2,944 healthy controls. CFTR (3.60%), BRCA1 (1.80%), MSH2 (1.80%) and BRCA2 (1.54%) are identified as the top germline mutated cancer genes. Integration of immunohistochemistry and gene expression analysis also supported CFTR as a powerful prognostic marker for PAAD. To investigate the molecular mechanism, reduced CFTR levels can result from LOH events or epigenetic modifications, such as DNA methylation. By comparing them to the known CF and pancreatitis-related mutations, we discovered 14 novel mutations predicted to be deleterious. Interrogation of the biological properties of down-regulated CFTR and nine CFTR mutants revealed a gain of function in pancreatic cell line proliferation assays. To characterize the drug sensitivity based on germline molecular subtyping, we performed drug screening on 23 organoids using a library of 63 chemicals. In particular, the CFTR mutant exhibited functional responses to CFTR modulator treatment.

W9-6: Characterizing Cytosine Methylation of Polymorphic Human Transposable Element Insertions Using Human Pangenome Resources

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Cytosine methylation is an important epigenetic modification that plays a crucial role in genomic regulation. The second generation sequencing-based, conventional bisulfite-conversion methods to interrogate cytosine methylation require comparing bisulfited-treated reads with reference genome for methylation calling. Therefore, it cannot characterize the methylation of non-reference regions. Taking advantage of the recent improvement of the 3rd generation sequecing, we investigated the methylation pattern of human lymphoblastoid cell lines (LCLs) of non-reference insertions from the human population, with a focus on polymorphic transposable elements. We first characterized whole-genome CpG methylation using both SMRT and nanopore technology and benchmarked their performance against WGBS using five human LCLs included in the draft Human Pangenome Reference. Both methods are highly correlated with the conventional WGBS results across the genome. The level of differences between PacBio and ONT on the same sample is comparable with that of two replicates of WGBS of the same sample. Using long-read data from the draft Human Pangenome Reference, we characterized CpG methylation of non-reference insertions, especially polymorphic transposable elements (TEs). We focused to address two questions: do newly inserted TEs adopt the methylation pattern of their genomic context? Do methylation spread from new TE insertions to their flanking regions? We found that most non-TE insertions exhibit DNA methylation pattern

consistent with their genomic context, but TE insertions are consistently methylated, with a few exceptions. We also found limited methylation spreading from Alu/L1 insertions to their flanking genomic regions. We also investigated INDEL frequency in both hypermethylated and hypomethylated CpG islands, and found INDELs are enriched in hypermethylated CpG islands. Our work demonstrated the methylation calling cabability of the 3rd generation sequencing and its unique advantage in characterizing epigenomic features within non-reference positions.

Workshop 61: New Frontiers in Cancer Metastasis Research (Room 2H)

Chairs: Chonghui Cheng and Jing Yang

W61-1: RNA Metabolism in Breast Cancer Metastasis

Chonghui Cheng
Breast Center, Baylor College of Medicine, Houston, TX, USA

RNA splicing is pivotal in post-transcriptional gene regulation, yet the exponential expansion of intron length in humans poses a challenge for accurate splicing. Here, we identify hnRNPM as an essential RNA-binding protein that suppresses cryptic splicing through binding to deep introns, maintaining human transcriptome integrity. Long interspersed nuclear elements (LINEs) in introns harbor numerous pseudo splice sites. hnRNPM preferentially binds at intronic LINEs to repress pseudo splice site usage for cryptic splicing. Remarkably, cryptic exons can generate long dsRNAs through base-pairing of inverted ALU transposable elements interspersed among LINEs and consequently trigger an interferon response, a well-known antiviral defense mechanism. Significantly, hnRNPM-deficient tumors show upregulated interferon-associated pathways and elevated immune cell infiltration. These findings unveil hnRNPM as a guardian of transcriptome integrity by repressing cryptic splicing and suggest that targeting hnRNPM in tumors may be used to trigger an inflammatory immune response, thereby boosting cancer surveillance.

W61-2: Epithelial-Mesenchymal Plasticity in Carcinoma Metastasis

Jing Yang
Department of Pharmacology and of Pediatricts, Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA

Breast tumors are often detected through manual palpation due to their apparent “hardness” compared to normal tissue. Increase in tissue stiffness is correlated with distant metastasis and poor outcome in breast cancer patients. Several studies, including ours, show that increasing matrix stiffness can induce Epithelial-Mesenchymal Transition (EMT) and cancer cell invasion in human and mouse 3D mammary epithelial organoids, suggesting that mechanical properties of extracellular matrix (ECM) directly regulate tumor metastasis. Using 3D reconstituted extracellular matrixes that recapitulate the range of physiological stiffness from normal mammary glands to breast tumors, we identified TWIST1 as a key player driving EMT and invasion in response to increasing ECM stiffness. High stiffness releases TWIST1 from its cytoplasmic anchor protein G3BP2, so it can enter into the nucleus to drive EMT-associated transcription. I will present our recent progress in understanding the Twist1/G3BP2 mechanotransduction pathway that senses and

transmits mechanical cues from extracellular matrix in the tumor microenvironment to promote EMT and invasion during tumor progression.

W61-3: Orchestrated Ecosystem of Circulating Tumor Cell-Immune Cell Interactions in Metastasis

Huiping Liu
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Cellular plasticity and stemness properties enable dynamic changes of circulating tumor cells (CTCs) during cancer dissemination, such as aggregation or cohesion of single CTCs into multicellular CTC clusters. CTC clusters possess 20-100 times higher metastatic propensity than the single CTCs. To characterize the CTC clusters, we have established multiple CTC analysis technologies including FDA-approved CellSearch, flow cytometry, and immunohistochemistry using patient blood and tissue sections collected at the pre-treatment baseline and after-therapy timepoints. Our previous work demonstrated that stemness glycoproteins on breast cancer stem cells, such as CD44, CD81, and ICAM1 drive CTC aggregation in metastatic breast cancer, triple negative breast cancer (TNBC) in particular. Recent studies further identified immune cells in the CTC clusters and these immune cells contribute to the seeding of the CTCs into distant organs. More specifically, we measured the glycosylation patterns in CTCs and its association with clinical outcomes, We found that chemotherapy-evasive CTC clusters are relatively quiescent with a specific loss of terminal sugar residues α2,6-sialic acids in glycoproteins. CTCs showed dynamic hypo-sialylation in the blood with loss of the sialyl-transferase ST6GAL1, promoting CTC cluster formation with cellular quiescence (proliferative dormancy) and evading chemotherapy in breast cancer. Seeded tumor cells regained ST6GAL1 to enable metastatic colonization. Many adhesion proteins and stemness drivers as glycoprotein substrates of ST6GAL1 drive CTC clustering and metastatic seeding. Neutralizing antibodies against these clustering drivers inhibit CTC cluster formation and improve therapy response, thereby blocking lung metastasis in TNBC.

W61-4: Regulation of Breast Cancer Metastasis Organotropism

Guohong Hu[1], Qiuyao Wu[1]
[1] *Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai, China.*

Tumor cells disseminate to various distant organs from the primary sites, while different tumor types and subtypes display distinct preference of target organs for metastatic colonization. However, the regulatory mechanism leading to metastasis organotropism remains largely elusive. Previously we have discovered a tumor-secreted protein DKK1 with Janus-faced roles in metastatic colonization of breast cancer in lung bone, explaining the choice of metastasis target organs by tumor cell expressing varied levels of DKK1. Here we further studied the metastasis preference of various breast cancer subtypes, with the focus to investigate the reason of bone proclivity of luminal cancers. We found an estrogen receptor-regulated factor SCUBE2 with a role to support the survival of disseminated tumor cells in bone. Such an effect of SCUBE2 is dependent on bone-specific microenvironment. Targeting SCUBE2 effectively suppresses bone recurrence. Therefore, our studies highlight the

roles of tumor-microenvironment crosstalk in organ-specific metastasis, and provide a rationale to treat metastasis in various distant sites.

W61-5: Lymphotoxin-β Promotes Breast Cancer Bone Metastasis Colonization and Osteolytic Outgrowth

Hanqiu Zheng[1], Xuxiang Wang[1], and Tengjiang Zhang[1]
[1]*State Key Laboratory of Molecular Oncology and Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing, 100084, China*

Bone metastasis is a lethal consequence of breast cancer. Here, we used single-cell transcriptomics to investigate the molecular mechanisms underlying bone metastasis colonization, the initial and rate-limiting step in the metastatic cascade. We identified lymphotoxin-β (LTβ), highly expressed in tumor cells when they are in the bone microenvironment, associated with poor bone metastasis-free survival, and capable of promoting tumor cell colonization and outgrowth in multiple breast cancer models. Mechanistically, tumor-derived LTβ activates osteoblasts through NF-κB2 signaling to secrete CCL2/5, which facilitates tumor cell adhesion to osteoblasts and accelerates osteoclastogenesis, leading to bone metastasis progression. Blocking LTβ signaling with a decoy receptor significantly suppressed bone colonization and metastatic progression *in vivo*, whereas clinical sample analysis revealed significantly higher LTβ expression in bone metastases than in primary tumors. Our findings highlight LTβ as a bone niche-induced factor that promotes tumor cell colonization and osteolytic outgrowth and underscore its potential as a therapeutic target for patients with bone metastatic disease.

W61-6:Next Generation Rat Models of Breast Cancer：

Yi Li
Breast Center, Baylor College of Medicine, Houston, TX, USA

As the first mammal to be domesticated for research purposes, rats served as the primary animal model for various branches of biomedical research, including breast cancer studies, up until the late 1990s and early 2000s. However, as genetic engineering of mice, but not rats, became routine, mice gradually supplanted rats as the preferred rodent model. But recent advances in genetic manipulations of rats have rekindled the significance of rats as a critical model in exploring various facets of breast cancer research. This is particularly pronounced in the study of the formation and progression of the estrogen receptor-positive subtype, which remains challenging to model in mice. Here, I will first discuss our progress in building rat models using intraductal injection of retrovirus/lentivirus to deliver oncogenic drivers into mature mammary glands. Then, I will describe our recent success in building rat models using somatic genome editing powered by CRISPR/Cas9. Furthermore, I will comment on potential applications of these rat models in areas of breast cancer research that have continued to challenge the mouse model community.

Day 1, July 26 (Friday)

Concurrent Workshops 10-19 3:15 – 4:45 pm

Workshop 10: RNA Modifications (Room 2A)

Chair: Hansen He

W10-1: Noncanonical RNA Caps
Xuemei Chen
School of Life Sciences, Peking University, Beijing, 100871
Beijing Advanced Center of RNA Biology, Peking University, Beijing, 100871

In eukaryotes, messenger RNAs (mRNAs) harbor a 5' methylguanosine (m^7G) cap, which stabilizes mRNAs and assists with their processing, nuclear export and translation. In recent years, it has come to be realized that many cellular metabolites such as NAD, FAD, dpCoA, UDP-glucose, and UDP-GlcNAc, can be present at the 5' end of RNA in viruses, prokaryotes, and all major lineages of eukaryotes (plants, fungi, and animals). We developed methods to quantify these RNA caps using LC-MS and enzymatic methods, and to profile noncanonically capped RNAs in the transcriptome. We also discovered various decapping enzymes with different substrate preferences and product types. I will discuss our work on the development of various methods to identify and quantify noncanonically capped RNAs with specificity and sensitivity. I will also discuss decapping mechanisms that regulate the levels of noncanonically capped RNAs.

W10-2: RNA Adenine Methylation in Neurological Diseases Arising from CAG Repeat Expansions
Yinsheng Wang
Department of Chemistry, University of California Riverside
900 University Avenue, Riverside, CA 92508 USA

Microsatellite repeat expansions within genes contribute to a number of neurological diseases. Accumulation of toxic proteins and RNAs with repetitive sequences, and/or sequestration of RNA-binding proteins by expanded repeat-containing RNAs are thought to be important contributors to disease etiology. In this presentation, I will discuss our discovery about the implications of RNA methylation in CAG repeat expansion diseases. In particular, we found that the adenosine in CAG repeat RNA can be methylated to *N*1-methyladenosine (m^1A) by TRMT61A, and the ensuing m^1A can be demethylated by ALKBH3. We also observed that m^1A/rA ratio in CAG repeat RNA increases with repeat length in a mouse model of Huntington's disease and a Drosophila model of SCA3, which is attributed to diminished expression of ALKBH3 elicited by the repeat RNA. Additionally, TDP-43 binds directly with m^1A in RNA at high affinity, which stimulates the cytoplasmic mis-localization and formation of gel-like aggregates of TDP-43, resembling the observations made for the protein in neurological diseases. We also found that m^1A in CAG repeat RNA contributes to CAG repeat expansion-induced neurodegeneration in *Caenorhabditis elegans*.

Moreover, we observed that N^6-methyladenosine, which also forms in CAG repeat RNA, contributes to repeat-associated non-AUG (RAN) translation. Together, our study offers a new paradigm about the mechanism through which nucleotide repeat expansion contributes to neurological diseases and reveals a novel pathological function of m^1A and m^6A in RNA. These findings may provide an important mechanistic basis for the therapeutic interventions of neurodegenerative diseases emanating from nucleotide repeat expansions.

W10-3: Single-Cell Sequencing Techniques for Mapping RNA Methylation

Yungui Yang
Beijing Institute of Genomics, Chinese Academy of Sciences, China National Center for Bioinformation
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RNA methylation, characterized by dynamic reversible nature, regulates RNA processing and metabolism, and is widely involved in various physiological and disease processes such as development and differentiation. Current RNA methylation sequencing technologies have promoted the exploration of its biological functions, but are bottlenecked technically by the requirement of a large amount of RNA input, and the depiction of RNA methylation characteristics only at an average level from thousands or millions of cells. We have developed several single-cell transcriptome sequencing techniques that enable depicting the heterogeneity and states of single cells. I will present our recent development of single-cell m^6A sequencing (scm^{6}A-seq), single-cell RNA m^5C sequencing (scm^{5}C-seq) and split-single-cell m^6A sequencing (sscm^{6}A-seq) approaches. These techniques show potency in decoding cellular heterogeneity at the epigenetic level, and further deciphering the fine regulatory mechanisms of epigenetics at the molecular level while achieving more precise cell grouping and subgroup identification

W10-4: Epitranscriptome Engineering Boosts Crop Productivity

Guifang Jia
Department of Chemical Biology, Synthetic and Functional Biomolecules Center, College of Chemistry and Molecular Engineering, Peking-Tsinghua Center for Life Sciences, Beijing Advanced Center of RNA Biology, Peking University
Beijing 100871, P. R. China.

RNA contains more than 150 modifications; however, the functions of most of them remain unclear. We have characterized the writer, eraser, and reader proteins of plant RNA methylation to understand their function in plant development and growth. I will present our recent discoveries on manipulating crop productivity through epitranscriptome engineering. We found that transgenic expression of the active RNA demethylase FTO increases crop yield and biomass in field trials, induced by RNA demethylation-mediated chromatin openness. I will also show how plants regulate chromatin state through RNA methylation, providing insights into our understanding of the mechanism of RNA demethylation-mediated chromatin openness in epitranscriptome-engineered crops.

W10-5: Functional Epitranscriptome Landscape in Cancer

Housheng Hansen He

Princess Margaret Cancer Center, University Health Network, Toronto, Ontario, Canada;

Comprehensive m^{6}A epitranscriptome profiling of primary tumors remains largely uncharted. Here, we profiled the m^{6}A epitranscriptome of 10 non-neoplastic lung (NL) tissues and 51 lung adenocarcinoma (LUAD) tumors, integrating the corresponding transcriptome, proteome and extensive clinical annotations. We identified distinct clusters and genes that were exclusively linked to disease progression through m^{6}A modifications. In comparison with NL tissues, we identified 430 transcripts to be hypo-methylated and 222 to be hyper-methylated in tumors. Among these genes, EML4 emerged as a novel metastatic driver, displaying significant hyper-methylation in tumors. m^{6}A modification promoted the translation of EML4, leading to its widespread overexpression in primary tumors. Functionally, EML4 modulated cytoskeleton dynamics through interacting with ARPC1A, enhancing lamellipodia formation, cellular motility, local invasion, and metastasis. Clinically, high EML4 protein abundance correlated with features of metastasis. METTL3 small molecule inhibitor markedly diminished both EML4 m^{6}A and protein abundance, and efficiently suppressed lung metastases *in vivo*.

Workshop 11: Targeting Immune Suppressive Myeloid Cells for the Cancer Treatment (Room 2B)

Chairs: Bin Zhang, Bin Zheng

W11-1: Targeting MDSC Metabolism to Enhance Cancer Immune Therapy

Bin Zheng[1]

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Myeloid-derived suppressor cells (MDSCs) are one of the major types of immune cells that contribute to tumor-induced immune suppression and escape from immune elimination. Importantly, MDSCs have been suggested to contribute to resistance to immune checkpoint blockade therapies in various cancer types. Hence, targeting MDSCs represents an attractive approach to modulating tumor immunity to improve current cancer immunotherapies.

We previously reported that phenformin, a mitochondrial respiratory chain complex I inhibitor, selectively reduced the accumulation and activities of immune suppressive neutrophils, and enhanced the effects of anti-PD-1 on inhibiting tumor growth in mouse models of melanoma. The selective sensitivity of MDSCs to phenformin has inspired us to systematically profile metabolic characteristic of MDSCs and to identify additional metabolic vulnerabilities in these cells.

Through this approach, we recently discovered itaconate as a critical immuno-metabolite selectively secreted from MDSCs to suppress CD8$^+$ T cell function and promote tumor growth. Itaconate is a metabolite produced from the Krebs cycle intermediate cis-aconitate by the activity of immune-responsive gene 1 (IRG1). We demonstrated that MDSCs secreted itaconate that can be taken up by CD8+ T cells and suppress their proliferation, cytokine production, and cytolytic activity. Metabolite profiling, stable-isotope tracing, and metabolite supplementation studies indicated that itaconate suppressed biosynthesis of aspartate and serine/glycine in CD8+ T cells to attenuate their proliferation. Moreover, host deletion of Irg1 in

mice bearing allografted tumors resulted in decreased tumor growth, inhibited the immune suppressive activities of immune suppressive neutrophils, promoted anti-tumor immunity of CD8+ T cells, and enhanced the anti-tumor activity of anti-PD-1 antibody treatment.
In summary, our findings establish IRG1 as a myeloid-selective target to promote anti-tumor immunity and enhance the efficacy of immune checkpoint protein blockade.

W11-2: The Role of Myeloid Cells in Promoting Esophagus Cancer Progression and the Identification of Therapeutic Targets

Yi Zhang[1]
[1]*Cancer Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, 450052, China.*

Myeloid cells differentiate from granulocyte-macrophage progenitor cells (GMP). In the context of malignant tumors, the bone marrow is continually exposed to various cytokines and other mediators, resulting in the generation of abnormal myeloid cells such as myeloid-derived suppressor cells (MDSCs), rather than classic monocytes or polymorphonuclear leukocytes. Using esophageal squamous cell carcinoma with distinct Chinese characteristics as a research model, MDSCs were identified as the primary immunosuppressive cell population that promotes the progression and resistance to immunotherapy in esophageal cancer. Additionally, innovative identification has revealed that immunosuppressive MDSCs highly express the specific GPCR, GPR84. The aggregation and function of MDSCs were reversed by inhibiting GPR84 activity using a selective inhibitor synthesized by our team. Targeting GPR84 was found to significantly enhance the efficacy of immunotherapy. Furthermore, experiments on the interaction between MDSCs and other stromal cells demonstrated that cancer cell genes such as MAEL, NEDD9, and DACH1 are significant mechanisms for the accumulation of MDSCs. Moreover, exosomes derived from cancer-associated fibroblasts also play an important role in generating and facilitating the immunosuppressive function of MDSCs. Additionally, a distinct subpopulation of tumor-associated macrophages (TAMs), known as $TREM2^+$ macrophages, has been identified in lung cancer models. TREM2 is responsible for regulating the metabolic reprogramming of macrophages, which contributes to treatment resistance in lung cancer patients. Targeting $TREM2^+$ macrophages or combining with immune adjuvants can reverse the aggregation of macrophages by promoting the accumulation of pro-inflammatory macrophages, thereby enhancing the efficacy of immunotherapy for non-small cell lung cancer patients. In conclusion, the distribution of myeloid cell subpopulations plays a pivotal role in determining the effectiveness of immunotherapy. And modifying the distribution characteristics of myeloid cells represents a crucial strategy for devising integrated immunotherapeutic protocols.

W11-3: Immunosuppression of Erythroid Progenitor Differentiated Myeloid Cells in Cancer

Bo Zhu[1,2], Haixia Long[1,2], Qingzhu Jia[1,2], Zheng Jin[1,2], Qi-Jing Li[3,4]
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It has long been appreciated that distant tumors, acting as an "acquired organ", interfer with the bone marrow ecosystem. However, the role of the tumor induced extramedullary hematopoiesis (EMH) in the regulation of anti-tumor immunity remains elusive. Here, we reported that tumors exploit anemia to trigger extramedullary erythropoiesis, resulting in a significant enrichment of $CD45^+$ erythroid progenitor cells ($CD71^+Ter119^+$; EPCs) within EMH organs, particularly in the spleen. Notably, these EPCs fail to mature into functional erythrocytes, but execute potent immunosuppression, impairing the systemic immune response of $CD8^+$T cells majorly through reactive oxygen species. Single-cell transcriptomic studies showed that $CD45^+$EPCs lose key transcription factors for erythroid differentiation (i.e., Gata1, Klf1), while gaining myeloid lineage-specifying transcription factors such as PU.1. Lineage tracking experiments further revealed that, while infiltrating tumors, splenic $CD45^+$EPCs fully trans-differentiates into the myeloid lineage, generating an erythroid-myeloid hybrid population termed erythroid differentiated myeloid cells (EDMCs). These EDMCs possess multifaceted machinery to curtail T cell-mediated anti-tumor responses and attenuated the efficacy of immune checkpoint therapy. In conclusion, we revealed a feed-forward mechanism by which tumors hyjack anemia-triggered extramedullary erythropoiesis, blunting RBC lineage and further deepening anemia progression. This ultimately results in the continuous production of EDMCs and systemic immunosuppression, including the dimishing of anti-tumor surveillance.

W11-4: The Mechanism of Itaconate Intercellular Transport

Xinjian Li[1]
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Itaconate is an immunoregulatory metabolite produced from *cis*-aconitate in mitochondria via a decarboxylation reaction catalyzed by the mitochondrial enzyme immune-responsive gene 1 (IRG1; also known as aconitate decarboxylase 1, ACOD1). Our previous finding demonstrated that itaconate is a lysosomal inducer that promotes antibacterial innate immunity (***Mol Cell* 2022**). Although many studies reported that itaconate production is almost exclusively confined in activated myeloid cells, including macrophages and neutrophils, however, the mechanisms underlying the itaconate generated in activated macrophages and neutrophils spreads into the non-myeloid cells remain unknown. To address this question, our group developed a fluorescent itaconate biosensor BioITA (***Nat Commun* 2022**). Utilizing BioITA as a tool for readout, we performed CRISPR screens from a custom-designed library containing thousands of gRNAs targeting genes encoding plasma membrane proteins and found that ATP-binding cassette transporter G2 (ABCG2) and SLC13A3 are the exporter and importer of itaconate, respectively. Functional studies revealed that ABCG2-mediated itaconate export is a key regulatory mechanism that limits TFEB-dependent lysosomal biogenesis and antibacterial innate immunity in inflammatory macrophages (***Cell Metab* 2024**) and SLC13A3-mediated itaconate uptake improves hepatic antibacterial innate immunity (***Dev Cell* under revision**).

Taken together, these findings decipher the mechanism of itaconate intercellular transport and characterize the role of itaconate intercellular transport in antibacterial innate immunity.

W11-5: Discovery of Deaminase Functions by Structure-Based Protein Clustering
Kevin Zhao, Qi Biodesign

Workshop 12: Molecular Mechanism of DNA Repair (Room 2C)

Chairs: Dong Wang, Shan Zha

W12-1: Molecular Basis of Transcription-Coupled Repair
Dong Wang[1,2,3]
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Transcription-coupled nucleotide excision repair (TC-NER) is a conserved repair pathway that deals with bulky lesions that block transcription. It requires coordination of core transcription-coupled repair factors to recognize and distinguish lesion-induced transcription arrest from other non-lesion transcription arrest. Here we will present our recent research that leads to new mechanistic understanding of conserved mechanism of eukaryotic transcription-coupled repair. We will present new structure and function studies that reveal important functional interplays among core TC-NER factors during repair that is likely conserved from yeast to human. This study enhances our knowledge of molecular basis of transcription-coupled repair.

W12-2: Fanconi Anemia (FA) Proteins are Potential Targets for Overcoming PARP Inhibitor Resistance
Dongyi Xu[1], Zuer Lu[1]
[1] *School of Life Sciences, Peking University, Beijing, China;*

Mutations in the BRCA1 or BRCA2 genes are linked to the development of breast, ovarian, prostate, and other cancers. Tumors harboring these mutations exhibit heightened sensitivity to PARP inhibitors (PARPi). Unfortunately, resistance to PARPi frequently develops in patients with advanced cancers. Beyond the restoration of BRCA1/2 expression or function through secondary mutations, the loss of 53BP1 or its downstream effectors represents a primary mechanism of PARPi resistance. Addressing PARPi resistance has thus become a crucial issue in clinical settings.
BRCA1 and 53BP1 antagonistically regulate the pathway selection for double-strand break repair. While BRCA1 promotes homologous recombination (HR) by recruiting CtIP, 53BP1 suppresses HR and favors non-homologous end joining (NHEJ). In cells mutated for BRCA1, HR deficiency can be compensated by disrupting 53BP1. However, the mechanisms by which CtIP is recruited in cells doubly mutated for BRCA1 and 53BP1 remain unclear. Our previous studies have shown that BRCA1 and 53BP1 also antagonistically influence the pathway choice for stalled fork restart.

Importantly, Fanconi anemia (FA) proteins contribute to BRCA1-dependent break-induced replication. Specifically, ubiquitinated FANCD2-I recruits the SLX4 complex redundantly with BRCA1. Here, we discovered that FA proteins also recruit CtIP and promote HR in a parallel pathway with BRCA1. Disruption of FA proteins significantly suppresses HR in cells with dual knockouts of BRCA1 and 53BP1, thereby restoring PARPi sensitivity. Thus, FA proteins represent promising targets for overcoming PARPi resistance.

W12-3: Structural Characterisations of SLF1 Complexes In Replication Coupled DNA Repair

Qian Wu[1]*, Emma L. Ryder[1], Nazia Nasir[1], Amy E. O. Durgan[1], Stephanie Tye2,3, Xiaodong Zhang2,3
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Unrepaired DNA damage during DNA replication threatens genome stability. SLF1 plays a crucial role in the DNA damage response by interacting with SLF2, RAD18, and nucleosomes, recruiting the SMC5/6 complex to DNA damage sites. The structural mechanisms of how SLF1 mediates these interactions remain unclear, and no protein tools are available to modulate its function. Our studies reveal the structural mechanisms of SLF1's interactions and its role in recognizing new nucleosomes during replication. Furthermore, high-affinity small protein tools called Affimer were isolated against SLF1, offering the potential for developing inhibitors to modulate DNA damage response.

W12-4: Profiling Instability at Repetitive Regions

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DNA damage and its improper repair are the major source of genomic alterations responsible for many human diseases, particularly cancer. To aid researchers in understanding the underlying mechanisms of genome instability, a number of genome-wide profiling approaches have been developed to monitor DNA damage and repair events. Highly repetitive regions, particularly ribosomal DNA (rDNA), centromere and telomeres are are inherently prone to instability but have an essential role in cellular functions and senescence. However, due to their repetitive nature, the standard alignment against the reference genome often yields limited information, making these regions to be largely overlooked or excluded in prior research. To fill in this gap, we collected all public available damage sequencing data and designed a computational workflow to explore genome instability at highly repetitive regions. Then we created DNA Damage Atlas (DDA), the first large-scale repository of DNA damage and repair information. DDA offers a user-friendly interface and encompasses analyses of highly repetitive regions. Taking rDNA as an example, we

found several strong strand-biased DNA end signals at the replication fork barrier near the 3' end of the pre-rRNA coding region and they are replication-dependent and conserved in mouse and human. Collectively, DDA will be a beneficial resource that caters to the diverse needs of researchers for elucidating the intricate mechanisms of DNA damage and repair, as well as facilitating an exploration of their correlations with somatic variations identified across a broad spectrum of diseases.

W12-5: The Role of Pre-Rrna in DSB Repair
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In response to DNA double-strand breaks (DSBs), DNA damage repair factors are recruited to DNA lesions and form nuclear foci. However, the underlying molecular mechanism remains elusive. Here, by analyzing the localization of DSB repair factors in the XY body and DSB foci, we demonstrate that pre-ribosomal RNA (pre-rRNA) mediates the recruitment of DSB repair factors around DNA lesions. Pre-rRNA exists in the XY body, a DSB repair hub, during meiotic prophase, and colocalizes with DSB repair factors. Moreover, pre-rRNA-associated proteins and RNAs, such as ribosomal protein subunits, RNase MRP and snoRNAs, also localize in the XY body. Similar to those in the XY body, pre-rRNA and ribosomal proteins also associate with DSB repair factors at DSBs in somatic cells. RNA polymerase I inhibitor treatment that transiently suppresses transcription of rDNA but does not affect global protein translation abolishes foci formation of DSB repair factors as well as DSB repair. The FHA domain and PST repeats of MDC1 recognize pre-rRNA and mediate phase separation of DSB factors, which may be the molecular basis for the foci formation of DSB repair factors during DSB response.

Workshop 13: Neuroprotection and Drug Development in Cerebral Functions and Brain Diseases (Room 2D)

Chairs: Hong-Shuo Sun, Heyu Ni

W13-1: Thrombosis and Stroke - Novel Mechanisms and Therapies
Heyu Ni, University of Toronto / Canadian Blood Services Centre for Innovation

W13-2: From Alchemy to Angong Niuhuang Wan for Stroke Treatment: A Cross-talk between Traditional Chinese Medicine and Modern Science
Jiangang Shen, University of Hong Kong

W13-3: Chloride Channels in Vascular Remodeling and Thrombosis
Guanlei Wang, Sun Yet-Sen University

W13-4: Discovery of Triptolidiol as a Direct NLRP3 Inhibitor by Decreasing K63-specific Ubiquitination
Ying Wang, University of Macau

W13-5: MAM, a Natural Programmed Cell Death (PCD) Inducer
Xiuping Chen, University of Macau

W13-6: Updates on Ion Channels in Neuroprotection and Drug Development
Hong-Shuo Sun, University of Toronto

Workshop 14: Ubiquitylation and Diseases (Room 2E)

Chairs: Yong Wan, Jianping Jin

W14-1: Direct Proteasomal Degradation of Target Proteins by Non-Ubiquitin PROTAC, Nutac
Xiao-Bo Qiu[1]
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Proteolysis targeting chimera (PROTAC) technology uses heterobifunctional protein degrader molecules consisting of a target-protein binder and a ubiquitin ligase binder. Once the target protein and ubiquitin ligase are brought into close physical proximity, the target protein is ubiquitinated by the ubiquitin ligase and then degraded by the ubiquitous 26S proteasome. But there are more than 600 different ubiquitin ligases, which are regulated extensively and sophistically, adding tremendous uncertainty to the success of PROTAC approaches. We show here that the target protein can be brought to Rpn13, a substrate receptor subunit of the 26S proteasome, by a heterobifunctional small molecule, and is then degraded by the proteasome independently of its ubiquitination. We have obtained a small molecule ligand that binds Rpn13 following a high-throughput screening. After linking this Rpn13 ligand to the molecule binding the target protein BRD4 or PD-L1 to form a tripartite non-ubiquitin proteolysis targeting chimera (NuTAC), the target degradation can be triggered by this NuTAC molecule, leading to potent tumor repression in mice. Thus, NuTAC may target any cellular proteins for degradation using only one proteasome-binding ligand without reversible ubiquitination.

W14-2: Splicing Factor SRRM2 Promotes Colorectal Cancer Growth by Activating the Mtor/S6K Pathway
Hai Rao[1]
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The mTOR pathway plays a critical role in cell growth and metabolism homeostasis, and its deregulation has been associated with many tumor growth. The ribosomal protein S6 kinase (S6K) are mTOR pathway major effectors that regulates cell growth and translation efficiency, but the regulation mechanisms of S6K are unclear. In this study, we identify a splicing factor SRRM2 that activates the mTOR/S6K pathway via regulating S6K1 and S6K2. We further show that SRRM2 regulates S6K1 and S6K2 expression through pre-mRNA alternative splicing and proteasome degradation pathway. Moreover, SRRM2 is highly expressed in colorectal cancer (CRC) tissues and associated with poor prognosis. SRRM2 promotes CRC cell and tumor growth in vitro and vivo. Collectively, these data revealed the oncogenic role of SRRM2 in CRC through activating the mTOR/S6K pathway and established SRRM2 as CRC potential therapeutic target.

W14-3: Ubiquitin Proteasome System and Host Antiviral Defense

Wenchun Fan[1]
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The ubiquitin-proteasome system (UPS) plays a pivotal role in maintaining cellular homeostasis by regulating protein turnover through targeted ubiquitination. The UPS is integral to host antiviral defense mechanisms, providing a multifaceted approach to combat viral infections. UPS contributes to antiviral defense by degrading viral proteins, modulating the expression of host restriction factors, and regulating antiviral immune signaling pathways. In turn, viruses have evolved strategies to manipulate the UPS to improve their replication and evade host antiviral immune responses. My lab focuses on dissecting the interaction mechanisms between positive-stranded RNA viruses and the host UPS. We employ loss-of-function and gain-of-function approaches to identify host E3 ubiquitin ligases that function as either host factors or restriction factors for RNA viruses. In my talk, I will share our recent findings on the role of the E3 ubiquitin ligase TRIM32 in restricting alphaviruses, which are transmitted by mosquitoes and can cause encephalitis and arthritis. Our findings reveal that TRIM32 acts as a novel intrinsic restriction factor suppressing alphavirus infection, and provides insight into the interaction between alphaviruses and the host UPS.

W14-4: Ubiquitylation-mediated Nutrient Sensing in Health and Disease

Feng Rao[1]
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The cellular ubiquitylation modification system is well known to regulate protein stability and other properties, and is altered in a variety of diseases, thus amenable to therapeutic targeting. How external stimuli such as nutrient signals utilize this modification to orchestrate physiological homeostasis remains under-explored. In this talk, I will introduce two E3 ligases systems that respond to two essentially nutrients: glucose and fatty acids. The sensing pathway and receptors will be described along with functional consequences of such nutrient perception. Furthermore, we demonstrate that disrupting signaling components in glucose and fatty acid sensing pathways leads to metabolic imbalance and disease progression in animal models.

W14-5: Proteasome Regulation in Health and Disease

Xing Guo[1]
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The ubiquitin-proteasome system is responsible for degradation of the majority of cellular proteins in eukaryotes. Sitting at the core of this pathway is the 26S proteasome, a gigantic ATP-driven protein machine that is essential for every aspect of cell biology. Often dubbed as the cellular trashcan, the proteasome has been widely misconceived as being static, constitutively active and solely afloat in the cytosol. Our work has contributed to understanding how the assembly, activity and substrate selectivity of the proteasome can be spatiotemporally regulated by proteasome-interacting proteins and chemical modifications (esp. phosphorylation) in response to various signals, reshaping the local or global proteome. We also recently characterized a special pool of proteasomes that associate with cellular membranes via N-myristoylation, which play surprisingly important roles in embryonic

development, tumorigenesis and viral replication. These findings call for rethinking current strategies for targeted protein degradation, and have fertilized an “old field” from which new research and therapeutic tools may sprout.

W14-6: Ubiquitylation and R-loop Regulation
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R-loops are three-stranded nucleic acid structures formed between a DNA-RNA hybrid and a displaced single-stranded DNA on genomes. They are usually formed during transcription and play prominent roles in various biological processes. Dysregulations of R-loop have been linked to several human diseases. Therefore, comprehending molecular mechanisms by which R-loops are formed and cleared will significantly improve our understanding of several related diseases, and potentially find new therapeutic targets. Protein ubiquitylation plays important roles in many aspects of biological activities. However, how protein ubiquitylation functions in R-loop regulation is still unclear. Protein ubiquitylation is a specific process that is mainly controlled by ubiquitin ligases. The roles of ubiquitin ligases in R-loop regulation will be discussed.

Workshop 15: DNA Metabolic Pathways: Mechanisms and Implications in Diseases (Room 2F)

Chairs: Guo-Min Li and Daochun Kong

W15-1: Repair of dsDNA Breaks in the Context of Chromatin
Daochun Kong
Peking University, China

W15-2: Novel Roles of a Mitotic Checkpoint Protein in Radiotherapy
Chun-Yuan Li
Chinese Institutes for Medical Research, Beijing

W15-3: Chromatin-based Regulation of DNA Replication Initiation
Haizhen Long, *Shenzhen Bay Laboratory, China*

W15-4: Role of DNA Mismatch Repair in Replication Stress Response
Guo-Min Li, *Chinese Institutes for Medical Research, Beijing*

W15-5: VGLL3 Promotes DNA Repair and Cellular Resistance to Genotoxic Agents
Caixia Guo, *Beijing Institute of Genomics, Chinese Academy of Sciences*

Workshop 16: The Roles of Thyroid Hormones and Agonists in Metabolism and Development (Room 2G)

Chairs: Hao Ying, Yun-Bo Shi

W16-1: Thyroid Hormone Actions on the Pathogenesis and Treatment of MASH

Paul Yen[1]
[1]*Duke-NUS Medical School, Singapore*

W16-2: Thyroid Control of Glucose Homeostasis via a Liver-Gut Axis
Ying Yan[1], **Jingjing Jiang**[2]*
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[2]*Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, 200032.*

Thyroid hormone regulates systemic glucose metabolism through incompletely understood mechanisms. Here, we show that improved glucose metabolism in hypothyroid mice after T3 treatment is accompanied with increased glucagon-like peptide-1 (GLP-1) production and insulin secretion. By using mice lacking hepatic thyroid hormone receptor β (TRβ), we demonstrate that TRβ-mediated hepatic TH signaling is required for both the regulation of GLP-1 production and the glucose lowering effects of T3. Mechanistically, T3 suppresses Cyp8b1 expression, resulting in increased the levels of Farnesoid X receptor (FXR)-antagonistic bile acids, thereby potentiating GLP-1 production and insulin secretion by repressing intestinal FXR signaling. T3 correlates with both plasma GLP-1 and fecal FXR-antagonistic bile acid levels in people with normal thyroid function. Thus, our study reveals a role for hepatic thyroid hormone signaling in glucose homeostasis through the regulation of GLP-1 production via bile acid-mediated FXR antagonism.

W16-3: Growth Hormone Can Greatly Ameliorate the Memory Impairment and Locomotor Dysfunction Caused by a Mutant Thyroid Hormone Receptor
Xiaochun Teng[1]
[1]*Department of Endocrinology and Metabolism, the first affiliated hospital, China Medical University*

Background. Patients with resistance to thyroid hormone alpha (RTHα) exhibitneurological abnormalities. Since these patients have severe thyroid hormone resistance, thyroid hormone therapy is poor. Currently, there is no effective treatment to ameliorate the neurological damage caused by this gene mutation.
Objective. Growth hormone (GH) play crucial roles in brain development, significantly affecting human growth, development, and cognitive function. The promoter region of the Gh gene contains response elements for thyroid hormone receptors (TRs). We hypothesize that Ghtreatment might ameliorate the memory impairment and locomotor dysfunction caused by a mutant thyroid hormone receptor.
Results. To generate the ThraE403X mouse model, traditional homologous gene targeting techniques was used. These mutant mice exhibited reduced body length and weight, decreased motor abilities, and lower serum levels of GH and IGF-1, as well as decreased GH and IGF-1 levels in hippocampal tissue. Transcriptomic and proteomic analyses indicated downregulation of downstream signaling pathways of the GH/IGF-1 axis in the hippocampus and cortex of mutant mice, along with developmental, differentiation, and functional impairments. Additionally, mutant mice displayed weakened long-term potentiation (LTP) and impaired hippocampus-dependent contextual fear memory. The expression of marker genes for mature neurons, oligodendrocytes, and astrocytes in the hippocampus and cortex was

reduced in mutant mice.Mutant mice were subcutaneously injected with rhGH at a dose of 8 μg/g/day from postnatal 3 days to 21 days.Following rhGH treatment, there was a significant improvement in body length and weight in mutant mice, with heterozygous mutants returning to wild-type levels, while homozygous mutants did not fully recover to wild-type levels. Serum and hippocampal GH and IGF-1 levels increased to wild-type levels post-treatment. Hippocampal LTP results suggested an enhancement in learning and memory abilities in heterozygous mutants, with homozygous mutants showing a trend towards recovery, although not statistically significant. Contextual fear conditioning experiments indicated an improvement in learning and memory abilities in heterozygous mutants, though not to wild-type levels. Nissl staining of the hippocampus revealed an increased thickness of the CA1 region and a higher number of pyramidal cells, along with increased cortical thickness post-treatment. Proteomic analysis of the cortex after treatment indicated partial recovery of GH/IGF-1 downstream signaling pathways, as well as components of the neuronal cytoskeleton, axons, and myelin. Furthermore, there was partial improvement in GH and LTP pathway proteins, and the levels of marker gene proteins for mature neurons, oligodendrocytes, and astrocytes in the hippocampus and cortex.
Conclusions. ThraE403X mutation resulted in developmental and functional impairments in the cerebral cortex and hippocampus of mice, as well as disruption of the GH-IGF1 axis. Administration of rhGHwas able to restore the GH-IGF1 axis and partially ameliorate the developmental and functional deficits in the cortex and hippocampus caused by the ThraE403X mutation. These findings suggest that rhGH treatment may have potential value in addressing RTHα-related neurological developmental abnormalities and dysfunctions.

W16-4: Thyroid Hormone in Skeletal Muscle Stem Cells and Muscle Regeneration

Zhuoyang Li[1], **Yuying Li**[1]*, Hao Ying[1]*
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The skeletal muscle (SM) is a well-known target tissue for thyroid hormone (TH), where TH plays a crucial role in regulating SM development, maintenance, and regeneration. Thyroid dysfunction patients commonly exhibit symptoms like muscle atrophy and weakness. Muscle stem cells (MuSCs) are primarily responsible for postnatal SM growth, maintenance, and injury-induced muscle regeneration. They must exit quiescence, re-enter the cell cycle, proliferate, differentiate, and self-renew for muscle repair.Complex regulatory networks of intrinsic and extrinsic factors govern the progression of the myogenic lineage. Defects in these processes can lead to muscle loss in patients with various muscular dystrophies. The intracellular TH concentration is influenced by local levels of TH transporters, deiodinase activity, and TH receptors (TR). During regeneration, the TH level in SC is precisely regulated by deiodinases and TH promotes MuSC differentiation by inducing myogenic factor expression via TR. In addition, TRamutant mice exhibit impaired muscle regeneration after injury. Interestingly, our recent findings suggest that TR has a significant role in maintaining MuSC homeostasis and the immune microenvironment. Specifically, TR regulates the dynamic changes of macrophages during skeletal muscle regeneration, which is crucial for proper muscle repair and regeneration.TR knockout mice in myeloid cells ($TR^{\Delta Mo)}$ have impaired muscle regeneration.Given the extensive

metabolic regulatory role of TH, we are investigating the mechanism of TH in remodeling macrophage metabolism, which can regulate the immune microenvironment, promote muscle regeneration, and potentially open new avenues for muscle-related disease treatment.

W16-5: Comparative Analysis of Transcriptome Profiles Reveals Stage- and TR-Dependent Gene Regulation Programs Underlying the Initiation of Tail Regeneration in Xenopus Tropicalis

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Studies in mammals have revealed that many organs lose regenerative ability soon after birth when thyroid hormone (T3) level is high. This suggests that T3 may play an important role in organ regeneration. Intriguingly, T3 peaks during amphibian metamorphosis, which is very similar to postembryonic development in human, a period of several months around birth. In addition, at least some organs, such as heart and tail, also lose regenerative ability during metamorphosis. Unfortunately, it is difficulty to study mammalian postembryonic development as the embryos and neonates depend on maternal supply for survival. Thus, the ability to easily manipulate frog development make frog a valuable model to address how organs gradually lose their regenerative ability during the period of high levels of T3. Here we have taken advantage of Xenopus tropicalistail as a model to explore regenerationmechanism asit has a remarkabledevelopmental stage-dependent regenerative ability.

Previously, we demonstrated that X. tropicalis tadpoles lose their capacity to regenerate tail at the climax of metamorphosis when tail resorption begins, suggesting that T3-induced metamorphic program inhibits tail regeneration. Interestingly, by using mutant tadpoles lacking both TRa and TRb (TRDKO), the only receptor genes in vertebrates, we found that TRDKO tadpoles retained regenerative capacity at the climax of metamorphosis (stage 60/61).Thisindicates that TR-mediated, T3-induced gene regulation program is responsible not only for tail resorption but also for the loss of tail regeneration capacity. By using stage-matched premetamorphic (stage 56) and metamorphosis climax(stage 61) wild-type (WT) and TRDKO animals for RNA-seq analyses to investigatethe gene regulation programs underlying the initiation of tail regeneration, i.e., wound healing and blastema formation, we discovered that GO (gene ontology) terms related to inflammatory response, metabolic process, cell apoptosis, and epithelial cell migrationwerehighly enriched among commonly regulated genes during wound healing (6 hours post amputation (hpa) vs 0 hpa) at either stage 56 or 61 or with either WT or TRDKO tadpoles, which was consistent with the morphological changes, i.e., both regenerative (WT 56, TRDKO 56, TRDKO 61) and nonregenerative animals (WT 61)could completethe wound healing process. Interestingly, ECM-receptor interaction and cytokine-cytokine receptor interaction are significantly enriched among regulated genes in 3 regenerative groupsat blastema period (24 hpa vs 6 hpa) but not in the non-regenerative group. In addition, the regulated genes specific in nonregenerative group were highly enriched with genes

involved in cellular senescence. Our results suggest that TR-mediated, T3-induced gene regulation changedthe permissive environment during the initial period of regeneration and affected the patterning/outgrowth period of the regeneration process. Further studies, particularly onthe regenerative microenvironment that may depend on ECM-receptor interaction and cytokine-cytokine receptor interaction, should provide novel insight onthe developmental regulation of regenerative capacity and offer potential new avenues for regenerative medicines.

W16-6: Thyroid Hormone Action in Adipose Tissues

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Thyroid hormone (TH) is essential for normal metabolic homeostasis. TH action is mediated primarily via its nuclear receptor TRα or TRβ. It has been shown that TRβ-selective agonists exhibit beneficial effects on whole-body metabolism. We show that systemic administration of T3, the active form of TH, affects both white adipose tissues (WAT) and whole-body metabolism. Our data from mice lacking adipocyte TRβ suggest that TRβ is the major isoform that mediates the T3 action on WAT and T3 regulates the expression of genes involved in multiple metabolic pathways in WAT, including glucose uptake and usage, de novo fatty acid synthesis, and both UCP1-dependent and -independent thermogenesis. Moreover, mice with adipocyte TRβ deficiency are susceptible to diet-induced obesity and metabolic dysregulation. Mechanistically, ChREBP acts to mediate the T3 effect on glucose and lipid metabolism and energy dissipation. Thus, our study suggest that TH is able to regulate systemic metabolic homeostasis through its action on the WAT and adipocyte TRβmay sever as a potential target for metabolic diseases. Lastly, our preliminary data indicate that the beneficial effect of TRβ-selective agonist can be mediated by not only the T3 action in hepatocytes and adipocytes but also hepatokines or adipokines-regulated inter-organ communication.

Workshop 17: Structural Biology in the Era of Cryo-EM II (Room 2H)

Chairs: Peijun Zhang, Yifan Cheng

W17-1: Cryo-EM Structures of Endogenous Kv1 Channels in the Mouse Brain

Shengjie Feng[1,4], Hao Wu[2], Peng Jin[1], Yuan-Hung Lin King[1], Yongqiang Wang[2], Juyeon Ko[1], Adeline Yang[3], Chao Chen[1], Yuh-Nung Jan[1,3], Lily Jan[1,3] and Yifan Cheng[2,3]
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Voltage gated potassium channels (Kvs) play a crucial role in regulating the electrical activity of neurons. To achieve precise control and diversity in neural function, Kvs form hetero-tetramers with different compositions of α subunits and auxiliary subunits in various types of neurons. However, the exact stoichiometry of endogenous Kv channels in the brain is largely unknown due to technical limitations. In this study, we

developed a novel method to determine the high-resolution structures of endogenous potassium channels in mouse brains. Our findings revealed the hetero-tetrameric structures of endogenous Kv1.6-containing complexes and showed that Kv1.6 can interact with multiple members of the Kv1 family, including Kv1.1, Kv1.2, Kv1.3, Kv1.4, and Kv1.5. This study uncovered the diverse compositions of endogenous Kv1 channels in the brain. Our research contributes to a better understanding of the role of native ion channels in neuronal activity.

W17-2: Visualizing the Translation Landscape in Human Cells at High Resolution

Wei Zheng[1], Yuekang Zhang[1], Jimin Wang[1], Shuhui Wang[1], Pengxin Chai[1], Elizabeth J Bailey[1], Wangbiao Guo[2,3], Swapnil C Devarkar[1], Shenping Wu[4], Jianfeng Lin[1], Kai Zhang[1], Jun Liu[2,3], Ivan B Lomakin[5], **Yong Xiong**[1]

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Obtaining comprehensive structural descriptions of macromolecules within their natural cellular context holds immense potential for understanding fundamental biology and improving health. Here, we present the landscape of protein synthesis inside human cells in unprecedented detail obtained using an approach which combines automated cryo-focused ion beam (FIB) milling and *in situ* single-particle cryo-electron microscopy (cryo-EM). With this *in situ* cryo-EM approach we resolved a 2.16 Å consensus structure of the human 80S ribosome and unveiled its 21 distinct functional states, nearly all higher than 3 Å resolution. In contrast to *in vitro* studies, we identified protein factors not enriched on purified ribosomes and revealed new biological functions. We also uncovered a new interface between adjacent translating ribosomes, providing structural insight into a helical polysome structure that was previously recognized but poorly understood. Finally, we resolved high-resolution structures from cells treated with homoharringtonine and cycloheximide, and identified numerous polyamines bound to the ribosome with functional consequences. Taken together, our work represents a significant advancement toward detailed structural studies inside cells, an approach with the potential to transform the fields of molecular biology, cell biology, and pharmacology.

W17-3: Structure-guided Discovery of Protein and Glycan Components in Native Mastigonemes

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Mastigonemes, the hair-like lateral appendages lining cilia or flagella, participate in mechanosensation and cellular motion, but their constituents and structure have remained unclear. Here we report the cryo-EM structure of native mastigonemes isolated from *Chlamydomonas* at 3.0 Å resolution. The long stem assembles as a

super spiral with each helical turn comprising four pairs of anti-parallel mastigoneme-like protein (Mst1). A large array of arabinoglycans, which represent a common class of glycosylation in plants and algae, are resolved surrounding the type II poly-hydroxyproline helix in Mst1. The EM map unveils a mastigoneme axial protein (Mstax) that is rich in heavily glycosylated hydroxyproline and contains a PKD2-like transmembrane domain. Mstax, with nearly 8000 residues spanning from the intracellular region to the distal end of the mastigoneme, provides the framework for Mst1 assembly. Our study provides insights to the complexity of protein and glycan interactions in native bio-architectures.

W17-4: Structural and Functional Study of Transporters in Combination of Cryo-EM and MD Simulations

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[3]*These authors contributed equally.*

Vesicular monoamine transporters (VMAT1/2) are responsible for loading and packaging monoamine neurotransmitters into synaptic vesicles, including serotonin (5-HT), dopamine (DA), norepinephrine, and histamine. Dysregulation of VMAT2 within the central nervous system can lead to schizophrenia, mood disorders, and Parkinson's disease, due to the imbalances of these monoamine neurotransmitters. Medications such as tetrabenazine (TBZ) and valbenazine (VBZ) targetting VMAT2 are approved for treating chorea associated with Huntington's disease and Tardive Dyskinesia. Our cryo-EM studies and molecular dynamics (MD) simulations on VMAT2 bound to drug inhibitors (TBZ and VBZ) and substrates (5-HT and DA), unveil the inhibition mechanism of VMAT2, alternating flipping mechanism of substrates during loading, translocation, and release, as well as the interplay between protonation of crucial acidic residues and substrate release. These findings enhance the understanding of VMAT-mediated monoamine neurotransmitter transport, fostering drug development for neurological and neuropsychiatric disorders, with a specific emphasis on VMATs.

W17-5: Chromatin Higher-Order Structure by Cryoem

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The histone variant CENP-A is the epigenetic determinant for the centromere, where it is interspersed with canonical H3 to form a specialized chromatin structure that nucleates the kinetochore. How nucleosomes at the centromere arrange into higher-order structures is unknown. By applying Single particle analysis on reconstituted centromeric chromatin, we demonstrate that the human CENP-A-interacting protein CENP-N promotes the stacking of CENP-A-containing mononucleosomes and nucleosomal arrays through a previously undefined interaction

between the α6 helix of CENP-N with the DNA of a neighboring nucleosome. We describe the cryo-EM structures and biophysical characterization of such CENP-N-mediated nucleosome stacks and nucleosomal arrays and demonstrate that this interaction is responsible for the formation of densely packed chromatin at the centromere in the cell. These results provide the first evidence that CENP-A, together with CENP-N, promotes specific chromatin higher-order structure at the centromere.

Workshop 18: Late Break-out Transcription (Room 3A)

Chairs: Bing Li, Guohong Li

W18-1: Mechanisms of Enhancer Assembly and Dynamics in Disease Progression

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Effective gene regulation is crucial for cellular function, and dynamic control of enhancer repertoires drives stage-specific transcription during tissue development and disease progression. Our research program aims to comprehensively understand the regulation of enhancer dynamics in response to signaling and its impact on gene regulation. By doing so, we seek to develop innovative approaches for preventing and treating enhancer-related diseases. Our research spans multiple levels, from enhancer chromatin organization to the coordination of enhanceosome components and the molecular interactions driving enhancer assembly.

Our previous studies have revealed that enhancer dynamics can be induced by 1) acute hormone stimulations, which can build up the active enhancer machinery in just minutes at many chromatin sites to turn on gene expression, and 2) chronic disease progression towards endocrine therapy resistance, which reprograms the ERα cistrome to evade endocrine therapies in breast cancer. These novel observations highlight the contributions of enhancer dynamics in both healthy and disease conditions. While enhancer activation relies on proper enhancer assembly, the molecular mechanisms underlying this process, including protein-DNA and protein-protein interactions, remain unclear. Our research has uncovered important principles of enhancer assembly, including combinatorial interactions of multiple transcription factors on hormone-regulated enhancers, and phase-separated condensation mediated by multivalent interactions of hormone receptors. To further investigate enhancer assembly, we employ diverse genetics, genomics, and imaging-based approaches, including the LacO arrays/LacI-fluorescence proteins system and single-molecule tracking (SMT) imaging to study condensation of enhanceosome components and their contributions to enhancer assembly.

By unraveling the intricate interplay between phase separated condensation and enhancer mechanisms, our research offers insights into gene regulation during disease progression and paves the way for the development of targeted therapeutic interventions.

W18-2: Pathogenic Germline Variants in Asian PAAD

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Putting pancreatic cancer screening into perspective for high-risk individuals could significantly reduce cancer morbidity and mortality. Previous studies have clarified the somatic mutations in PAAD. In contrast, the prevalence of mutations in PAAD predisposition genes has not been well investigated, especially in the Asian population. Here we revealed a comprehensive germline mutation landscape in 1,123 cancer patients compared to 2,944 healthy controls. CFTR (3.60%), BRCA1 (1.80%), MSH2 (1.80%) and BRCA2 (1.54%) are identified as the top germline mutated cancer genes. Integration of immunohistochemistry and gene expression analysis also supported CFTR as a powerful prognostic marker for PAAD. To investigate the molecular mechanism, reduced CFTR levels can result from LOH events or epigenetic modifications, such as DNA methylation. By comparing them to the known CF and pancreatitis-related mutations, we discovered 14 novel mutations predicted to be deleterious. Interrogation of the biological properties of down-regulated CFTR and nine CFTR mutants revealed a gain of function in pancreatic cell line proliferation assays. To characterize the drug sensitivity based on germline molecular subtyping, we performed drug screening on 23 organoids using a library of 63 chemicals. In particular, the CFTR mutant exhibited functional responses to CFTR modulator treatment.

W18-3: CTCF Mutation at R567 Causes Developmental Disorders via 3D Genome Rearrangement and Abnormal Neurodevelopment

Jie Zhang[1,3], Gongcheng Hu[2,3], Yuli Lu[1,3], Huawei Ren[2,3], Yin Huang[2], Ning Ma[2], Wei Zhang[2], Zhichao Miao[2], **Hongjie Yao**[1,2 *]

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CCCTC-binding factor (CTCF) mediates chromatin insulation and long-distance chromatin interactions in the specific context. The three-dimensional genome structure organized by CTCF is required for development. Clinically identified mutations in CTCF have been linked to adverse developmental outcomes. Nevertheless, the underlying mechanism remains elusive. In this study, we explored the regulatory roles of a clinically relevant R567W point mutation, located within the 11th zinc finger of CTCF, by introducing this mutation into both murine models and human embryonic stem cell-derived cortical organoid models. Mice with homozygous CTCF^{R567W} mutation exhibit growth impediments, resulting in postnatal mortality, and deviations in brain, heart, and lung development at the pathological and single-cell transcriptome levels. This mutation induces premature stem-like cell exhaustion, accelerates the maturation of GABAergic neurons, and disrupts neurodevelopmental and synaptic pathways. Additionally, it specifically hinders CTCF binding to peripheral motifs upstream to the core consensus site, causing alterations in local chromatin structure and gene expression, particularly at the clustered protocadherin locus. Comparative analysis using human cortical organoids mirrors the consequences

induced by this mutation. In summary, this study elucidates the influence of the $CTCF^{R567W}$ mutation on human neurodevelopmental disorders, paving the way for potential therapeutic interventions.

W18-4: Dynamic Regulation of FACT on Macroh2a-Nucleosome at Transcription

Dengyu Ji[1†], Xue Xiao[2†], Anfeng Luo[1†], Wei Li[2*], **Ping Chen**[1*]
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The FACT (Facilitates chromatin transcription) complex has been revealed to play a pivotal role in almost all chromatin-related processes, including transcription, replication and repair. Our previous study has revealed the two-face functions of FACT in breaking nucleosome and maintaining its integrity at nucleosome level, and has revealed the regulations of mono-ubiquitination of H2AK119 and H2BK120 on the function of FACT. However, how the function of FACT is regulated by the histone variant macroH2A still remains elusive. We observed that while the integration of macroH2A into nucleosome does not affect the stability or folding dynamics, it notably hinders the maintenance function of FACT. FACT effectively diminishes the stability of macroH2A-nucleosomes and expedites their depletion subsequent to the initial unfolding process. We identify the residue S139 in macroH2A as a critical switch to modulate the FACT's function in nucleosome maintenance. Further genome-wide analyses demonstrated that FACT-mediated depletion of macroH2A-nucleosome allows the correct localization of macroH2A, while the S139 mutation reshapes macroH2A distribution and influences the stimulation-induced transcription and cellular response in macrophage. Our findings provide mechanistic insights into the intricate interplay between macroH2A and FACT at the nucleosome level, and elucidate their collective role in transcription process.

W18-5: Harmony in Chaos: Embracing the Disorder in Transcription Regulation

Jingdong Xue, Yixuan Pan and **Bing Li***
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The architecture of biological molecules is fundamentally linked to the orchestration of life's processes. Within this molecular landscape, intrinsically disordered regions (IDRs) of proteins, particularly those within transcriptional and epigenetic regulatory complexes, challenge the paradigm of structure-function relationships. Despite accounting for up to half of these complexes, IDRs remain enigmatic, often referred to as "dark matter" due to our limited understanding of their diverse roles. Recent research has uncovered the propensity of IDRs to undergo phase separation, forming biomolecular condensates that have been implicated in various regulatory functions. Yet, the significance of IDRs extends well beyond their ability to drive liquid-liquid phase separation (LLPS). In fact, attributing their function exclusively to LLPS

overlooks their broader impact, especially in relation to the large assembly of RNA polymerase II (Pol II) elongation and termination complexes.

In this presentation, I will elucidate our latest findings on the versatile functions of intrinsically disordered regions (IDRs) in epigenetic regulation, both within and outside the context of phase-separated states. Our approach leverages precisely engineered reconstitution systems to unravel the IDR-driven regulation of transcription. First, I will demonstrate how the condensation of transcription factors and oncogenic fusion proteins dictates their DNA-binding specificity and how promoter condensates guide chromatin remodeling complexes and transcription machinery to specific genomic loci. Then, I will discuss the role of IDRs under non-liquid-liquid phase separation (LLPS) conditions, particularly their involvement in coordinating the dynamic 'modify-on-the-go' actions of the histone deacetylase complex Rpd3S during RNA polymerase II (Pol II) transcriptional elongation. Lastly, I will present overarching principles that govern the actions of mobile epigenetic modifying complexes as they accompany the processive transcription machinery.

Workshop 19: Chemical Biology Approaches to Cell Signaling (Room 3B)

Chairs: Zhong-Yin Zhang

W19-1: Advancing Drug Discovery by Targeting Protein Tyrosine Phosphatases
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Aberrant cellular signaling stemming from altered protein tyrosine phosphorylation is a major contributing factor to human diseases including cancer, diabetes, neurodegenerative and autoimmune disorders. Consequently, anomalous cellular events driven by defective protein tyrosine phosphorylation afford tremendous opportunities for targeted intervention. Success for such targeted approach is evident by the abundance of kinase-based therapeutics that have become important treatment modalities. Given the reversible nature of protein tyrosine phosphorylation, the ability to selectively modulate signaling pathways through inhibition of protein tyrosine phosphatases (PTPs) holds enormous therapeutic potential. However, despite increasing interest in the PTPs, they still remain largely an underexploited target class. Among major factors that contribute to the difficulty of PTP-based drug discovery are incomplete understanding of how PTP malfunction causes diseases and insufficient target validation. In addition, there is the general lack of PTP-specific small molecule probes for functional interrogation, target validation, and therapeutic development. In this presentation, I describe our recent work on oncogenic PTPs that yield new insights into their roles in tumorigenesis. Improved knowledge of the PTP-mediated disease mechanisms is essential for designing new therapeutic strategies. I also discuss several approaches for the acquisition of highly potent and selective PTP inhibitors with efficacious *in vivo* activity. Potent and specific PTP inhibitors facilitate functional analysis of the PTPs in complex signal transduction pathways and may constitute novel therapeutics for a wide range of human diseases.

W19-2: Gentle Dyes for Imaging Mitochondrial Structure and Insulin Secretion
Zhixing Chen*
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Phototoxicity has become a prevailing issue in the super-resolution era when boosted illumination is applied, compromising the physiological relevance of the recorded data. We advocate leveraging chemical approaches to tackle phototoxicity. By exploiting chemical motifs such as triplet state quenchers and biocompatible auxiliaries, we systematically upgrade the commonly used fluorescent markers toward alleviated phototoxicity. These gentle dyes can be directed to various cellular targets spanning mitochondria, DNA, cytoskeleton, insulin granule, and specific proteins, enabling time-lapse super-resolution imaging with minimal photodamage. For example, PK Mito Orange probe is a mitochondrial inner membrane stain that enables 30 frames of STED recording and multi-color imaging of mitochondrial components. PK Zinc dyes enable multiplexed imaging of insulin secretion in isolated islets. These biocompatible probes, with high specificity and gentle behavior under excitation light, promise to offer reliable spatial-temporal information in the era of 4D multiplexed nanoscopy.

W19-3: Multimodal Metabolic Nanoscopy for Studying Aging and Diseases
Lingyan Shi
Shu Chien-Gene Lay Department of Bioengineering, University of California San Diego 9500 Gilman Drive, #0412, La Jolla, CA 92093-0412, USA

Metabolism is a complex biochemical process in living organisms that involves different biomolecules and consists of various reaction steps. To understand the multi-step biochemical reactions involving various components, it is essential to elucidate in-situ dynamics and the correlations between different types of biomolecules at subcellular resolution. In this context, we have developed and integrated deuterium-probed picosecond stimulated Raman scattering (SRS), multiphoton fluorescence (MPF), and second harmonic generation (SHG) into a single nanoscopy system to study metabolic changes in aging and diseases.
By developing A-PoD and PRM algorithms, our current multimodal metabolic nanoscopy provides super-resolution with hyperspectral volumetric imaging capability. Combined with deuterated molecules (glucose, amino acids, fatty acids, water molecules, etc.) as probes, the metabolic heterogeneity of the brain, adipose tissue, liver, muscle, retina, kidney, lung, and ovaries (in Human, Mouse, and Drosophila tissues) is quantitatively imaged. One of our significant findings unveiled that lipid turnover diminishes more rapidly in aged female Drosophila compared to males. Meanwhile, dietary restriction, downregulation of the insulin/IGF-1 signaling (IIS) pathway, or activation of AMPK, notably changed lipid metabolism in aged or Alzheimer's brains. This platform equips researchers to quantitatively visualize various molecular events within the same region of interest, offering tools for early-stage disease detection, prognosis, and treatment, as well as fostering a deeper mechanistic understanding of the fundamentals of aging and diseases.

W19-4: Development and Applications of Tools for Regulation of Protein Glycosylation
Yun Ge
Institute of Chemical Biology, Shenzhen Bay Laboratory, Shenzhen, China,

Glycosylation is the most abundant and diverse post-translational modification of proteins, encompassing N-glycosylation, O-GlcNAcylation, and other types. Notably, the nutrient sensor O-GlcNAc is dynamically and reversibly regulated by OGT and OGA to fine-tune protein functions. Current approaches for modulating O-GlcNAc in live cells have limited specificity and spatiotemporal resolution. To address this, we designed a nanobody-directed O-GlcNAc eraser to dissect distinct O-GlcNAc functions on specific substrates through protein engineering. The modularity and specificity of this tool are well demonstrated at the proteome level. Additionally, we utilized a small-molecule responsive intein for controllable activation of OGA in a dose-dependent and spatiotemporally resolved manner, showcasing its potential for tracking O-GlcNAc homeostasis and combinatory cancer therapies. We envision that these tools will achieve precise regulation of O-GlcNAc in live cells, enhancing our understanding of O-GlcNAc functions. Furthermore, we have engineered N-glycosylation on proteins, particularly nanobodies, to confer additional desired functions. We equipped an anti-CD47 nanobody with high-mannose-type N-glycosylation and loaded it onto cancer cell-derived vesicles. In two mouse tumor models, this tripartite ensemble of high-mannose glycan, anti-CD47 nanobody, and cellular vesicles has been validated to significantly activate antitumor immunity and suppress tumor growth. In summary, chemical biology strategies for glycosylation regulation enable us to study and leverage glycan functions in biological processes.

W19-5: Chemical Tools for Probing the Biological Functions of Protein PTMs

Shixian Lin
Life Sciences Institute, Zhejiang University, Hangzhou 310058

Post-translational modifications (PTMs) alter the molecular structure, localization, function and stability of proteins, and play central roles in a wide range of signaling pathway and biological process. Nowadays, various PTMs, such as phosphorylation, acetylation, glycosylation, can be efficiently discovered by proteomic studies. However, it is still challenging and laborious to address the function of any particular modification by biochemical and genetic approaches. In this talk, I will present recent developments in the investigation of the biological functions of protein PTMs and the discovery of protein PTMs using genetically encoded unnatural amino acids as chemical tools.

Day 2, July 27 (Saturday)

Concurrent workshops 20-29, 1:30-3:00pm

Workshop 20: Cancer Systems Biology (Room 2A)

Chair: Jiyang Yu

W20-1: Spatially Decoding the Tumor Microenvironment to Explore Immune Escape around Cancer-Paracancer Interaction Zone

Youqiong Ye[1], Zhenzhen Xun[1], Jintong Shi[1], Xia Wei[1], and Yanhua Du[1]

[1]*Shanghai Institute of Immunology, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China.*

The tumor microenvironment (TME) represents a complex network in which tumor cells communicate not only with each other but also with stromal and immune cells. The cell type location and intercellular interactions of TME on spatial level, is critical factor influencing the outcome of immune checkpoint blockade (ICB) therapy. Recent advances in spatial transcriptomics (ST) have revolutionized the molecular understanding of the TME at the spatial level. It remains challenging to delineate pathology-relevant and cellular localizations, and interactions exclusive to a spatial niche (e.g., tumor boundary). Here, we develop Cottrazm (Construction of Tumor Transition Zone Microenvironment), integrating spatial transcriptomics (ST) with hematoxylin and eosin histological image, and single-cell transcriptomics to delineate the tumor boundary connecting malignant and non-malignant cell spots in tumor tissues, deconvolute cell-type composition at spatial location, and reconstruct cell type-specific gene expression profiles at sub-spot level (*Nat Commun* 2023a). Using Cottrazm in pan-cancer analysis revealed that the cellular composition, gene expression, and interactions of the tumor spatial microenvironment in the tumor and paracancer interaction zone are significantly different from those inside the tumor (*Cancer Res* 2024a). Revealing the interaction between components in the cancer-paracancer interaction zone to regulate immune escape from a spatial perspective. Tumor-associated fibroblasts and *SPP1*$^{+}$ macrophages are enriched in the tumor and paracancer interaction zone and interact to form a connective tissue barrier, affecting T cell infiltration and leading to tumor immune therapy tolerance (*Nat Commun* 2022, *J Hepatol* 2023). This highlights novel therapeutic strategies that could be used to disrupt cell-cell interactions around transition zone and could be combined with immune checkpoint inhibitors to improve therapeutic efficacy.

W20-2: Deciphering the Tumor Cell State Dynamics under Drug Treatment

Jin Gu[1]

[1] *MOE Key Lab of Bioinformatics, BNRIST Bioinformatics Division, Department of Automation, Tsinghua University, Beijing, China*

Understanding the heterogeneity and dynamic plasticity of tumor cells is crucial for overcoming drug resistance. Single-cell technologies enable the analysis of cell states at a given condition or time point, but it is still challenging to catenate static tumor cell snapshots to characterize their dynamic responses after drug treatment. Here, we propose scStateDynamics, an algorithm to infer tumor cell state dynamics and identify common drug effects by modeling single-cell level gene expression changes. We first

demonstrate its reliability of inferring cell state dynamics on both simulated data and the data with lineage tracing information. By applying to several real tumor drug treatment datasets, we show scStateDynamics can identify more subtle cell subclusters with different drug responses beyond static transcriptome similarity. Further, scStateDynamics can also identify the common drug effects by extracting cluster-shared components from the cell-level expression changes.

W20-3: Comprehensive Hidden Driver Analysis of Medulloblastoma

Xinran Dong[1]
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Hidden drivers may not be genetically or epigenetically altered or differentially expressed at the mRNA or protein levels, but, rather, act as multi-functional regulators via post-translational modification or other mechanisms. Conventional approaches based on genomics or differential expression are limited in exposing such hidden drivers. Here, we present a comprehensive algorithm and toolkit NetBID2 (data-driven network-based Bayesian inference of drivers, version 2), which reverse-engineers context-specific interactomes and integrates network activity inferred from large-scale multi-omics data, empowering the identification of hidden drivers that could not be detected by traditional analyses. We deploy NetBID2 Viewer, Runner, and Cloud apps with 145 context-specific gene regulatory and signaling networks across normal tissues and paediatric and adult cancers to facilitate end-to-end analysis, real-time interactive visualization and cloudbased data sharing.
Medulloblastoma (MB) is a highly malignant embryonic brain tumor with distinct cytogenetic heterogeneity in children. Despite elucidating divergent regulatory underpinnings in MB molecular subtypes, the hidden oncogenic signaling drivers with minimal differential expression remain obscure. We constructed MB specific regularoty network with 763 transcriptomic profiles and found out driver candidates using NetBID2. Specifically, we delineated unique upregulated attributes of ribosomal pathways in the *MYC*-amplification featured Group 3. *NPM1*, a ribosomal regulator, was identified as a promising targetable driver. The proposed gene activity values of *NPM1* displayed superior prognostic efficacy compared to conventional expression levels in assessing outcomes for *MYC*-amplified MB patients. Together, our findings provide new approaches for exploring the hidden regulators in MB.

W20-4: Dissection of The Translatomes and Design of New Translation Regulation Languages

Xuerui Yang[1], Fajin Li[1], Ding Wu[2], and Xu Chen[1]
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The process of RNA translation is controlled by sophisticated interplay between the RNA sequences and the translation regulatory machinery. Therefore, cell-specific RNA translatome and its regulation are deeply encoded in the RNA sequence language. The data of translatome, generated by ribosome profiling, provides profiles of translation landscapes with codon resolution at different physiological states. However, it has remained a major challenge to dissect the translatomes and interpret the intrinsic RNA language structure for translation. Rapidly developing artificial intelligence models have greatly improved their analytical power and generalization

ability. Among them, recently emerging are large fundamental language models pretrained on massive RNA sequences. We have been aiming to fully utilize the resolution and generalization ability brought by the latest large RNA language models, for interrogating the process of RNA translation and guiding the design of artificial mRNAs. Specifically, we have used large-scale translatome data, covering various cells and physiological states, for fine-tuning of the RNA language models, thereby generating the first RNA language model specifically for RNA translation. The training process also repaired and eventually reconstructed the translatome datasets. Such an insightful resource can serve as a reference for elucidating the mode of translation regulation under particular experimental or physiological conditions. For example, deep mining of this data helped us elucidate the complete and detailed machinery of RocA as a potent antitumor drug via its interference with the dynamics of translation elongation. In addition, supported by this RNA translation language model and the reconstructed translatome data, we built an AI framework to establish prediction models, from mRNA sequences to the general translation efficiency. Finally, with cell-specific or universal translation optimization as the goal, we built generative learning models for high-throughput de novo design of artificial mRNA CDS and UTR sequences. These artificial mRNA sequences have demonstrated significantly improved RNA stabilities and translation efficiencies.

W20-5: Single-Cell Systems Pharmacology Identifies Development-Driven Drug Response and Combination Therapy in B Cell Acute Lymphoblastic Leukemia

Xin Huang[1]

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Leukemia can arise at various stages of the hematopoietic differentiation hierarchy, but the impact of developmental arrest on drug sensitivity is unclear. Applying network-based analyses to single-cell tran- scriptomes of human B cells, we define genome-wide signaling circuitry for each B cell differentiation stage. Using this reference, we comprehensively map the developmental states of B cell acute lympho-blastic leukemia (B-ALL), revealing its strong correlation with sensitivity to asparaginase, a commonly used chemotherapeutic agent. Single-cell multi-omics analyses of primary B-ALL blasts reveal marked intra-leukemia heterogeneity in asparaginase response: resistance is linked to pre-pro-B-like cells, with sensitivity associated with the pro-B-like population. By targeting BCL2, a driver within the pre-pro-B- like cell signaling network, we find that venetoclax significantly potentiates asparaginase efficacy in vitro and in vivo. These findings demonstrate a single-cell systems pharmacology framework to predict effective combination therapies based on intra-leukemia heterogeneity in developmental state, with potentially broad applications beyond B-ALL.

W20-6: Systems Biology Exploration of Drug Resistance in Acute Myeloid Leukemia

Hong Wang[1], Long Shen[1], Yang Yang[1], Lele Xie[1], Dong Zhang[1] Tao Cheng[1], jianxiang Wang[1] and Chunliang Li[2]

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Background: FLT3 mutant relapsed/refractory acute myeloid leukemia (AML) patients often initially respond to FLT3 inhibitors (FLT3i) but relapse due to residual tumor cells. Understanding the mechanisms of resistance is essential to improve treatment outcomes.
Results: Using a systems biology approach, we conducted single-cell transcriptomics, immune repertoire sequencing, and deep proteomics profiling with our in-house developed ultrasensitive method on paired pre-treatment and FLT3i-relapsed bone marrow samples from patients enrolled in a FLT3i clinical trial. We also performed multi-omics analyses and CRISPR-Cas9 functional genomics screening on FLT3i-resistant cell and/or PDX models. Our analysis reveals a differentiation blockage of primitive multipotent cells in relapsed FLT3-ITD cells. Systemic multi-omics integration shows down-regulation and reduced activation of the aryl hydrocarbon receptor (AHR), a critical transcription factor promoting hematopoietic stem cell differentiation, in FLT3i-resistant cells. AHR knockout activated the STAT5 signaling pathway and promoted cell differentiation both in vitro and in FLT3-ITD patient samples. Combining AHR agonists with FLT3i enhanced therapeutic efficacy against AML in pre-clinical assays.
Conclusions: Our analyses reveal differentiation blockage of primitive multipotent cells as a therapeutic resistance mechanism upon FLT3i treatment and identify AHR as a master regulator of this process. AHR is a potent biomarker and therapeutic target for FLT3 mutant AML-relapsed patients. The combination of AHR agonists with FLT3i shows promise in improving therapeutic outcomes for AML.

Workshop 21: Giant Cells in Cancer (Room 2B)

Chairs: Jinsong Liu, Tao P. Wu

W21-1: YAP-Mediated Induction of Proliferative Polyploid Giant Cancer Cells: A Driver of Recurrence in High-Grade Serous Ovarian Cancer with Implications for Adverse Prognosis

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High-grade serous ovarian cancer (HGSOC) is the most prevalent form of ovarian malignancy. Polyploid giant cancer cells (PGCCs) are generated by diploid cancer cells in response to the stress of the tumor microenvironment and cancer treatment, resulting in an abnormal number of chromosomes exceeding two sets. Our study revealed that chemotherapy induces pgcc formation in HGSOC, with pgcc indicating a favorable prognosis. However, proliferative pgcc may contribute to ovarian cancer recurrence, suggesting a poor prognosis. We observed an increase in Yap expression during PGCCs formation, and found that Yap knockdown inhibited the development of proliferative pgcc. In HGSOC, high levels of Yap expression are strongly correlated with the presence of proliferative PGCCs and the likelihood of tumor recurrence. Therefore, it appears that Yap plays a role in the formation of proliferative

pgccPGCCs, making it a potential new prognostic indicator and therapeutic target for ovarian cancer patients.

W21-2: Retrotransposable Elements Mediate the Drug-Tolerant Persistence in Breast Cancer Chemo-Treatment

Zijian Zhang [1], Yiyang Wang [2,3,] Xinlong Luo [1], Xiaomei Zhan [1], Xiaopeng Zhu [4], Dan Qi [5,6], Erxi Wu [5,6], Jun Ding [2,3,7,8], **Tao P. Wu** [1,9,10,11,*]

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The emergence of acquired drug resistance through treatment remains a critical threat to efficient cancer therapies, which most often lead to relapse and metastasis. The development of resistance is an evolutionary adaptation process that heavily depends on tumor heterogeneity (underpinned by cellular plasticity). Therefore, a longitudinal deconvolution of treatment adaptation is critical to uncover the driving-force mechanisms underpinning resistance adaptation. In the clinic, chemotherapy remains the mainstream treatment for TNBC, and one of the primary chemo-agents is doxorubicin. Although the initial responsive rate of doxorubicin-based chemotherapies is up to 70%, it is well recognized that TNBC cells usually generate an adaptive response and result in acquired drug-resistance and multi-drug resistant phenotypes. Although numerous mechanisms of chemo-resistance evolution have been proposed, most of these studies focused on the initial and terminal states, from which we could not deconvolute the adaptation routes and might miss the transient fate-switching events. We hypothesize that the claudin-low TNBC chemo-resistant cells may derive from the early-stage reversible chemo-tolerant persistent state, and early-stage state-switching mediated by epigenetic landscape reprogramming might determine the adaptation through treatment. We developed an in vitro "pulsing-treatment" model to test the hypothesis, which could mimic the clinical treatment and provide therapeutically relevant insights into the acute drug-induced stress response and regrowth. Unexpectedly, we found that the human endogenous retrovirus-like elements (HERVs) show an aberrant bursting in the adaptation. To further interrogate the adaptation, we did the longitudinal single-cell multi-omics sequencing. With a novel bioinformatic workflow, we integrated the HERV's expression information with protein-coding genes profiling and chromatin accessibility. The preliminary result indicated that a subpopulation of HERV-high

cells might transiently mediate the persistence of cancer cells at the early stage of the treatment, which coupled with whole transcriptomic and chromatin landscape reprogramming, and molecular subtype switching. In this study, we employed a new strategy to investigate the longitudinal adaptation route through treatment and uncover a non-canonical element, which shed new light on drug resistance research and novel target screening.

W21-3: Role of the CTCF/p300 Axis in Osteoporosis/Chondrogenic Differentiation of Polypoid Giant Cancer Cells with Daughter Cells

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Polyploid giant cancer cells (PGCCs) have the properties of cancer stem cells (CSCs). PGCCs with daughter cells (PDCs) undergo epithelial–mesenchymal transition and are shown with enhanced cellular plasticity, which may be exploited therapeutically by trans-differentiation into post-mitotic and functional cells. The purpose of this study was to elucidate the mechanisms underlying osteo/chondrogenic differentiation of PDCs. PDCs recovered from cobalt chloride treatment had strong capacities of invasion and migration. When cultured in osteochondrogenic medium, the stemness of PDCs decreased and the expression of osteochondrogenic markers increased. This osteo/chondrogenic process was regulated by the transforming growth factor-β pathway in a time-dependent manner. A concurrent increase in the expression of histone acetyltransferase p300 and the transcription factor CCCTC-binding factor (CTCF) was noted. Coimmunoprecipitation assays revealed that p300 acetylated the osteo/chondrogenic marker RUNT-related transcription factor 2 (RUNX2). Analysis of the chromatin immunoprecipitation sequencing datasets revealed that the CTCF binding sites and histone H3 lysine 27 acetylation (H3K27ac) were both enriched in the promoter region of E1A-associated protein p300 (P300). Four predicted binding sites between CTCF and P300 were validated using a dual-luciferase reporter assay. We then examined the interaction between CTCF and H3K27ac and found that these two proteins had a combined effect on the transactivation of P300. In summary, CTCF, in synergy with H3K27ac, amplified the expression of P300, facilitating acetyl group transfer to RUNX2. This acetylation process stabilized RUNX2 and promoted osteo-/chondrogenic differentiation, thereby reversing PDC malignancies.

W21-4: Giant Cells: toward A Unified Understanding of Embryogenesis and Tumorigenesis on the Organismal Level

Jinsong Liu,

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Traditionally, complexity of cancer has been explained as genetic-based diseases characterized by somatic mutations. However, despite its wide acceptance, this simple concept cannot explain cancers' numerous oncogenic driver mutations, structural genomic changes in normal, benign and embryonic tissues, nuclear atypia, and presence of tissue from different germ layers in certain cancer types. Due to these pitfalls, the cancer research community has moved back to understand the complexity via the big data, omics, and AI-based technology. In searching for a new paradigm to explain the complexity of tumors and guide future research. Here I proposed a new hypothesis based on the morphologic similarities and differences to their tissue origins and their correlation with early embryogenesis and organ development to explain human tumors' complexity and origin. I consider cancer a embryogenic and developmental disease of sexual or asexual origin. In tumors of sexual origin, the super-giant egg cell undergoes cleavage after fertilization to form blastomeres that generate genomic chaos via the rapidly increasing genomic contents of the zygote via reset of whole genome to new embryonic genome. The compacted morula embryo then forms a blastocyst, resulting in embryonic transformation to form a blastocyst composed of inner cell mass and the trophectoderm. After implantation, the trophectoderm develops into a placenta, and the inner cell mass develops into an embryo or a differentiated tumor resembling embryonic or fetal organ tissue if one or more germ layers continues their growth but fail to complete differentiation at a specific stage of development. In tumors of asexual origin, the somatic cells reset their genome via whole genomic duplication, giving rise to polyploid giant cancer cells (PGCCs), yielding a somatic equivalent of blastomeres via creating an embryonic genome. This process leads the development of high-grade malignant tumor, a process analogous to blastomere-mediated pre-embryogenesis via dysregulated or without differentiation. This new hypothesis not only explains the different tumor entities and their benign and malignant natures but also provides a unified explanation of the tumorigenesis and embryogenesis. The evidence to support this hypothesis will be presented from the pathology, developmental, and genetic perspectives. The switch from conceiving cancer as a disease of cell proliferation to a super-giant cell-mediated embryogenesis provides a new paradigm to decode tumors' complexity and understand their origins on the organismal level.

Workshop 22: Autophagy in Health and Diseases (Room 2C)

Chairs: Qing Zhong, Jun-Lin Guan

W22-1: Assembly and Disassembly of SNARE Complex in Autophagosome-Lysosome Fusion

Qing Zhong[1]

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Autophagy is a highly regulated cellular degradation system that engulfs cytosol, organelles, protein aggregates and invading microorganisms into a double-membrane vesicle termed the autophagosome, then delivers cargo to endolysosomes for degradation. Autophagy dysfunction has been implicated in a broad spectrum of

human diseases, including cancers, neurodegeneration, infectious diseases, metabolic diseases and aging. My lab focuses on the biochemical mechanisms of autophagosome biogenesis, substrate recruitments and autophagosome fusion with lysosomes. I will report our recent progress in dissecting biochemical mechanisms of autophagic SNARE recruitment, tethering factors and SNARE complex assembly and disassembly, and the connection of this fusion mechanism to neurodegeneration diseases

W22-2: Genetic Analysis of Complex Roles of Autophagy in Mouse Models of Breast Cancer and Lymphangiosarcoma

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Autophagy is a highly conserved cellular process for maintenance of cellular homeostasis, and dysfunction of autophagy is implicated in a variety of diseases including cancer, autoimmune disorders and neurodegenerative diseases. In cancer, paradoxical observations of pro-tumorigenic and tumor suppressive roles of various autophagy genes present a major challenge in this field, likely due to the broad impact of autophagy on many cellular processes and the potential role of various autophagy genes in non-canonical functions. Thus, it is crucial to understand the underlying mechanisms to guide clinical applications and reap full benefit of targeting autophagy. FIP200 (FAK-family Interacting Protein of 200 kDa) was initially identified in my laboratory and subsequently shown to be a component of the ULK1/Atg13/FIP200 complex essential for the induction of autophagy. Our previous studies showed that conditional knockout (cKO) of *Fip200* decreased mammary tumor development, growth, and metastasis driven by PyMT oncoprotein, providing the first evidence for a pro-tumorigenesis role for autophagy in animals with intact immune systems. Given the increasing evidence for non-canonical autophagy functions of different autophagy genes, we also took a rigorous genetic approach to generate *Fip200-4A* mutant knock-in allele that blocks its canonical autophagy function specifically to reveal both autophagy and non-autophagy functions of FIP200 in vivo. In this presentation, I will discuss my laboratory's studies of the complex roles and mechanisms of FIP200 and other autophagy genes in breast cancer and the progression of vascular malformation to lymphangiosarcoma in mouse models of these diseases.

W22-3: Neuronal and Glial Autophagy: Selective Eater and Neuroprotection

Zhenyu Yue, PhD[1]
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Autophagy-lysosome pathway plays a critical role in maintaining cellular homeostasis by removing aggregated proteins, damaged organelles, and invading bacteria. Autophagy is a highly coordinated process of autophagosome synthesis and degradation, which can be activated in response to tissue injuries and cellular stresses. Mendelian variants of autophagy related genes (ATG) are associated predominantly with brain developmental and degenerative diseases, underscoring the significant function of autophagy in central nervous system. Despite the recent advance in cell

biology of autophagy, the detailed functions and regulation of autophagy in CNS remains largely elusive. I will speak about our recent study in systematic profiling and characterization of autophagy cargo and neuronal functions controlled by autophagy. Dysfunctional autophagy has been implicated in the pathogenesis of Alzheimer's disease (AD) and Parkinson's disease (PD). I will also present our recent study in understanding microglial autophagy in neuroprotection by using AD and PD mouse models. I will discuss the function of microglial autophagy in regulating the homeostasis of amyloid plaques and alpha-synuclein and preventing senescence; removal of senescent microglia is a promising therapeutic strategy.

W22-4: Sensing Lysosomal Stress by the Master Transcription Regulator of Autophagy, TFEB

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Autophagy, a cellular catabolic pathway mediated by lysosomal degradation, plays important roles in multiple physiological and pathological processes. Given the crucial role of the lysosome in autophagy and other cellular processes, cells have developed a plethora of strategies to cope with lysosomal malfunction or stress. However, how exactly lysosome stress (LS) is sensed by downstream molecular entities is largely unknown. Transcription Factor EB (TFEB) is a master regulator of autophagy and lysosomal biogenesis. TFEB can be dephosphorylated and activated under various stressful conditions, often via suppression of the nutrient-sensing kinase mTORC1. In this talk, I will present our work on the activation of TFEB by LS. LS triggers TFEB activation, likely as a compensatory response. Intriguingly, we found that LS does so independent of mTORC1 suppression but instead requires an enhanced protein phosphatase 2A (PP2A)-B55α activity. LS perturbs the acidic environment of the lysosome, leading to the attenuation of the enzymatic activity of lysosomal acid ceramidase (ASAH1) hence an increase of cellular ceramides, which are known PP2A stimulators. Interestingly, ASAH1 is a transcriptional target of TFEB, thus these two proteins form a feedback loop to modulate the autophagy-lysosome pathway. Therefore, this newly discovered role of ASAH1 in mediating TFEB activation represents a definitive mechanism for the sensing of lysosomal stress.

W22-5: p62/SQSTM1 Sequestration is Critically Regulated by Ubiquitylation and Phosphorylation in Response to Oxidative Stress

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The ubiquitin-binding protein, p62 (SQSTM1), among its numerous functions, critically regulates both proteostasis and redox balance, by sequestering certain proteins in aggregates for autophagic degradation. One of the client proteins sequestered by p62 is Kelch-like ECH-associated protein 1 (Keap1), a negative regulator of the antioxidant response that suppresses the antioxidant transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2). This sequestration function of p62 relies on its dimerization via the hydrogen bond between lysine (K)7 and aspartate (D)69 residues in the N-terminal Phox and Bem1p (PB1) domain, which is negatively regulated by the ubiquitin E2 ligase TRIM21 that ubiquitylates K7 and

disrupts the K7-D69 dimer formation. In addition, p62 sequestration and protein clearance activity is positively regulated via phosphorylation at multiple sites: S403, S349, and S407. We examined the molecular relationship between TRIM21-mediated p62 K7 ubiquitylation and S403 phosphorylation. In response to oxidative stress induced by free fatty acids, TRIM21 is oxidized and oligomerized via disulfide bond formation in the N-terminal cysteine cluster (C92, 111, and 114). TRIM21 oxidation and oligomerization abolish its E3 activity hence relieve its inhibition of the p62 dimerization, which facilitates p62 S403 phosphorylation and subsequent aggregation, thereby promotes cellular antioxidant capacity. Therefore, p62 antioxidant sequestration is positively regulated by S403 phosphorylation and negatively regulated by K7 ubiquitylation, which provides a delicate mechanism to critically balance the cellular antioxidant capacity.

W22-6: Truncated Oxidized Phospholipids Mediate Synchronized Ferroptosis and Contribute to Acute Kidney Injury

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Synchronized ferroptosis is suggested to contribute to nephron loss in acute kidney injury (AKI). However, the underlying mechanism of the synchronized ferroptosis for renal tubular injury remains elusive. We report that truncated oxidized phospholipids or platelet-activating factor (PAF) mediate synchronized ferroptosis and contribute to acute kidney injury (AKI). PAF caused the permeabilization of biomembranes and signalled the cell death of neighbouring cells. PAF-acetylhydrolase (II) (PAFAH2), an enzyme of the phospholipase A2 (PLA2) family that specifically removes the truncated acyl chain from phospholipids, and antibody against PAF that binds and neutralizes PAF were able to suppress synchronized ferroptosis. Genetic or pharmacological inhibition of PAFAH2 increased PAF production, augmented PAF mediated synchronized ferroptosis and exacerbated I/R-induced AKI. Our findings uncover a novel mechanism for synchronized ferroptosis and suggest a promising new strategy for therapeutic intervention of AKI.

Workshop 23: Basic and Clinical Research of Liver Diseases (Room 2D)

Chairs: Yinying Lu and Ning Zhang

W23-1: Regulatory Roles of Tissue-Specific Genes in Liver Tumor Heterogeneity

Junfang Ji, Zhejiang University

W23-2: The Role and Mechanism of FSTL1 in Liver Fibrosis

Jianhua Rao, Wenzhu Li, Yongquan Chi, Junda Li, Shanke Sun, Wei Xu, Long Zhang, Feng Cheng, Xuehao Wang, Ling Lu

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Objective: Follistatin-like protein 1 (FSTL1) is widely recognized as a secreted glycoprotein, but its roles have not been documented in liver fibrosis. Herein, we

aimed to characterize the clinical significance, roles and mechanisms of FSTL1 in the development of liver fibrosis.
Design: Expression analysis was conducted with human liver samples obtained from 33 patients with liver fibrosis and 18 individuals without fibrosis serving as controls. Myeloid-specific FSTL1-knockout ($FSTL1^{M\text{-}KO}$) mice were constructed to explore the function and mechanism of macrophage FSTL1 in 3 murine models of liver fibrosis induced by carbon tetrachloride (CCl_4) injection, bile duct ligation (BDL) or a methionine- and choline-deficient (MCD) diet. In addition, We investigated the accuracy of circulating Follistatin-like protein 1 (FSTL-1) as a non-invasive biomarker for diagnosing liver fibrosis in patients.
Results: FSTL1 expression was significantly elevated in macrophages from fibrotic livers of both humans and mice. Myeloid-specific FSTL1 deficiency effectively attenuated the progression of liver fibrosis. In $FSTL1^{M\text{-}KO}$ mice, the microenvironment that developed during liver fibrosis showed relatively less inflammation. $FSTL1^{M\text{-}KO}$ macrophages exhibited suppressed M1 polarization and NF-kB pathway activation *in vivo* and *in vitro*. Furthermore, this study showed that, through its FK domain, FSTL1 bound directly to the N- or C-terminus of PKM2. Interestingly, FSTL1 promoted PKM2 phosphorylation and nuclear translocation, reduced PKM2 ubiquitination to enhance PKM2-dependent glycolysis, and increased M1 polarization. Clinically, plasma FSTL-1 levels in liver fibrosis patients were 2.6 times higher than in non-fibrosis patients, with high diagnostic accuracy (AUROC, 0.85).
Conclusion: Macrophage FSTL1 promotes the progression of liver fibrosis by inducing M1 polarization and inflammation based on the intracellular PKM2 reprogramming function of macrophages. Circulating FSTL-1 is a high diagnostic accuracy and reliable non-invasive biomarker to identify liver fibrosis, and has potentially reducing the need for liver biopsy to identify patients.

W23-3: Co-option of Tumor Clonal Evolution and Immunosuppressive Myeloid Cells at Single Cell Resolution

Ruidong Xue, Peking University First Hospital

W23-4: Cancer B Profiling Reveals Extra-Follicular Pathway Associated with Therapeutic Opportunity

Qiang Gao, Fudan University

W23-5: Engineered CRISPR Systems for Disease Treatment and Diagnostics

Xue Gao[1, 2]
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[2]*Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA*

The recent discovery of the CRISPR genome editing systems has been revolutionizing both basic biological research and the treatment of human genetic disorders. However, there are remaining challenges in improving the precision and multiplexity of the current CRISPR systems for genome manipulation. In this seminar, I will overview our recent development of highly specific and powerful genome-editing tools for the treatment and diagnostics of diseases and the discovery of new potential small-molecule drugs. First, I will introduce the development of high-precision and

multiplex CRISPR genome-editing strategies for safe and effective molecular therapy to treat genetic disorders; Second, I will describe the application of these advanced CRISPR tools for fungi genome engineering to enable the production of novel small molecules with therapeutic potential; Finally, I will present the improved ultrasensitive viral detection by using the engineered CRISPR systems to advance timely and accurate diagnostics for the current and future pandemics and epidemics.

Workshop 24: Non-coding RNAs (Room 2E)

Chairs: Xiang-Dong Fu

W24-1: A Nuclear Function of MIWI/piRNAs for Meiotic Exit
Mofang Liu

W24-2: From DNA to Life: Decode the Noncoding Genome
Xiaohua Sheng

W24-3: Enhancer-Promoter RNA Interactions in Transcriptional Regulation
Yuanchao Xue

W24-4: Enhancer RNAs in Gene Regulation
Wenbo Li

W24-5: Genomic Landscape of Regulated Alternative Polyadenylation
Xiang-Dong Fu

Workshop 25: DNA Replication and Repair Interface and Synthetic Lethality (Room 2F)

Chairs: Xiaoqi Liu, Jianjian Li

W25-1: Dynamic Tumor Microenvironment under Radio-Immunotherapy
Jian Jian Li
Department of Radiation Oncology, University of California Davis School of Medicine

Radiotherapy (RT), currently utilized in treating over half of cancer patients worldwide, offers the advantage of localized tumor control with fewer systemic side effects compared to chemotherapy. However, there is a significant need to enhance the therapeutic efficacy of RT. Additionally, evidence suggests that only a minority of solid cancer patients derive benefits from combining RT with immune checkpoint blockade (ICB) targeting various immune checkpoint receptors. To achieve a substantial synergy between RT and ICB, it is essential to investigate the dynamics of radiation-induced biological responses within the ecology of irradiated tumor microenvironment (EITME). Through studies involving radioresistant cancer cells, recurrent tumors after RT in mice, and recurrent tumors from RT-treated patients, we have revealed mechanistic insights into the EITME. These findings indicate that the ecological evolution within the ITME can be driven by integrated multiple mechanisms which include stem cell repopulation, rewiring mitochondrial

metabolism, immune evasion with IC regulation, and communication between tumor cells and stromal cells. Specifically:

1. Mitochondrial oxidative respiration promotes cell cycle G2/M progression and radiation-induced DNA repair.
2. Radiation-repopulated cancer stem cells cause acquired tumor resistance and aggressive phenotype.
3. Radiation awakens OXPHOS to boost ATP generation for cancer cell proliferation.
4. Radiation-enhanced FAO metabolites fuel tumor growth with CD47-mediated immune evasion.

Overall, these results highlight a dynamic ecology in the tumor microenvironment treated under radiotherapy, contributing to the acquired tumor resistance and immune evasion, ultimately leading to the failure of cancer control. An integrated therapeutic approach targeting key molecules within the multiple pathways in radio-immunotherapy is suggested as a potential strategy to address these challenges.

W25-2: Enhancing the Efficacy of Radiotherapy in Castration-Resistant Prostate Cancer

Xiaoqi Liu, Jianlin Wang
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Radiotherapy is the major approach to manage prostate cancer (PCa), including castration-resistant prostate cancer (CRPC), with limited success. Therefore, it is of high clinical relevance to understand the underlying molecular mechanisms for radio-resistance and develop new approaches to enhance the efficacy of radiotherapy. The histone H3-H4 chaperone anti-silencing factor 1A (ASF1A) interacts with newly synthesized H3-H4, hands over the histones to the histone chaperone CAF-1 to facilitate nucleosome assembly after DNA replication or repair. ASF1A is also required for DNA damage checkpoint recovery after damaged DNA is repaired. Moreover, ASF1A is directly involved in non-homologous end joining (NHEJ) repair via promoting MDC1 phosphorylation by ATM. As such, depletion of ASF1A enhances the efficacy of radiotherapy. Herein, we have identified cyclin-dependent kinase 1 (CDK1) and polo-like kinase 1 (PLK1) as two kinases that phosphorylate ASF1A. Our data show that CDK1-dependent phosphorylation of ASF1A at S16 promotes subsequent phosphorylation of ASF1A at S166 by PLK1, and that CDK1/PLK1-phosphorylation of ASF1A drives its protein degradation. Thus, we aim to test if pre-administration of docetaxel will enhance the efficacy of radiotherapy in CRPC. Mechanistically, docetaxel administration-associated mitotic arrest results in activation of both CDK1 and PLK1, thus ASF1A degradation, eventually contributing to improved efficacy of radiotherapy. The study was accomplished using biochemical analyses of signaling intermediates and employing genetic strategies with culture systems and xenograft models. We dissected the importance of CDK1/PLK1-associated phosphorylation of ASF1A, thus offering an approach to enhance the efficacy of radiotherapy. This contribution is significant as it provides evidence that pre-administration of docetaxel will enhance the efficacy of subsequent radiotherapy and that the pS166-ASF1A epitope is a prognostic biomarker for radiotherapy.

W25-3: DNA-damage Tolerance as a Potential Therapeutic Target

Wei Xiao[1,2], Li Fan[1,2], and Josephine Rybchuk[1]
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The DNA replication block represents one of most dangerous class of DNA damage as it, if remained unrepaired, causes repliucation fork arrest and collapse, leading to double-strand breaks and cell death. Eukaryotic cells have evolved a comprehensive network of DNA-damage tolerance (DDT) that is regulated through sequential post-translational modifications of proliferating cell nuclear antigen (PCNA). When budding yeast cells encounter replication blocks, its PCNA-K164 residue is monoubiquitinated by a ubiquitin E2-E3 complex Rad6-Rad18, which recruits translesion DNA synthesis (TLS) polymerases to bypass the lesion often associated with increased mutations. Monoubiquitinated PCNA can also be polyubiquitinated by another E2-E3 complex Mms2/Ubc13-Rad5, which leads to error-free lesion bypass by utilizing the newly synthesized sister chromatid as a template, a process known as template switching. Meanwhile th same PCNA-K164 residue can be sumoylated by a SUMO E2-E3 complex Ubc9-Siz1 in the absence of DNA damage, which recruites a DNA helicase Srsr2 and prevents undesired homologous recombination. To further explore regulatory mechanisms of yeast DDT, we recently defined a novel PCNA-binding domain in both Rad5 and Rad18. Interestingly, Rad5 preferentially binds ubiquitinated PCNA while Rad18 does not, which unveals yet another signal transduction cascade within DDT. We also performed genoe-wide screens and identified novel mutations that can rescue the severe DNA-damage sensitivity of *rad5* and *rad18* null mutants. Genes involved in the DDT network, including sequential ubiquitinations and sumoylation of PCNA, are highly conserved in eukaryotes, from yeasts to human, and mutations in genes like *XPV/POLH* and *UBC13* have been associated with cancer. On the other hand, the synergistic interaction between TLS and erro-free lesion bypass pathway defects has been exploted for cancer chemotherapy. With investigation on how the three E2-E3 complexes coordinately regulate various DDT pathways, one can develop therapeutic strategies for cancer and other diseases based on manipulating the DDT network.

W25-4: Targeted Immunotherapy for Triple-Negative Breast Cancer Harboring 17p Loss

Xiongbin Lu, PhD
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Triple-negative breast cancer (TNBC) is a molecularly diverse and clinically heterogeneous disease. The challenges for developing novel treatment approaches for TNBC are the paucity of actionable targets and lack of targeted therapies. Breast cancer genomics revealed that heterozygous deletion of chromosome 17p is the most prevalent (53%) event in TNBC. Within the 17p deletion region is the tumor suppressor TP53 (encoding p53), whose deletion or mutation has been long known as a primary tumorigenic driver. However, it remains ambiguous whether the deletion event, which includes as many as 200 genes, impacts tumorigenesis beyond TP53 loss alone. For an accurate inference of the immune and stromal cell types and their specific activity levels from cancer tissue transcriptomics data, we developed a novel semi-supervised deconvolution method, by which we demonstrated that heterozygous

deletion of 17p is tightly correlated with poor cytotoxicity of tumor infiltrating lymphocytes (TILs) and poor clinical outcomes in patients with TNBC. This result suggests that the selective advantage due to 17p loss reflects the combined impact of TP53 loss and the reduced dosage of co-deleted genes.
In the TP53-neighboring region, we identified POLR2A as a collateral vulnerability target in the TNBC tumors with 17p loss, suggesting that inhibition of POLR2A may be a precision therapy approach for TNBC. To accelerate the translational development of our important finding, we used α-amanitin, a natural small compound isolated from Amanita phalloides, to specifically inhibit POLR2A (Kd ∼10−9 M). However, free form of α-amanitin causes liver toxicity via the interaction with the hepatocyte-specific OATP1B3 transporter, limiting its clinical applications. To overcome the toxicity of α-amanitin, we collaborated with Heidelberg Pharma to develop α-amanitin-based antibody-drug conjugates (ADC). This approach inhibits the specific uptake of α-amanitin into hepatocytes and increases tumor-specific targeting using tumor-specific monoclonal antibodies. This type of ADC showed significant efficacy in inhibiting the growth of TNBC tumors with heterozygous 17p loss and enhanced the efficacy of immune checkpoint blockade therapy. In summary, we found that heterozygous deletion of 17p not only leads to immune evasion and tumor progression in TNBC, but also confers therapeutic vulnerabilities, which can be utilized to develop novel targeted cancer therapy.

Workshop 26: Structural Biology in the Era of Cryo-EM III (Room 2G)

Chairs: Peijun Zhang, Yifan Cheng

W26-1: Conserved Function of Drg GTPases in Promoting Protein Synthesis in Translation

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Maintaining proper protein homeostasis is essential for cell physiology. The ribosome and GTPases, which are two of the most ancient and critical cellular molecules, are central players in protein synthesis and its regulation. Here we report the discovery of a new general translation factor that targets stalled ribosomes and promotes protein synthesis in an evolutionarily conserved manner. We show that the essential bacterial Obg GTPases are distant homologs of eukaryotic and archaeal Drg proteins and serve critical roles in promoting efficient protein translation in stalled ribosomes. Through *in vivo* characterization, including cross-species complementation of cells where ribosomes are induced to stall by addition of either the antibiotic anisomycin or exogenous mRNA harboring a long poly(A) sequence, we demonstrate that a conserved function of Drg proteins is to alleviate ribosomal stalling during translation. Our data show that bacterial Obg rescues stalled ribosomes in both Saccharomyces cerevisiae and human cells lacking endogenous Drgs, as does supplementation of the respective endogenous Drg proteins from yeast and human cells. Furthermore, the

presence of ObgE and GTP stimulates peptidyl transfer, the key catalytic function of the ribosome, suggesting a possible molecular mechanism of this GTPase to enhance translation in stalled ribosomes. This discovery shows that the Drg protein is a new general translation factor that directly affords cells from three domains of life a new form of translation regulation.

W26-2: **Molecular Architecture of Coronavirus Double Membrane Vesicle Pore Complex**

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Coronaviruses remodel the intracellular host membranes during replication, forming double-membrane vesicles (DMVs) to accommodate viral RNA synthesis and modifications. SARS-CoV-2 non-structural protein (nsp) 3 and 4 are the minimal viral components required to induce DMV formation and to form a double-membrane spanning pore, essential for the transport of newly synthesized viral RNAs. The mechanism of DMV pore complex formation remains unknown. Here we describe the molecular architecture of SARS-CoV-2 nsp3-4 pore complex, as resolved locally up to 3.9 Å resolution by cryo-electron tomography and subtomogram averaging within isolated DMVs. The structures uncover an unexpected stoichiometry and topology of the nsp3-4 pore complex comprising of twelve copies each of nsp3 and nsp4, organized in four concentric stacking hexamer rings, mimicking a miniature nuclear pore complex. The transmembrane domains are interdigitated to create a high local curvature at the double-membrane junction, coupling double-membrane reorganization with pore formation. The ectodomains form extensive contacts in a pseudo-12-fold symmetry, belting the pore complex from the inter-membrane space. A central positively charged ring of arginine residues coordinates the putative RNA translocation, essential for virus replication. Altogether, our work establishes a framework for understanding DMV pore formation and RNA translocation, providing a structural basis for the development of new antiviral strategies to combat coronavirus infection.

W26-3: **Structural Insight into the Invasion Machinery of Apicomplexan Human Parasiteses**

Stella Y. Sun[1,2], Li-Av Segev-Zarko[3], Muyuan Chen[5,6], Grigore Pintilie[2], Chiyong Kim[3,4], Sophia Staggers[1], Michael Schmid[5], Steven Ludtke[6], Elizabeth Egan[4], John Boothroyd[3], Wah Chiu[2,3,5]

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Host cell invasion by intracellular, eukaryotic parasites within the phylum Apicomplexa is a remarkable and active process involving the coordinated action of apical organelles and other structures. *Toxoplasma gondii* and related apicomplexan parasites possess a specialized apical complex that mediates invasion. This complex includes subpellicular microtubules (SPMTs), conoid fibrils (CFs), secretory organelles (micronemes and rhoptries), and structural features like apical rings. We used electron cryogenic tomography to determine the molecular structures of SPMTs and CFs in vitrified parasites and liberated cytoskeletons generated by gentle detergent lysis. Subvolume densities from tomograms were computationally segmented and processed to derive averaged density maps at subnanometer resolutions, relating them back to their in situ architecture. This multiscale approach can determine cellular filamentous structures. An intraluminal spiral lines the interior of the 13-protofilament SPMTs, revealing their preferred orientation relative to the parasite's long axis. Each CF comprises 9 tubulin protofilaments with a comma-shaped cross-section and associated components. The use of a tubulin protofilament building block with different accessory proteins illustrates the adaptability of the apicomplexan cytoskeleton. We also reveal a dramatic change in the shape of the anteriorly located apical vesicle upon its apparent fusion with a rhoptry in stimulated parasites. Additionally, a previously proposed rosette structure involved in rhoptry secretion is associated with multiple apical vesicles, suggesting a mechanism for enabling repeated invasion attempts. Using the same approach, we demonstrate that *Plasmodium falciparum* merozoites also possess an apical vesicle beneath a rosette, indicating evolutionary conservation of this subcellular organization.

W26-4: **Structural Basis of The Chloroplast Protein Import System**

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Chloroplast is a fundamental organelle essential for plant photosynthesis and photoautotrophic growth. It was suggested that chloroplasts have originated from a monophyletic endosymbiotic event, during which a cyanobacterium was engulfed by a eukaryotic cell more than a billion years ago. During evolution, most of the endosymbiont genes were transferred to the host nuclear genome and only about 100 genes were retained in the chloroplast. An estimated 2000-3000 proteins need to be transported into the chloroplast upon synthesis in the cytosol as precursor proteins (preproteins), which contain N-terminal transit peptides that direct them to the chloroplast. Thousands of chloroplast proteins are imported via the TOC-TIC supercomplex, a process driven by an ATPase motor. We investigate the structural basis of the chloroplast protein import system, illuminates the composition, assembly, and potential working mechanism of the chloroplast protein translocons and import motor.

W26-5: **Structures, Recruitment and Regulation of Master Kinases in DNA Damage Signalling**

Xiaodong Zhang
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DNA double-strand breaks (DSB) are one of the most severe types of DNA damage and cells have evolved several repair pathways including homologous recombination to ensure the damages are corrected promptly, especially before DNA replication and cell division. Homologous recombination utilises homologous sequences in sister chromatins and is thus error free. Homologous recombination requires coordinated actions, orchestrated by master kinases ATM (its orthologue Tel1 in yeast) and ATR (Mec1 in yeast) through phosphorylating a myriad of substrates that lead to the coordinated actions of modulating cell cycle progression with repair. Not surprisingly, these master kinases are highly regulated, both in terms of their recruitment and activation, involving a number of distinct macromolecular complexes and inter-dependent post-translational modifications. Our research focuses on elucidating the structures and mechanisms of these large signalling complexes. I will discuss our recent progress in elucidating these systems and our current mechanistic understanding of their regulation and recruitment.

Workshop 27: Neurodevelopment and Associated Disorders

(Room 2H)

Chairs: Guo-li Ming, Zhiheng Xu

W27-1: Engineering Human Brain Organoids for Modeling Brain Development and Neural Regeneration

Guo-li Ming
Departments of Neuroscience & Psychiatry; University of Pennsylvania, School of Medicine 415 Curie Blvd, CRB111, Philadelphia, PA, 19104

Human Induced pluripotent stem cells (hiPSCs) have the potential to generate all cell types of a human body under 2D culture conditions or form organ like structures-organoids, under 3D culture conditions. These hiPSC based model systems offer unique advantages in understanding molecular and cellular mechanisms governing embryonic tissue development and in modeling developmental disorders. I will discuss our recent work in developing protocols for generating brain-region specific organoids by first specifying hiPSCs into brain region specific neural stem cell. I will also discuss our work on using these organoid models to understand molecular and cellular mechanisms underlying human brain development and the regenerative capacity after injury by transplanting human forebrain organoids into the rodent brains.

W27-2: Unilateral Disturbed Function of Striatal DRD1 MSN Underlies Autism-like Behaviors in *Sh3rf2* Null Mice

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Autism spectrum disorder (ASD) occurs in more than 1% of the population, and unilateral hemisphere dysfunction has been detected in autistic children.
However, the underlying mechanism remains to be explored. *SH3RF2* mutations were found in patients with ASD and *Sh3rf2* haploinsufficiency leads to autism-like behaviors in mice. In this study, we revealed that *Sh3rf2*$^{-/-}$ (null) mice also exhibit autism-like symptoms, including social deficits and repetitive stereotyped behaviors. We disclosed that *Sh3rf2* is specially expressed in the striatal medium spiny neurons (MSNs), and dendrite complexity, spine density and synaptic transmission are altered predominantly in the DRD1 MSNs of the left striatum in *Sh3rf2*$^{-/-}$mice. Notably, autism-like behaviors could be rescued by pharmacogenetic inhibition of DRD1 MSNs in the left dorsomedial striatum (DMS). Mechanistically, we identified 222 potential SH3RF2-interacting proteins, with 15.3% of them encoded by ASD risk genes. We found that SH3RF2 is localized in the postsynaptic density (PSD) and can interact with both PP1 and CaMKII. Loss of SH3RF2 perturbs the CaMKII/PP1 switch, leading to hyperactivity of CaMKII and increased Ser831-phosphorylated and PSD-localized AMPA receptor subunit GluR1, particularly in the left striatum of *Sh3rf2* null mice. Together, our results unveil that *Sh3rf2* plays important roles in dendritic development, synaptic plasticity, and normal functional lateralization of the striatum. Moreover, AMPA receptor dysfunction of DRD1 MSNs in the left DMS is associated with autism-like behaviors. Thus, our study of unilateral hemisphere dysfunction in mouse model provides new insights into the pathogenesis of ASD.

W27-3: Human Ipsc-based 2D and 3D Disease Modeling and Therapeutic Development

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The iPSC technology has provided a great platform for disease modeling and cell therapy development. We have used the human iPSC (hiPSC) platform to model neurological disorders and develop cell therapies for these debilitating diseases. We have developed 2D and 3D models from human iPSCs to study neurological disorders including Alexander disease, Alzheimer's disease, and Canavan disease. 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.

W27-4: KCTD10 p.C124W Variant Contributes to Schizophrenia by Attenuating LLPS-mediated Synapse Formation

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KCTD10 is a member of the KCTD family associated with neuropsychiatric disorders, and its rare de novo variant p.C124W was identified in schizophrenia cases. KCTD10 functions as a substrate recognition receptor within the RING-type ubiquitin ligase complex, and it plays a crucial role in brain development, with brain-specific knockout mice exhibiting motor deficits. However, the pathogenesis of KCTD10 mutation remains unexplored. Here, we unveil that KCTD10 C124W heterozygous mice exhibit pronounced synaptic abnormalities and schizophrenia-analogous behaviors, including deviant prepulse inhibition patterns, compromised social engagements, and heightened anxiety indices, reminiscent of patients' situation. Mechanistically, KCTD10 exhibits distinct liquid-liquid phase separation (LLPS) propensity, orchestrated by the IDR sequence. p.C124W mutation impairs KCTD10's LLPS capacity, which in turn leads to reduced RHOB degradation and ultimately excessive RHOB accumulation in synaptosome. Importantly, neither KCTD10 with IDR deletion nor p.C124W mutation can ameliorate the synaptic abnormalities caused by *Kctd10* deficiency. Thus, our results indicate that LLPS is involved in the pathogenesis of KCTD10-related brain disorder, and underscore the therapeutic promise of RHOB in diseases caused by mutations of KCTD10 or RHOB.
Key words: neuropsychiatric disorder, KCTD10, liquid-liquid phase separation (LLPS), synaptic abnormalities.

W27-5: Non-epithelial Radial Glia Cells in the Subventricular Zone of Medial Ganglionic Eminence Maintains GABAergic Neuron Production during Human Brain Development

Longzhong Jia[1], Xiaohan Li[1], Yiming Yan[1], Jianbin Guo[2], Borui Shang[1], Weichao Wang[1], Zhiyan Liao[1], Yan Yu[1], Jiayi Su[1], Tao Wang[1], Ruijuan liang[1], Li Gu[1], Zhen Long[1], Yashan Dang[1], Lianyan Li[1], Lan Zhu[2], **Da Mi**[1]*

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The medial ganglionic eminence (MGE), a transitory structure of the fetal brain, serves as a pivotal source of striatal and cortical GABAergic inhibitory neurons, cholinergic neurons and glial cells in the forebrain. The subventricular zone (SVZ) of the human

MGE (hMGE) undergoes massive expansion, comprising a high density of progenitor cells hypothesized to drive the extended production of neurons. However, the progenitor cell diversity of the hMGE and its functional roles in the generation of distinct neuronal cell types remains elusive. In this study, we performed spatial and single-cell transcriptomics to delineate spatially and molecularly segregated progenitor populations that give rise to a variety of neurons and glial cells in the developing hMGE across gestational weeks (GW) 10 to 39. We showed that the hMGE SVZ populates with a large number of non-epithelial radial glia cells (SVZ RGCs) that are evolutionarily conserved across primate species but absent in rodents. The SVZ RGCs are characterized by unique molecular features, mitotic behavior and spatial distribution pattern. We discovered that distinct populations of hMGE progenitor cells exhibit temporally and spatially restricted transcriptional patterns that lead to different classes of postmitotic neurons and glial cells. Notably, we identified that SVZ RGCs are the main source of cortical GABAergic inhibitory neurons but not cholinergic neurons. Finally, we observed that SVZ RGCs, intermediate progenitor cells, newborn neurons, and blood vessels collectively assemble the specialized germinal compartments (GCs) that are involved in the generation, specification and migration of cortical GABAergic inhibitory neurons. Together, our findings reveal a novel progenitor cell type in the SVZ of the hMGE and shed light on the distinctive mechanisms underlying the generation and diversification of MGE-derived neurons during human brain development.

Workshop 28: HIV Infection, Viral Persistence and Inflammation (Room 3A)

Chairs: Liang Shan

W28-1: Immune Regulation of HIV Reservoirs
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Despite effective antiretroviral therapy (ART), HIV mainly persists in a small pool of latently infected, resting memory CD4+ T cells in people living with HIV (PLWH). HIV proviruses can be found in all subsets of memory CD4+ T cells in individuals on ART. The stability of viral reservoir is mainly due to the long half-lives of memory $CD4^+$ T cells and their capacity to clonally expand. No broadly applicable strategy can clear latent HIV reservoirs, which is the major barrier to a cure. In order to purge latent HIV reservoir, it is important to understand of the immunobiology of viral reservoirs. Our studies revealed key transcription factors including Bach2 and TCF1, which drive initial seeding and long-term maintenance of HIV reservoir in $CD4^+$ T cells, respectively. Blocking these key factors leads to significant reductions in HIV reservoir size and HIV remission in experimental animal models. In addition, we identified a novel innate immune receptor, which can be activated to specifically eliminate $CD4^+$ T cells harboring residual viruses. Taken together, these studies help establish novel immunological approaches for HIV eradication.

W28-2: HIV-1 Infection Induces T Cell Transdifferentiation
Kai Deng

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Human CD4 is the primary receptor for HIV-1 entry. However, the viral accessory proteins Nef and Vpu downregulate surface CD4 on the infected T cells. It remains unclear whether surface CD4 expression is ever fully restored after some infected T cells survive and enter a latent state. Surprisingly, by tracking the fate of HIV-1-infected $CD4^+$ T cells, we found that some of these cells transdifferentiated into $CD8^+$ T cells. Single-cell RNA sequencing and T cell receptor sequencing data suggested that these induced CD8+ T cells (iCD8) predominantly derived from regulatory $CD4^+$ T cells, retaining regulatory characteristics post-conversion. Mechanistically, HIV-1 Vpr specifically induced the generation of iCD8 cells via TGF-β1 signaling. Further ex vivo evidence showed that $CD8^+$ T cells from untreated or ART-suppressed people living with HIV-1 (PLWH) contained actively transcribed viral RNA or intact proviral DNA. More importantly, significantly higher frequencies of TCR overlap between $CD4^+$ and $CD8^+$ T cells, as well as class II-restricted antigen-specific CD8+ T cells were detected exclusively in PLWH. This strongly indicates CD4-to-CD8 conversion in vivo driven by HIV-1. Together, these findings reveal a novel mechanism by which HIV-1 causes the loss of $CD4^+$ T cells and expand the heterogeneous spectrum of the viral latent reservoir. These insights must be accounted for in future efforts to achieve a functional cure for HIV-1.

W28-3: HIV Rectal Transmission and Gut Microbiome

Qingsheng Li

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Receptive anal intercourse is a primary way through which HIV is transmitted among men who have sex with men (MSM). However, the early events during HIV rectal transmission are not well understood. To address this knowledge gap, we examined SIV viral RNA in the rectum, draining and distant lymph nodes, as well as non-lymphatic tissues like the brain, lungs, and kidneys of Indian rhesus macaques at various time points after intrarectal SIV exposure. Our findings showed that the virus rapidly disseminated to distant tissues and organs within one day post-inoculation. To investigate the impact of a high-fat diet (HFD) on the susceptibility of the host to HIV-1 rectal transmission, we used a double humanized bone marrow, liver, thymus (dHu-BLT) mouse model. We discovered that feeding an HFD led to changes in the composition of gut microbes with elevated inflammation and increased the host susceptibility to rectal HIV infection.

W28-4: Immunopathogenesis and Immunotherapy in HIV Infection

Liang Cheng

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HIV/AIDS continues to be a major global public health issue, and currently there is

no vaccine or cure. Our previous work has been focused on understanding the HIV-pDC-IFN axis in AIDS pathogenesis and developing novel stratagems for HIV cure. We revealed that aberrant activation of pDC (plasmacytoid dendritic cells) and persistent IFN-I signaling during chronic HIV infection are the leading causes of chronic inflammation, CD4+ T-cell depletion, immune exhaustion, and AIDS development. Depleting pDC or blocking IFN-I signaling during chronic HIV infection promotes CD4+ T cell recovery, enhances anti-HIV immunity and accelerates HIV clearance. Furthermore, we proved that IFN-I signaling impaired the mitochondrial activity of CD8+ T cells during chronic HIV-1 infection with effective anti-retroviral therapy. Reprogramming immunometabolism by transient inhibition of glycolysis with 2-Deoxy-D-Glucose (2-DG) rescued mitochondrial activity, reversed aberrant immune activation, and enhanced functions of CD8+ T cells from HIV-infected hosts. In combination with HIV-1 reservoir activating agent, 2-DG reduced HIV-1 reservoir size in hu-mice and inhibited HIV-1 amplification in cells from PLWH. In addition, we have developed a novel fusion protein hyperIL-15×sCD4-Fc, which was constructed by fusing hyperIL-15 and sCD4 (the extracellular domain of HIV receptor protein CD4) to human IgG1-Fc as heterodimer. We proved the multifunctionality of the novel fusion protein: the hyperIL-15 can reactivate HIV reservoir in latently infected cells, and the sCD4 can target the reactivated reservoir cells which expressing HIV membrane protein, and at the same time hyperIL-15 and Fc would boost anti-HIV immunity to eradicate reservoir cells though enhancing the function of cytotoxic lymphocytes and through antibody-dependent cellular cytotoxicity. Our studies thus advanced our understanding of HIV immunopathogenesis and provided potential targets for future treatment of AIDS.

W28-5: Characterize Immune Pathogenesis and Antibody Responses in SARS-CoV-2

Zheng Zhang

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Virus acute and chronic infection, such as SARS-CoV-2 and HBV, respectively, has seriously endangered human health, and there is still a lack of effective therapeutic strategy. Although vaccines and neutralizing antibodies have been authorized for emergency use of SARS-CoV-2, the recent increasing prevalence of mutant strains has caused serious threats and challenges. We used single-cell omics technology to deeply reveal the immune pathogenesis of SARS-CoV-2 infection, and comprehensively characterized neutralization antibody and SARS-CoV-2-specific T cell responses for the development of future vaccine against COVID-19. In addition, although effective treatments are available for chronic hepatitis B (CHB), functional cure is hardly achieved. We also dissected the unknown immune factors responsible for the functional cure in a unique cohort of pediatric CHB patients with IFNα

treatment, which had a higher rate of functional cure. The most significant alterations occurring in a specific type of immune cell called antibody-secreting cell in responders after IFN-α treatment. Particularly, the early generation of HBV-specific IgG3 are robustly associated with the functional cure in these pediatric CHB patients. These findings significantly expanded our understanding on the immunological factors regarding the B cell responses during the critical period of HBsAg seroconversion, which may greatly facilitate the development of more effective treatments.

Workshop 29: The Role of Circular RNAs in Disease Development (Room 3B)

Chairs: Burton B Yang

W29-1: Epigenetic Regulation of LncRNA and CircRNAs in Pancreatic Cancer
Yangchao Chen
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Long non-coding RNA HOX Transcript Antisense RNA (HOTAIR) is overexpressed in multiple cancers with diverse genetic profiles. Since HOTAIR heavily contributes to cancer progression by promoting tumor growth and metastasis, HOTAIR becomes a potential target for cancer therapy. However, the underlying mechanism leading to HOTAIR deregulation is largely unexplored. We demonstrated that the establishment of CDK9/RNA PolII/H3K4me3/DNA methylation feedback promoted HOTAIR expression by RNA elongation enhancement in cancer.
Circular RNA is another type of non-coding RNA implicated in multiple cancers. In the past years, we performed genome-wide identification of circRNAs with critical roles in pancreatic cancer. We demonstrated that circRTN4 and circFOXK2 played important roles in pancreatic cancer and further explored their underlying mechanisms. The upregulated circRTN4 promotes tumor growth and liver metastasis in PDAC through the novel circRTN4-miR-497-5p-HOTTIP pathway. In addition, circRTN4 stabilizes RAB11FIP1 to contribute epithelial-mesenchymal transition.

W29-2: Circular RNA SCMH1 Suppresses Kynurenine 3-Monooxygenase Expression to Inhibit Mitophagy and Functional Recovery Following Stroke
Honghong Yao[1]
[1]*Department of Pharmacology, School of Medicine, Southeast University, Nanjing, China;*

Metabolic dysfunction is considered a key event after ischemic stroke. However, the underlying mechanisms of metabolic disorders in cerebral ischemia are unknown. In this study, the kynurenine pathway, which is the major pathway of tryptophan metabolism, was identified after ischemic stroke through coordinated metabolomic

and RNA-seq analysis. Circular RNA SCMH1 (circSCMH1) is a circular RNA that has been reported to play a role in brain repair after stroke, and was herein confirmed to be involved in kynurenine pathway regulation. Specifically, we found that circSCMH1 inhibited the kynurenine pathway and promoted mitochondrial fusion after cerebral ischemia. Mechanistically, circSCMH1 bound to STAT5B and inhibited its translocation to the nucleus, resulting in decreased expression of kynurenine 3-monooxygenase (KMO), a key enzyme in the kynurenine pathway. Our findings suggest that circSCMH1 inhibits post-stroke mitophagy via STAT5B mediated regulation of KMO, providing insight into the mechanism by which circSCMH1 promotes stroke recovery.

W29-3: Exercise-Induced Circular RNA circUtrn is Required for Cardiac Physiological Hypertrophy and Prevents Myocardial Ischemia-Reperfusion Injury

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Regular exercise training can bring cardiovascular health benefits and effectively reduce the risk of cardiovascular disease. Circular RNAs (circRNAs) play an important role in heart pathophysiological processes. However, the role of circRNAs in response to exercise training and the biological mechanisms which are responsible for exercise-induced cardiac protection in the heart remain largely unknown. RNA sequencing was used to profile the circRNA expression in adult mouse cardiomyocytes in response to exercise training. CircRNA circUtrn was found to be significantly increased in swimming-trained adult mouse cardiomyocytes. To study the role of circUtrn in cardiomyocytes, H9 human embryonic stem cell–induced cardiomyocytes (hESC-CMs) were used to examine the role of circUtrn in cardiomyocytes growth and survival. *In vivo*, adeno-associated virus-9 (AAV9) to overexpression circUtrn in mice hearts undergoing acute myocardial ischemia/reperfusion (I/R) (30min/24hours) or with pathological cardiac remodeling for 3 weeks after I/R. AAV9 to knockdown circUtrn in mice hearts undergoing swimming training-induced physiological cardiac hypertrophy. *In vitro*, overexpression of circUtrn promoted hESC-CMs growth and survival via protein phosphatase PP5. *In vivo*, circUtrn is required for exercise-induced physiological cardiac hypertrophy. CircUtrn inhibition abolished the protective effects of exercise on I/R remodeling. CircUtrn overexpression prevented myocardial I/R-induced acute injury and pathological cardiac remodeling. To investigate the regulation mechanism of circUtrn in cardiomyocytes, functional rescue experiments, RNA pull down, mass spectrometry, *in vitro* RNA transcription, RNA immunoprecipitation assay, RNA stability assay, protein degradation assay, and coimmunoprecipitation assays were conducted. Mechanistically, circUtrn directly bound to PP5 and regulated PP5 expression in a ubiquitin-proteasome-dependent manner to activate MAPK/ERK signaling. In conclusions, CircUtrn overexpression prevented myocardial I/R-induced

acute injury and pathological cardiac remodeling. Exercise-induced cardiac circUtrn elevation is a potential therapeutic strategy for cardiac therapy.

W29-4: The Roles Of Long Non-coding RNA in Brain Cancer Progression

Nu Zhang
Department of Neurosurgery, the 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

The immunosuppressive tumour microenvironment (TME) is a prominent feature of glioblastoma (GBM), the most lethal primary brain cancer resistant to current immunotherapies. The mechanisms underlying the highly immunosuppressive GBM-TME remain to be explored. We reported that the long non-coding RNA (LncRNA) H19 encodes an immune-related protein called H19-IRP. Functionally separated from H19 RNA, H19-IRP promotes GBM immunosuppression by binding to the CCL2 and Galectin-9 promoters and activating their transcription, thereby recruiting myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs), leading to T cell exhaustion and an immunosuppressive GBM-TME. H19-IRP is overexpressed in clinical GBM samples and acts as a tumour-associated antigen (TAA) presented by MHC-I molecules. A circular RNA vaccine targeting H19-IRP (circH19-vac) triggered a potent cytotoxic T cell response against GBM and inhibits GBM growth in vivo. Our results highlight the unrevealed function of H19-IRP in creating an immunosuppressive GBM-TME by recruiting MDSCs and TAMs, supporting the idea of targeting H19-IRP with a cancer vaccine for GBM treatment.

W29-5: Improving Cardiac Functions by Targeting Circnlgn and its Protein Coding Activity

Burton B Yang, William W Du, Hui Yuan, Kevin Y Du, Javeria Qadir,
Sunnybrook Research Institute, and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

We have previously reported that transgenic expression of the cardiac specific circular RNA (circRNA) circNlgn promoted cardiac remodeling and fibrosis by upregulation of SGK3 and ING4 expression through a Nlgn isoform Nlgn173 that was translated by circNlgn (Du et al, Circ Res, 2021). In this study, we developed an approach to target specifically the junction sequence of circNlgn by silencing circNlgn, resulting in enhanced cardiac function, decreased expression of SGK3 and ING4, and reduced cardiac remodeling and fibrosis. These results are similar to the siRNAs targeting SGK3 and ING4, which also resulted in enhanced cardiac function and decreased cardiac remodeling and fibrosis. We tested how these processes might be affected by cardiac drugs and found that norepinephrine increased circNlgn expression, while beta-blockers inhibited this process. Norepinephrine has additive effects with pressure overload and other stress treatments, whereas beta-blockers can prevent their effects leading to improved cardiac functions and decreased cardiac remodeling and fibrosis

by targeting Nlgn transcription and splicing. Different beta-blockers appear to function differently. Future studies may investigate how beta-blockers modulate circNlgn biogenesis and function.

Day 2, July 27 (Saturday)

Concurrent Workshops 30-39, 3:15 – 4:45 pm

Workshop 30: Developmental Biology and Stem Cell (Room 2A)

Chairs: Mai Har Sham, Ting Xie

W30-1: The Function of an Enhancer for the Posterior Development of Mouse Early Embryos

Naihe Jin
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The regulatory mechanisms governing cell fate determination, particularly lineage diversification during mammalian embryonic development, remain poorly understood with in-depth regulatory paradigms yet to be fully elucidated. Here, leveraging the epigenetic landscape of mouse gastrula, we identified p-Enh, a pre-marked enhancer in primitive streak region, as pivotal regulator for posterior tissue development in mouse embryos. Morphological and single-cell transcriptomic analyses confirmed embryonic lethality phenotype with disrupted posterior tissue development trajectories in p-Enh-KO embryos. Molecularly, apart from regulating the neighboring coding-gene *Cdx2 in cis*, we found that p-Enh can also modulate the global transcriptome and epigenomic landscape through the transient production of chromatin-binding eRNA *in trans*. Further investigation revealed p-Enh-eRNA participate in the regulatory cascades of TGF-β signaling by colocalizing with TFs such as SMAD4. Chemical modulation of TGF-β signaling or over-expression of nuclei-resident eRNAs can morphologically rescue the posterior development in *in vitro* gastruloids. Thus, we propose that the broadly distributed p-Enh transcripts within the nucleus serve as essential coordinators to prime the posterior development of mouse embryo.

W30-2: Development of Sensory and Non-Sensory Structures in Mammalian inner Ear

Mai Har Sham
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Mammalian cochlear epithelium differentiates into distinct sensory and non-sensory domains with characteristic cell types during development. While the sensory hair cells have been extensively studied, there is limited understanding of the development of non-sensory cell types which are also essential for hearing functions. By mouse mutants and single cell transcriptomic studies, we have demonstrated that Sonic hedgehog (Shh) signaling is responsible for controlling the spatiotemporal differentiation of both sensory hair cells and non-sensory marginal cells of the cochlear epithelium.
We show that Sufu, a negative regulator of Shh signaling, is essential for controlling the timing and progression of sensory hair cell (HC) differentiation. Removal of *Sufu*

leads to abnormal Atoh1 expression and a severe delay of HC differentiation due to elevated *Gli2* mRNA expression. Later in development, HC differentiation defects are restored in the *Sufu* mutant by the action of Spop which promotes Gli2 protein degradation. Deletion of both *Sufu* and *Spop* results in robust Gli2 activation, exacerbating HC differentiation defects. We further demonstrate that Gli2 inhibits HC differentiation through maintaining the progenitor state of $Sox2^{+}$ prosensory cells. The spatiotemporal dynamic change of Sox2 expression level is controlled by Shh signaling through Gli2.
The marginal cells (MCs) are derived from the cochlear epithelium and constitute the epithelial layer of the stria vascularis (SV), an intricate non-sensory structure responsible for generating endolymph in the mammalian cochlea. We show that Ptch1/Shh signaling is essential in regulating marginal cell differentiation and SV formation. High levels of Gli2 lead to progenitor maintenance and inhibition of Gli2 is a prerequisite for marginal cell differentiation and SV assembling. We further reveal an early specification event in the cochlear non-sensory epithelium before evident differentiation process. Our results provide mechanistic insight for the function of Shh signaling in regulating the development of mammalian cochlear sensory and non-sensory structures.

W30-3: Niche Control of Stem Cell Self-Renewal and Differentiation
Ting Xie
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Stem cells in adult tissues undergo self-renewal and generate differentiated cells that replenish the lost cells caused by natural turnover, injury or disease. The molecular mechanisms regulating stem cells are also critical for regenerative medicine and fighting against cancer and aging. The Drosophila ovary is an excellent model system to elucidate niche structures and functions as well as the molecular mechanisms underlying self-renewal, differentiation, and aging. We are among the first to demonstrate that the Drosophila ovary harbors two separate niche compartments for controlling self-renewal and stepwise differentiation, respectively, which are known as the stem cell niche and the differentiation niche. In addition, we have also elucidated how the two niches work together to control stem cell development and thus tissue regeneration by identifying various pathways and factors involved, including BMP, Hh, Wnt, Netrin, Notch, cadherin-mediated cell adhesion, epigenetic factors, non-coding RNAs, RNA modifications and various protein complexes. In this presentation, I will report our recent progress in understanding stem cell-niche communications and niche regulation.

W30-4: Decoding the Hedgehog Signal in Development
Jin Jiang
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Hedgehog (Hh) morphogen controls organ development and adult tissue homeostasis in species ranging from *Drosophila* to human. Deregulation of Hh signaling contributes to numerous human diseases including birth defect and cancer. Hh exerts its biological influence through a signaling cascade culminating in the conversion of the latent transcription factors Cubitus interruptus (Ci)/Gli from their repressor forms

(Ci^R/Gli^R) into activator forms (Ci^A/Gli^A). Whereas the mechanisms underlying the regulation of Ci^R/Gli^R formation have been well studied, the mechanisms underlying Ci/Gli activation remain poorly understood, especially for Gli proteins whose activation is thought to occur at the tip of primary cilium. In *Drosophila*, the Ser/Thr kinase Fused (Fu) acts downstream of Smoothened (Smo) to convert full-length Ci (Ci^F) into Ci^A by antagonizing the inhibitory function of Sufu. In response to Hh, Smo induces Fu dimerization and trans-autophosphorylation, leading to Fu kinase activation. Activated Fu kinase directly phosphorylates Ci at multiple sites, priming CK1-mediated phosphorylation on adjacent sites. These phosphorylation events promote Ci activation by alleviating Sufu inhibition in a dose-dependent manner. We further show that the Fu/ULK family kinases ULK3 and STK36 act in parallel to phosphorylate Gli and promote Gli activation at ciliary tip. On-going work that provides further insight into how Fu/ULK family kinases regulate Ci/Gli ciliary localization and activation will also be presented.

W30-5: Investigation of Unpaired 2 as a Novel Cell Competition Factor

Yan Yan
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Cell competition is a process that viable cells are eliminated through interactions with their neighbors in a tissue composed of a seemingly homogenous cell population. It was originally discovered through studies of ribosome-deficient clones in imaginal discs of *Drosophila Melanogaster*. Cell competition has now been found in different types of tissues and organs across animal species as a general mechanism to maintain tissue fitness by removing unfit or precancerous cells. Using *Drosophila* wing imaginal discs as a model, we found that Unpaired 2 (Upd2), an Interleukin-6 (IL-6) family cytokine, is a previously unrecognized cell competition factor. While *upd2* loss-of-function mutants have a normal wing disc size, clones deficient in Upd2 production fail to survive in wing discs. Our studies demonstrate that a cytokine can function as an intercellular messenger to communicate the fitness level among cells.

W30-6: Mechanosensitive Osteolectin+ Cells in Skeletal Development and Homeostasis

Bo Shen
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Osteolectin, an osteogenic growth factor recently identified, exhibits expression in chondrocytes, osteoblasts, and bone marrow stromal cells (eLife 5:e18782). To discern the cellular origin of Osteolectin and trace changes in the distribution pattern of *Osteolectin*-expressing cells during development, we engineered a membrane-bound tdTomato reporter into the endogenous *Osteolectin* locus. Our findings reveal that *Osteolectin* is exclusively expressed by peri-arteriolar $LepR^+$ stromal cells in adult bone marrow. Employing fate-mapping mouse genetics, we previously identified *Osteolectin*-expressing stromal cells (Osteolectin$^+$ cells) as short-lived osteogenic progenitors responsible for adult bone formation (Nature 591:438). However, during neonatal development, *Osteolectin*-expressing cells display a broader distribution pattern. Through the use of *Osteolectin*-mTomato and *Gli1*-TriGFP reporter alleles, we have observed significant overlap between *Osteolectin*-expressing cells and *Gli1*-expressing cells in the neonatal growth plate.

Utilizing a dual-recombinase lineage tracing system for fate mapping Osteolectin^{+}Gli1^{+} cells *in vivo*, we have established that growth plate Osteolectin^{+}Gli1^{+} cells play a pivotal role in postnatal bone elongation. Importantly, these Osteolectin^{+} cells are also mechanosensitive: deletion of *Piezo1* from Osteolectin^{+} cells in neonatal bone marrow results in a significant reduction in bone length, underscoring the critical role of mechanical loading in postnatal bone elongation. Furthermore, the deletion of *Piezo1* from Osteolectin^{+} cells in adult bone marrow leads to a depletion of Osteolectin^{+} cells and a reduction in osteogenesis. In summary, our findings demonstrate that mechanical loading is essential for Osteolectin^{+} cells, not only in the maintenance of bone during adulthood, but also postnatal bone elongation during development.

Workshop 31: Mechanisms in Cancer Development and Therapy

(Room 2B)

Chairs: Shi-Yuan Cheng

W31-1: Targeting the Fluidic Force-Sensing Mechanism to Treat Brain Tumor Metastasis

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Biofluid flow generates fluid shear stress (FSS), a mechanical force widely present in tissue microenvironment. How brain tumor growth alters the conduit of biofluid, thereby impacting FSS-regulated cancer progression is unknown. Medulloblastoma (MB) is the most common malignant brain tumor in children. Dissemination of MB cells into the cerebrospinal fluid (CSF) initiates metastasis within the central nervous system. By simulating CSF dynamics based on magnetic resonance imaging of MB patients, we discovered that FSS is elevated at the cervicomedullary junction. MB-relevant FSS promotes metastasis along mouse spinal cords. Mechanistically, FSS induces metastatic cell behaviours, including weakened cell-substrate adhesion, increased motility, cell clustering, and plasma membrane localization of glucose transporter 1 (GLUT1) to enhance glucose uptake. FSS is perceived by mechanosensitive ion channel PIEZO2, which drives actomyosin contractility-dependent GLUT1 recruitment at the plasma membrane. Genetic targeting of PIEZO2 or pharmacologic inhibition of GLUT1 mitigates metastasis. Collectively, these findings define a targetable FSS-activated mechano-metastatic cascade for the treatment of MB metastasis.

W31-2: Epigenetic Regulation and Glioma Heterogeneity

Haizhong Feng

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Glioma heterogeneity remains poorly understood. Here, we identify the epigenetic regulator TRIM24 as a driver of glioma heterogeneity. Here, we identify epigenetic regulator TRIM24 as a driver of glioma progression. TRIM24 overexpression promotes $HRas^{V12}$ anaplastic astrocytoma progression into epithelioid GBM (Ep-GBM)-like tumors. Co-transfection of TRIM24 with $HRas^{V12}$ also induces Ep-GBM-like transformation of human neural stem cells with *TP53* knockdown. TRIM24 is highly expressed in clinical Ep-GBM specimens. Single-cell RNA-sequencing further indicates that TRIM24 overexpression impacts both intratumoral heterogeneity and tumor microenvironment. Mechanically, $HRas^{V12}$ activates PHAX and upregulates U3 snoRNAs to recruit Ku-dependent DNA-PKcs. Overexpressed TRIM24 is also recruited by PHAX to U3 snoRNAs, thereby facilitating DNA-PKcs phosphorylation of TRIM24 at S767/768 residues. Phosphorylated TRIM24 induces epigenome and transcription factor network reprogramming and promotes Ep-GBM-like transformation. Targeting DNA-PKcs with small molecule inhibitor NU7441 synergizes with temozolomide to reduce Ep-GBM tumorigenicity and prolong the survival of animals. Our findings provide new insights into epigenetic regulation of Ep-GBM-like transformation and glioma heterogeneity and suggest a potential therapeutic strategy for patients with Ep-GBM.

W31-3: Distinct Roles of TREM2 in Central Nervous System Cancer and Peripheral Cancers

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Glioblastomas (GBM) are incurable central nervous system (CNS) cancers characterized by substantial myeloid cell infiltration. Whether myeloid cell-directed therapeutic targets identified in peripheral non-CNS cancers are applicable to GBM requires further study. Here, we identify that the critical immunosuppressive target in peripheral cancers, triggering receptor expressed on myeloid cells-2 (TREM2), is immunoprotective in GBM. Genetic or pharmacological TREM2 deficiency promotes GBM progression in vivo. Single-cell and spatial sequencing reveals downregulated TREM2 in GBM-infiltrated myeloid cells. TREM2 negatively correlates with immunosuppressive myeloid and T cell exhaustion signatures in GBM. We further demonstrate that during GBM progression, CNS-enriched sphingolipids bind TREM2 on myeloid cells and elicit antitumor responses. Clinically, high TREM2 expression in myeloid cells correlates with better survival in GBM. Adeno-associated virus-mediated TREM2 overexpression impedes GBM progression and synergizes with anti-PD-1 therapy. Our results reveal distinct functions of TREM2 in CNS cancers and support organ-specific myeloid cell remodeling in cancer immunotherapy.

W31-4: RNA Splicing Decipher Developmental Hierarchies and Contribute to Glioma Heterogeneity

Shi-Yuan Cheng

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RNA alternative splicing (AS) is a critical mechanism that generates multiple transcripts from a single gene, thereby expanding the transcriptome and dictating cellular functions and signaling. Widespread alterations in RNA alternative splicing (AS) have been identified in adult gliomas. However, their regulatory mechanism, biological significance, and therapeutic potential remain largely elusive. Here, I will report our recent studies of dysregulated RNA AS that contributes glioma malignancy. We first showed that serine and arginine rich splicing factor (SRSF) 3 promotes cell proliferation, self-renewal of glioma stem cells (GSCs), and their in vivo tumorigenicity through regulates thousands of SRSF3-regulated AS events. We further investigated the functional impact of two SRSF3-regulated events in the transcription factor ETS variant 1 (*ETV1*) exon 7 and nudE neurodevelopment protein 1 (*NDE1*) exon 9, which affect oncogenic transcription and maintain efficient mitosis of GSCs, repectively. Next by using a computational approach with both bulk and single cell RNA-sequencing, we uncover a prognostic AS signature linked with neural developmental hierarchies. Using novel iPSC glioma models driven by glioma driver mutations, we show that this AS signature could be enhanced by EGFRvIII and inhibited by *in situ IDH1* mutation. Functional validation of two isoform switching events in *CERS5* and *MPZL1* shows regulations of sphingolipid metabolism and SHP2 signaling, respectively. Analysis of upstream RNA binding proteins reveals PTBP1 as a key regulator of the AS signature where targeting of PTBP1 suppresses tumor growth and promotes the expression of a neuron marker TUJ1 in glioma stem-like cells. Overall, our data highlights the role of AS in impacting glioma malignance and heterogeneity and its potential as a therapeutic vulnerability for treating adult gliomas.

W31-5: The Moonlighting Functions of Metabolic Enzymes

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We elucidated instrumental mechanisms of the Warburg effect, discovered the protein kinase and phosphatase activity of metabolic enzymes, and revealed the non-metabolic functions of metabolic enzymes in tumorigenesis. (1) Our work elucidated important mechanisms underlying the RTK-promoted Warburg effect, which are regulated by nuclear function of pyruvate kinase M2 (PKM2) and

mitochondrial function of phosphoglycerate kinase 1 (PGK1). RTK activation induces translocation of the glycolytic enzyme PKM2 into the nucleus, where it binds to and activates tyrosine-phosphorylated β-catenin, thereby upregulating expression of glycolytic genes and enhancing the glucose uptake and lactate production. In addition, we revealed that activation of RTKs, expression of K-Ras G12V and B-Raf V600E, and hypoxia induce the mitochondrial translocation of the glycolytic enzyme PGK1, which phosphorylates and activates pyruvate dehydrogenase kinase 1 (PDHK1) to inhibit mitochondrial pyruvate metabolism, thereby promoting the Warburg effect. (2) We discovered that metabolic enzymes (PKM2, PGK1, PCK1, HK2, and fructokinase KHK-A) can function as protein kinases to regulates cell cycle progression, mitochondrial function, autophagy, and de novo nucleic acid synthesis. (3) We discovered for the first time that metabolic enzymes can function as protein phosphatase. Fructose-1,6-bisphosphatase 1 (FBP1) dephosphorylates histone H3 at T11 and suppresses gene transcription. (4) Our work revealed that metabolic enzymes, including fumarase, acetyl-CoA synthetase 2 (ACSS2), and α-ketoglutarate dehydrogenase (KGDH) can possess nonmetabolic functions in regulation of instrumental cellular activities including DNA repair and gene expression. The discoverers of tumor-promoting functions of metabolic enzymes provide novel approaches for diagnosis and treatment of human cancer.

Workshop 32: Cellular Metabolism: Exploring Impact beyond the Cell (Room 2C)

Chairs: Qun-Ying Lei, Xiao-Wei Chen

W32-1: The Metabolic Origin of Aging
Li Qiang
Peking University

Aging and obesity share significant overlap in terms of comorbidities and pathological changes in adipose tissue, suggesting a common underlying mechanism. Adipose tissue, as a direct contributor to obesity, is also identified as a driving tissue in aging, exhibiting the earliest and most significant changes. This makes it a unique system for studying both aging and obesity. Unlike conventional static analyses, we introduced the concept of metabolic elasticity to describe the dynamic process of achieving metabolic homeostasis in aging. Our research revealed that metabolic elasticity is impaired in both aging and obesity, preceding the onset of aging markers. Furthermore, we recently identified IgG as an aging factor that accumulates in adipose tissue, promoting chronic inflammation and fibrosis, which in turn lead to metabolic decline. In summary, our work suggests that aging has a metabolic foundation, particularly rooted in adipose tissue. Addressing metabolic dysregulation before the onset of aging could provide an opportunity to maximize healthspan.

W32-2: Human Pluripotent Stem Cell, Organoids and Disease Modelling
Shuibing Chen
Weill Cornell

The major research interest in the Chen Laboratory is to manipulate stem cell fate using chemical and biological approaches and to generate functional tissues and organs that can be used for translational research. Our current main focus is on human pluripotent stem cells (PSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). We combine our knowledge of stem cell biology, developmental biology, chemical biology, medicine chemistry and tissue engineering to derive functional cells, tissues and organs from human pluripotent stem cells. Our long-term goal is to apply patient specific PSC-derived tissues or organs for replacement therapy and build up "disease in a dish" platforms for drug discovery.

W32-3: BCAA-BCKA Sensing beyond mTOR signaling
Qun-Ying Lei
Fudan University

Thousands of enzymatic reactions in the body generate approximately 200,000 metabolites, forming a complex dynamic network that regulates cellular behavior and life activities. Imbalance in this network can lead to the development of diseases, including tumors. In recent years, the non-canonical functions of metabolites have received increasing attention. In this report, we will briefly summarize the research progress of our research group on the plasticity of nutrition and cellular metabolism, focusing on recent research findings in branched-chain amino acid metabolism and cellular metabolic plasticity.

W32-4: PTEN and Prostate
Hong Wu,
Peking University
Abstract: To be updated

W32-5: Epigenome, Circadian Clock, and Neuroendocrine Regulation of Metabolism
Zheng Sun
Baylor

The circadian clock provides cue-independent anticipatory signals for diurnal rhythms of systemic insulin sensitivity and lipid metabolism. The central circadian clock is located in the hypothalamic suprachiasmatic nucleus (SCN), which comprises primarily GABAergic neurons. The SCN clock entrains the peripheral oscillators through neurohumoral mechanisms, which together regulates physiological diurnal rhythms of glucose tolerance and tissue-specific lipid metabolism. Disruption of the molecular circadian clock is associated with metabolic disorders. I will dissect the role of the circadian clock in the SCN, heart, and muscles in glucose and lipid metabolism in the context of diabetes, heart failure, and exercise performance. I will review neural and secreted factors in physiological diurnal rhythms of energy metabolism and their pathological implications in metabolic diseases.

Workshop 33: Liver and Cancer (Room 2D)

Chairs: Yu-Jui Yvonne Wan, Yilei Mao

W33-1: Hepatic Metabolizing Enzyme CYP3A1: a Key Regulator of Neurotoxicity Caused by Strychnine in Rats

Yuan Jiang[1], Lin Qin[1,2], Qianru Zhang [1,2], Yanliu Lu[1,2], Di Wu[1,2], Jian Xie[1], Daopeng Tan[1]*, **Yuqi He**[1,2]*

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In this study, we investigated the neurotoxic effects of strychnine, a major bioactive and toxic constituent of Semen Strychni, focusing on susceptibility mechanisms and markers related to individual differences in hepatic metabolism. Acute toxicity and oxidative stress levels of strychnine were assessed in rats through a single-dose administration of strychnine (2.92 mg/kg, i.g.). LC-MS/MS was employed to quantify the concentration of strychnine in plasma, hippocampus, striatum, and cortical tissues. The expression levels of CYP450 enzymes, UGT enzymes, and ABC transporter enzymes in the liver and duodenum were analyzed using proteomics. Potential susceptibility marker proteins were screened and validated at the animal level. Our findings revealed that strychnine induced morphological lesions in striatal vesicles in the striatum, as well as oxidative stress damage in the striatum and cortex, identifying these areas as primary toxic targets. Furthermore, the concentration of strychnine in the striatum and cortex demonstrated a close association with plasma drug levels, highlighting hepatic metabolism and intestinal absorption as key mechanisms of susceptibility to strychnine neurotoxicity. Plasma strychnine concentration exhibited a significant negative correlation with the expression levels of 19 enzymes, including CYP3A1, CYP3A18, CYP3A62, ABCA6, and UGT1A5, in the liver. Additionally, there was a significant positive correlation with the expression levels of three enzymes, including CYP51A1, in the duodenum. These findings identified 22 proteins as potential markers for susceptibility to strychnine-induced neurotoxicity. Phenobarbital sodium, an inducer, and ketoconazole, an inhibitor of the hepatic enzyme CYP3A1, can influence in vivo exposure to strychnine by regulating the expression level of *cyp3a1* in rat liver, thereby leading to variations in strychnine-induced neurotoxicity. These results indicate that CYP3A1 may be a susceptible biomarker for its neurotoxicity. These findings provide valuable insights into the neurotoxic susceptibility of strychnine and could inform its rational clinical use, potentially including applications involving Semen Strychni.

W33-2: Functional Metabolomics Characterizes the Contribution of Farnesoid X Receptor in Pyrrolizidine Alkaloid-Induced Hepatic Sinusoidal Obstruction Syndrome

Li Yang

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Herbal drug-induced liver injury has become a key issue in recent years. In addition, the unclear of its toxic mechanism and toxic targets further impair the reputation of herbal medicines and seriously limit their application. Hepatic sinusoidal obstruction syndrome (HSOS) caused by herbal medicines containing pyrrolizidine alkaloids (PAs), such as *Senecio scandens* and *Gynura japonica*, is a well-known, albeit rare lifethreatening disease. PA-induced HSOS cases have attracted worldwide attention since 1920s. However, the crucial metabolic variation and

biomarkers which can reflect these changes remain amphibious and thus to result in a lack of effective prevention, diagnosis and treatments against this disease. The aim of the study was to determine the impact of HSOS caused by PA exposure and to translate metabolomics-derived biomarkers into the mechanism.
Our previous studies have noted that PA exposure in rodents impaired bile acid homeostasis in a way quite different from those in drug-induced liver injury induced by other toxins and the hepatic expression of farnesoid X receptor (FXR, BR1H4), which is known to inhibit bile acid biosynthesis in hepatocytes, was decreased. In the present study, a combination of nontargeted metabolomics and targeted analysis of bile acids in clinical samples from two cohorts of PA-HSOS patients were performed to uncover the informative diagnostic biomarkers of PA-HSOS. As a result, cholic acid species (namely, cholic acid, taurine conjugated-cholic acid, and glycine conjugated-cholic acid) were identified as the candidate biomarkers (area under the ROC curve 0.968 [95% CI 0.908–0.994], sensitivity 83.87%, specificity 96.55%) for PA-HSOS. The increased primary bile acid biosynthesis and decreased liver expression of FXR were highlighted in PA-HSOS patients. Furtherly, a murine PA-HSOS model induced by senecionine (50 mg/kg, p.o.), a hepatotoxic PA, showed increased biosynthesis of cholic acid species via inhibition of hepatic FXR-SHP singling and treatment with the FXR agonist obeticholic acid restored the cholic acid species to the normal levels and protected mice from senecionine-induced HSOS.
This work elucidates that increased levels of cholic acid species can serve as diagnostic biomarkers in PA-HSOS. This study also translates metabolomics-derived biomarkers to the mechanism and lays the scientific basis for the clinical treatment of PA-HSOS by targeting FXR in clinics.

W33-3: Sphingolipids in MASH-HCC

Huiping Zhou[1], Jing Zeng[2], Grayson Way[1], Yunling Tai[1,] Xuan Wang[1], Derrick Zhao[1], Lianyong Su[1], Emily C. Gurley[1], Jiangao Fan[2], and Phillip. B Hylemon[1]
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Metabolic dysfunction-associated steatotic liver disease (MASLD) includes a spectrum of conditions from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and cirrhosis. MASLD is emerging as a significant risk factor for hepatocellular carcinoma (HCC) and is quickly becoming the leading cause of liver transplantation. Sphingolipids are important signaling moleculeas and play diverse roles in many physiological processes. Dysregulation in sphingolipid metabolism has been associated with the development of MASH-HCC. This study aims to provide detailed insights into sphingolipid profiles and the cell type-specific changes in key genes involved in sphingolipid metabolism in MASH-HCC progression.
Utilizing the well-established DIMOND mouse model, we analyzed total RNA sequencing data, NanoString nCounter® Gene profiling, single-nucleus RNA sequencing (snRNA-seq) data (GSE225381), and TCGA HCC patient data. Targeted lipidomics was used to profile sphingolipids in serum and liver. Univariate and multivariate Cox analyses were used to identify potential prognostic genes for MASH-HCC. Key findings revealed dysregulation in sphingolipid metabolism genes such as ceramide synthase 6 (Cers6), serine palmitoyltransferase long chain base subunit 2 (Sptlc2), sphingosine kinase 2 (SphK2), and sphingosine-1-phosphate receptors 1-3 (S1pr1-3), corresponding with significant changes in sphingolipid levels in serum and liver. Multivariate Cox analysis of the TCGA-LIHC cohort highlighted the prognostic importance of genes like CERS6, SPTLC2, and S1PR1.

Our study offers valuable insights into the role of sphingolipids in the progression from MASH to HCC. The distinct sphingolipid profiles identified could serve as crucial biomarkers for the diagnosis and prognosis of MASH-HCC.

W33-4: Discovery of a Novel Biomarker Predicting Liver Metastasis of Non-Functional Pancreatic Neuroendocrine Tumors

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Objective: Nonfunctional pancreatic neuroendocrine tumors (NF-PanNETs) exhibit varied clinical behaviors ranging from indolent to highly malignant. Current understanding of the molecular pathology of NF-PanNETs is insufficient for their clinical management, with a recognized challenge being the need to identify patients with aggressive tumors who should receive intensive therapeutic interventions. This study aims to explore pro-cancer regulatory mechanisms and to identify both potential therapeutic targets and prognostic biomarker(s) for NF-PanNETs.
Design: We generated single cell RNA-seq data from 10 clinically diverse patients, single cell ATAC-seq from 4 patients and then used this to construct a large-scale atlas providing an in-depth view of the NF-PanNET microenvironment. We then harnessed this atlas and employed various tumor models for follow-up analyses underlying NF-PanNET malignancy and to identify potential vulnerable therapeutic targets. Finally, we validated the prognostic relevance of simple IHC staining against a newly identified biomarker in a two-center cohort of 167 NF-PanNET patients.
Result: We mapped 6 major cell clusters (16 subclusters) within NF-PanNET samples and identified a pro-cancer subcluster. We discovered a specific transcription factor directly regulates the proliferative transcriptional program in these subcluster cells and promotes NF-PanNET growth. Chemically targeting this transcription factor confers benefit against pancreatic neuroendocrine tumors. We also show that the positivity of this subcluster marker by IHC staining is associated with several adverse prognostic clinical features and with poorer patient outcomes.

W33-5: Bacterial Immunotherapy using BCG as a Novel Option for HCC Treatment

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Hepatocellular carcinoma (HCC) continues to pose significant treatment challenges despite the utilization of immunotherapy. Bacillus Calmette-Guérin (BCG) has specific effects against tuberculosis and non-specific effects. Studies have revealed that these non-specific effects of BCG may be attributed to its trained immunity feature. Thus, BCG has been used successfully to treat non-muscle invasive bladder cancer. This study investigates whether BCG is a treatment option for orthotopic mouse HCC.
Two HCC models were produced using the hydrodynamic injection of myr-AKT1 plus NRasV12 or ΔN90-β-catenin plus NRasV12 and Sleeping Beauty transposase. Approximately 20-40% of HCC cases have β-catenin mutations, which tend to resist

immune checkpoint inhibitor treatment. The Pasteur strain of BCG was subcutaneously delivered ($1x10^6$ CFU, one time) when tumors had already been formed. The results showed that BCG treatment effectively reduced tumor burden and extended the survival time of HCC mice of both sexes. Moreover, BCG had much better effects than anti-PD-1 in reducing tumor load. In addition, BCG reduced hepatic fibrosis, improved liver function, and reduced hepatic cholesterol and triglyceride concentrations. Spatial transcriptomics analysis uncovered the effects of BCG inside the tumor, inducing enrichment of metabolic pathways and inhibiting cancer-related pathways. Multiplex immunohistochemistry also revealed that BCG treatment significantly increased CD4+ and CD8+ T cells and F4/80+ cells in both HCC models. *Ex vivo* trained immunity assays showed heightened IL-6 and TNFα production of monocytes isolated from the spleens and bone marrows of BCG-treated mice after the restimulation with LPS compared with those from untreated HCC mice, revealing the feature of trained immunity. Furthermore, blocking T cells, the trained immunity, or IFN-γ each could abolish the anti-HCC effects of BCG.
In summary, BCG is a promising HCC treatment option with its known safety profile. BCG engages T cells and monocyte-mediated trained immunity to combat HCC.

W33-6: Three-Dimensional Bioprinted Hepatorganoids & Primary Liver Cancer for Precision Medicine

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3D bioprinting is an innovative biofabrication strategy that enables the creation of bioactive artificial multicellular tissues/organs, offering a novel approach and method for developing new human tissue models that closely resemble real physiological conditions. On one hand, 3D bioprinting presents exciting prospects in simulating human normal tissue and organ physiological functions, making the regeneration of complex living tissues and automated production possible. At the same time, bioprinted disease tissue models, such as tumors, play a significant role in the treatment of certain diseases, novel drug screening, and toxicity prediction.
Here, we successfully printed a stable 3D liver organoid in vitro (3DP-HO model), which exhibited systematic liver functions both in vitro and in vivo. Through transplantation assays, we demonstrated the 3DP-HO model of liver tissues possessed in vivo hepatic functions and alleviated liver failure after transplantation, suggesting that 3D bioprinting could be used to generate human liver tissues as alternative transplantation donors for the treatment of liver diseases. The model may also serve as a rescue bridge for transitioning from liver failure to liver regeneration or act as a supplement towards extensive hepatectomy resection and temporary maintenance of the liver during a waiting period for transplantation.
Additionally, we achieved another breakthrough by extending 3D bioprinting using primary human HCC cells. We successfully established the patient-derived 3D bio-printed HCC (3DP-HCC) models, and after long-term culture, these models grew well and retained the features of parental HCCs. Furthermore, we demonstrated that 3DP-HCC models were capable of displaying drug screening results intuitively and quantitatively, making them suitable for evaluating the efficacy of multiple candidate drugs for HCC patients. Therefore, 3DP-HCC models are faithful in vitro models that are reliable in long-term culture and able to predict patient-specific drugs for personalized treatment.

Workshop 34: New Functions of Regulatory RNA (Room 2E)

Chairs: Lin He

W34-1: Cell Surface RNAs in Hematopoietic Cells
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Most RNAs in mammalian cells reside within the confinement of the cell membrane. Recently, a group of RNAs located on the outer cell surface was discovered. Because the outer cell surface is topologically different from the cytoplasm and nucleus where RNAs are produced, the existence of these cell surface RNAs raises key questions on their stability, functions, mechanisms of production and transportation. I will present our recent findings of cell surface RNAs in hematologic cells.

W34-2: Impact of Tandem Repeat Expansions on Gene Regulation and Human Diseases
Wei Li
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University of California, Irvine

The Genome Aggregation Database (gnomAD), widely recognized as the gold-standard reference map of human genetic variation, has largely overlooked tandem repeat (TR) expansions, despite the fact that TRs constitute ~6% of our genome and are linked to over 50 human diseases. Here, we introduce the TR-gnomAD (https://wlcb.oit.uci.edu/TRgnomAD), a biobank-scale reference of 0.86 million TRs derived from 338,963 whole-genome sequencing (WGS) samples of diverse ancestries (39.5% non-European samples). TR-gnomAD offers critical insights into ancestry-specific disease prevalence using disparities in TR unit number frequencies among ancestries. Moreover, TR-gnomAD is able to differentiate between common, presumably benign TR expansions, which are prevalent in TR-gnomAD, from those potentially pathogenic TR expansions, which are found more frequently in disease groups than within TR-gnomAD. Together, TR-gnomAD is an invaluable resource for researchers and physicians to interpret TR expansions in individuals with genetic diseases.

W34-3: A Micropeptide SPLIT Encoded by A Noncoding RNA Regulates Midbody Formation and Cytokinesis in Cancer Cells
Yue-Qin Chen
School of Life Science, Sun Yat-sen University, Guangzhou, China

Midbody functions during telophase to regulate the abscission step of cytokinesis. Dysregulation of formation and dissociation of midbody could result in the abnormal cytokinesis and causes various of disease including cancers. Due to the difficulty of capturing this critical point of cytoplasmic division, precise study of this biological process is not fully understood. In this present study, combining Ribo-seq, mass

spectrometry, and RNA-seq datasets, we identified a small peptide (85 aa in length) which was encoded by a non-coding RNA, we named this small peptide as SPLI. SPLIT is lower-expressed in various subtypes of cancer cells and confers a poor prognosis. The micropeptide is enriched in contractile ring-midbody region and regulates the process of formation and dissociation of midbody to maintain high rates of mitosis. Overexpression of SPLIT exhibited broad anti-cancer effects *in vitro* and *in vivo*. We further demonstrate its regulatory mechanism in cytokinesis, especially in cancer cells. Therefore, these findings reveal that SPLIT may act as a "brake" during mitosis, preventing cell division from becoming too rapid, leading to malignant cell proliferation and cancerous transformation. This study not only provides new ideas for further understanding of the mechanism of metaphase mitosis and dissociation, but suggests that exogenous introduction of micropeptide can inhibit the malignant proliferation of cancer cells.

W34-4: miRNA Dosage Control and its Applications in Cancer Therapy

Peng Du
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In mammals, miRNAs recognize target mRNAs through base pairing, forming a complex ‘multiple-to-multiple’ regulatory network. In contrast to studies that concentrate on individual miRNAs, emerging evidences suggest that global miRNAs serve as a ‘cellular buffer’ influencing cell fate. Our recent research has unveiled a miRNA dosage regulatory mechanism and its applications in stem cells. I will present our latest findings on a universal miRNA dosage control mechanism driven by 3’ terminal stability in cancer cells, along with its potential impacts on tumorigenesis and aging. Additionally, through cross-species bioengineering, we utilize plant-specific RNA-dependent RNA polymerase (RDR) to address the miRNA deficiency in cancer cells, leading to an extensive inhibition of tumor growth both *in vivo* and *in vitro*. Our research thus introduces an innovative gene therapy approach to combat tumors.

W34-5: Transposons, a Selfish Friend in Mammalian Preimplantation Development

Lin He
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Since the discovery of transposons, their sheer abundance in host genomes has puzzled many. While historically viewed as largely harmless 'parasitic' DNAs during evolution, transposons are not a mere record of ancient genome invasion. Instead, nearly every element of transposon biology has been integrated into host biology. My lab has discovered cell-type and context-dependent expression of many retrotransposon-derived transcripts in preimplantation embryos, and characterized their biological significance. Many retrotransposon elements, upon reactivation, can provide alternative promoter, cause alternative splicing, or generate alternative

polyadenylation signals to the proximal protein coding genes. Thus, these parasitic sequences in the mammalian genome can be exploited by the host genome to impose gene regulatory functions during defined developmental and pathological contexts. Hence, genome sequences introduced by transposon activities provide raw material for genome innovation and document the distinct evolutionary path of each species.

Workshop 35: Targeting DNA Repair for Cancer Therapy (Room 2F)

Chairs: Xiaohua Wu, Bing Xia

W35-1: Targeting EHMT1/GLP and EHMT2/G9a for Cancer Chemotherapy and Immunotherapy

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EHTM1 (GLP) and EHMT2 (G9a) are closely related protein lysine methyltransferases often thought to function together as a heterodimer to methylate histone H3 and non-histone substrates in diverse cellular processes, including transcriptional regulation, genome methylation, and DNA repair. Here we show that in addition to DNA damage, EHMT1/2 inhibitors cause reduced DNA replication fork speed, accumulation of single-stranded replication gaps, increased expression of STING, emergence of cytosolic DNA, and activation of the cGAS-STING pathway. Remarkably, EHMT1/2 inhibition substantially potentiates the efficacy of alkylating chemotherapy and anti-PD-1 immunotherapy in mouse models of tripe negative breast cancer. Mechanistically, the effects of EHMT inhibition on replication fork progression and alkylating agent sensitivity are largely caused by the loss of EHMT1-mediated methylation of DNA ligase I (LIG1), whereas the elevated STING expression is, at least part, caused by site-specific demethylation of its promoter mainly elicited by loss of EHMT2 function. Moreover, EHMTi-induced cytosolic DNA accumulation, which presumably activates the cGAS-STING pathway, results primarily from loss of EHMT1 function. These results reveal differential functions of the two EHMT paralogs and provide new paradigms and corresponding molecular basis for combination cancer therapies involving alkylating agents and immune checkpoint inhibitors.

W35-2: The Role of Histidine Phosphorylation in DNA Dealkylaiton Repair and Cancer

Yihan Peng[1], **Huadong Pei**[1]
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Histidine phosphorylation (pHis) is a poorly characterized regulatory phosphorylation event in mammalian cells. Although protein-His kinase NME1/2 was the first candidate metastasis suppressor gene identified, its detailed roles in cancer are still elusive. Here we show that NME1/2 is a key regulator of DNA dealkylation repair

and critical for protection against colitis-associated tumorigenesis. We find that an alkylating agent directly alkylates NME1/2, enabling NME1/2 autophosphorylation and the full activation. Activated NME1/2 phosphorylates ASCC3, which stimulates its helicase activity through relief of an autoinhibitory function inherent to its amino terminus, thereby facilitating the alkylation damage repair by AlkBH3 at single-stranded DNA. Moreover, Nme1/2 null mice showed DNA-alkylated lesion repair defects and colitis-associated colorectal cancer. Together, our study implicates a novel protein alkylation-histidine phosphorylation cascade that integrates histidine kinase activation, genome stability and tumor suppression.

W35-3: The CDK1-RNF40-PLK1 Cascade Regulates Cell Cycle-Dependent Centrosome Maturation and Chromosome Segregation

Jimin Li[1], Jianqiang Liang[1], Guifang Chen[1], Yunjing Guo[1], **Xuefeng Chen**[1,2]
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Centrosomes serve as the major microtubule organizing centers and form the poles of bipolar spindles to ensure proper chromosome segregation. Centrosome aberrations are commonly observed in human tumors. In the G2 to M phase, centrosomes must undergo a process called maturation to allow full activation of their microtubule organizing activity. Polo-like kinase 1 (PLK1) is the key kinase promoting centrosome maturation. However, how the cell cycle controls timely PLK1 centrosomal localization and centrosome maturation remains elusive.
Here, we found that the ubiquitin E3 ligase RNF40 localized to centrosomes where it was phosphorylated by CDK1 on T529/T557 in the G2 to M phase. This phosphorylation facilitates RNF40 interaction with PLK1, triggering timely PLK1 centrosomal localization, centrosome maturation, and proper chromosome segregation. Notably, we observed that RNF40 was acetylated on K517/K561 by PCAF in interphase before its phosphorylation, and the acetylation appears to suppress premature RNF40 phosphorylation by impeding the access of CDK1. However, RNF40 acetylation was gradually erased by HDAC1 upon entering the G2 phase, allowing subsequent RNF40 phosphorylation and centrosome maturation. As a result, blockage of its phosphorylation or mimicking constitutive acetylation impaired PLK1 localization and γ-tubulin recruitment and caused abnormal chromosome segregation phenotypes and increased chromosome instabilities. Importantly, these defects induce mitotic catastrophe in cancer cells and enhance their vulnerabilities to chemotherapeutic agents. Thus, our results unveil the CDK1-RNF40-PLK1 cascade as a novel signaling pathway regulating cell cycle-dependent PLK1 recruitment and centrosome maturation and offer alternative opportunities for targeting cancer.

W35-4: Unveiling Thioredoxin System's Impact on CHK1 Inhibitor Sensitivity via Redox-mediated Regulation of Ribonucleotide Reductase Activity

Junran Zhang
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The replication stress (RS) response protein ataxia telangiectasia and Rad3-related protein (ATR) and its main downstream factor, checkpoint kinase 1 (CHK1), play important roles in cell survival under RS. The inhibitors targeting CHK1 (CHK1i's) have been shown to be a powerful strategy for treating solid tumors and hematological malignancies in preclinical studies. However, in most clinical trials, including those for treating non-small cell lung cancer (NSCLC), which accounts for 85% of all lung cancer cases, CHKi's have failed to achieve their primary endpoints and have shown cumulative tissue toxicities in normal tissues. These findings significantly limit the clinical benefit of these agents. Thus, identifying novel combinational strategies that can enhance the sensitivity of tumor cells to CHKi's, while limiting their toxicities, might be the key to improve the efficacy of these compounds.

To explore novel combinational strategies that can overcome these limitations, we performed an unbiased high-throughput screen in a NSCLC cell line and identified thioredoxin1 (Trx1), a major component of the mammalian antioxidant-system, as a novel determinant of CHK1i sensitivity. We established a role for redox recycling of RRM1, the larger subunit of ribonucleotide reductase (RNR), and a depletion of the deoxynucleotide pool in this Trx1-mediated RS. In addition, we demonstrated that CHK1 inhibition abrogates the Trx1 or TrxR1 depletion-induced RRM2 expression and leads to the more profound dNTP pool scarcity. Further, the TrxR1 inhibitor auronafin, an anti-rheumatoid arthritis drug, shows a synergistic interaction with CHK1i via interruption of the deoxynucleotide pool. In summary, we propose that increased RRM1 oxidation following Trx system inhibition and the abrogation of RRM2 pathway by CHKi contributes to the synergistic interaction by the severe loss of RNR function. Our findings identify a new pharmacological combination to treat NSCLC that relies on a redox regulatory link between the Trx system and mammalian RNR activity.

W35-5: Regulation of The ATR-CHK1 Checkpoint Signaling

Xingzhi Xu[1]
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To maintain genome integrity upon replication stalling at damaged template strands, cells have evolved a complex mechanism known as the S phase checkpoint to detect and repair DNA damage that occurs during replication. This checkpoint is mediated by the ATR (ataxia telangiectasia and Rad3-related protein)-CHK1 (checkpoint kinase 1) pathway. CLASPIN is an essential mediator for ATR-dependent CHK1 activation upon replication stress. We have identified several novel regulatory mechanisms for this checkpoint activation, including ubiquitination/deubiquitination of CLASPIN, PARP1 UFMylation, and ASPM (a protein encoded by *primary microcephaly 5*)-dependent recruitment of RAD9/TopBP1. Our works have further elaborated the complicate yet delicate regulatory networks for replication fork stability during replication stress, thus safeguarding genome integrity.

W35-6: RNF126-Mediated MRE11 Ubiquitination Activates the DNA Damage Response and Confers Resistance of Triple-Negative Breast Cancer to Radiotherapy

Wenjing Liu1,[2], Xu Zhi[4], Dewei Jiang[1], and **Ceshi Chen**[1,2,3]

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Triple-negative breast cancer (TNBC) has higher molecular heterogeneity and metastatic potential and the poorest prognosis. Because of limited therapeutics against TNBC, irradiation (IR) therapy is still a common treatment option for patients with lymph nodes or brain metastasis. Thus, it is urgent to develop strategies to enhance the sensitivity of TNBC tumors to low-dose IR. Here, the authors report that E3 ubiquitin ligase Ring finger protein 126 (RNF126) is important for IR-induced ATR-CHK1 pathway activation to enhance DNA damage repair (DDR). Mechanistically, RNF126 physically associates with the MRE11-RAD50-NBS1 (MRN) complex and ubiquitinates MRE11 at K339 and K480 to increase its DNA exonuclease activity, subsequent RPA binding, and ATR phosphorylation, promoting sustained DDR in a homologous recombination repair-prone manner. Accordingly, depletion of RNF126 leads to increased genomic instability and radiation sensitivity in both TNBC cells and mice. Furthermore, it is found that RNF126 expression is induced by IR activating the HER2-AKT-NF-κB pathway and targeting RNF126 expression with dihydroartemisinin significantly improves the sensitivity of TNBC tumors in the brain to IR treatment in vivo. Together, these results reveal that RNF126-mediated MRE11 ubiquitination is a critical regulator of the DDR, which provides a promising target for improving the sensitivity of TNBC to radiotherapy.

Workshop 36: Coevolution of Cancer and Tumor Microenvironment (TME) in Tumor Progression (Room 2G)

Chair: Min Yu

W36-1: Advanced Proteogenomic Approaches for Tumor Antigen Discovery
Bin Zhang, Baylor College of Medicine

W36-2: Tumor Resident Microbes Regulate Metastatic Progression of Breast Cancer
Shang Cai, Westlake University

W36-3: Activated Type I IFN Microenvironment in Brain Metastasis
Qing Chen, Wistar Institute

W36-4: The Role of Lin28 in the Pre-metastatic Niche Formation and Immunosuppressive Tumor Microenvironment
Lixing Zhan, Shanghai Institute of Nutrition and Health

W36-5: Circulating Tumor Cells inform Mechanisms of Cancer Metastasis

Min Yu
Department of Pharmacology, University of Maryland School of Medicine, Baltimore, Maryland. Marlene and Stewart Greenebaum NCI Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland

Hematogenous metastasis is a complicated and inefficient multistep process by which tumor cells spread via blood circulation to form secondary tumors in distant organs. Only a very small fraction of the circulating tumor cells (CTCs) shed into the bloodstream is able to initiate a metastasis. Our research is focusing on understanding the molecular properties of these metastatic "seeds" and their interactions with the local organ microenvironment or "soil". To identify the metastasis-initiating CTCs, we *ex vivo* expanded CTCs derived from breast cancer patients and inoculated them into the bloodstreams of immunocompromised mice and identified metastases in the common sites of breast cancer. Importantly, the metastatic patterns in mice reflected those in the corresponding patients. Particularly, one CTC line showed a high capacity for brain metastasis in mice, which preceded the clinical detection of brain metastasis in the corresponding patient by one year. Via genetic, epigenetic and transcriptional analyses, we revealed genes associated with organotropic features and identified drivers for brain metastasis. To further elucidate the mechanisms of the pro-metastatic features of the CTCs, we analyzed the impact of hypoxia on CTC phenotypes. We discovered that hypoxia suppresses the tumor intrinsic interferon response in CTCs and such suppression last longer than the hypoxic exposure, showing a type of "hypoxic memory". Moreover, the hypoxic memory of prolonged suppression of tumor intrinsic interferon signaling is associated with enhanced tumorigenesis and metastasis, pointing to a novel mechanism underlying hypoxia associated pro-metastatic feature.

Workshop 37: RNA and Cytosolic DNA in Genome Maintenance (Room 2H)

Chairs: Li Lan, Zhongsheng You

W37-1: Targeting DNA Replication Stress and the ATR Checkpoint in Cancer Therapy

Lee Zou[1]
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In proliferating cells, the integrity of the genome is consistently challenged by intrinsic and extrinsic stresses during DNA replication. Replication stress, a collection of different types of interferences with DNA replication, is a major source of genomic instability. In highly proliferative cancer cells, replication stress is elevated by oncogenic events, driving genomic instability during tumorigenesis and tumor evolution. The ATR kinase is a master regulator of the replication stress response in human cells. My lab has extensively studied how ATR is activated by replication stress and how it functions to protect replication forks under stress. In addition, our recent studies reveal that ATR is activated by different types of replication stress in cancer cells, and it plays an important role in keeping cancer cells alive under stress. Excitingly, inhibition of ATR in cancer cells harboring high replication stress leads to

replication-associated DNA damage, which preferentially kills cancer cells over normal cells. These findings suggest that the ATR pathway is a promising target for cancer therapy in tumors harboring high replication stress. In my talk, I will discuss our recent findings on the sources of replication stress in cancer cells, the mechanisms by which ATR protects replication forks under replication stress, and how targeting ATR may benefit cancer therapy in specific oncogenic contexts.

W37-2: Cytosolic DNA, Ca^{2+} Signaling and Genome Maintenance

Zhongsheng You[1]
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Protecting the replication fork structure, especially under replication stress, is essential for maintaining genome integrity and stability. Defects in fork protection, often linked to mutations in tumor suppressor genes like BRCA1, BRCA2 and FANCD2, can lead to genomic instability and consequently, cancer formation. Such defects may also influence cancer progression by altering the tumor microenvironment through the generation of cytosolic DNA fragments that activate the innate immune response. Intriguingly, our recent findings indicate that cytosolic DNA, produced after replication stress, unexpectedly also supports replication fork protection. This protective effect is mediated through a complex signaling pathway that involves the activation of the cGAS/cGAMP/STING axis, Ca^{2+} release from the endoplasmic reticulum via the TRPV2 ion channel, activation of CaMKK2 and AMPK protein kinases, and the inhibition of the EXO1 nuclease. Disruption of this pathway results in excessive fork processing, chromosomal instability and heightened sensitivity to replication stress inducers. The links between innate immune factors, intracellular Ca^{2+} and genome maintenance in this novel signaling pathway may open new avenues for cancer treatment.

W37-3: Resolution of R-loop by BRCA1/2 and their Partners

Weixing Zhao[1], Wenjing Li[1], Yuxin Huang[1], Tzeh Foo[2], Bo Wu[1], Jae-Hoon, Ji[1,3], Qingming Fang[1,3], Boya Gao[4], Jingfei Xu[5], Rouf Maqbool[1], Tengyang Ni[1], Yuan He[5], Li Lan[4], Bing Xia[2]
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[5]*Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA.*

BRCA1 and BRCA2 are tumor suppressor genes widely recognized for their critical roles in the repair of double-strand DNA breaks, primarily through homologous recombination (HR). Mutations in these genes are strongly associated with high risks of breast and ovarian cancers. Beyond their established functions in DNA repair, BRCA1/2 also play crucial roles in maintaining genomic stability, including the resolution of R-loops. R-loops are three-stranded nucleic acid structures composed of a DNA-RNA hybrid and displaced single-stranded DNA. These structures can arise

during transcription and pose threats to genomic integrity by causing replication stress and genomic instability. We are exploring the intricate mechanisms by which BRCA1/2, along with their interacting partners, contribute to the resolution of R-loops. Additionally, we are investigating the pathological implications of impaired R-loop resolution due to BRCA1/2 dysfunction, which is linked to an increased risk of genomic alterations and cancer progression. The elucidation of these additional roles of BRCA1/2 enhances our understanding of their multifaceted functions in genomic maintenance and provides insights into potential therapeutic strategies that could leverage the disruption of R-loop dynamics in BRCA1/2-deficient cancers. By detailing these mechanisms, we underscore the importance of BRCA1/2 beyond traditional DNA repair, highlighting their broader implications in maintaining genomic stability.

W37-4: Targeting RNA Modifying Enzymes in Cancer Therapy

Li Lan[1]
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The treatment of many solid tumors presents significant challenges with chemotherapy, radiation therapy, and the limited effectiveness of immunotherapy. Targeted therapy offers a promising approach yet the lack of targets restricts the applicability across the spectrum of solid tumors. Our past studies revealed an mRNA-dependent DNA repair pathway coupling with mRNA modifications induced by damage at the transcribed regions of the genome contributes to cell survival and drug resistance in cancer cells specifically due to high level of transcription and DNA damage of cancer cells. We identified the RNA methyltransferase, TRDMT1, as the primary modifier of mRNA methyl-5-cytosine in the RDDR pathway. In-house cell-based and in vitro assays, we developed the first TRDMT1 inhibitor via a chemical screen. We will discuss how we use cancer cell lines, xenograft models, and patient specimens to evaluate cancers with genomic instability for identifying biomarkers and how this pathway crosstalk with anti-tumor immunity.

W37-5: TERRA-LSD1 Phase Separation Promotes R-Loop Formation for Telomere Maintenance in ALT Cancer Cells

Meng Xu[1], Dulmi Senanayaka[2], Astha Tripathi[1], Rongwei Zhao[1], Jason Tones[1], Rachel M. Lackner[3], Laurel N. Moneysmith[2], David M. Chenoweth[3], Nicholas J. Reiter[2], and **Huaiying Zhang**[1]*
1 Department of Biology, Carnegie Mellon University, Pittsburgh, PA 15213
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3 Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19014

Chromatin-associated RNAs play essential roles in maintaining genome stability. For example, telomere repeat-containing RNA (TERRA), a long non-coding RNA transcribed from chromosome ends, forms R-loops to promote homology-directed DNA synthesis in the alternative lengthening of telomere (ALT) pathway. Here we report that TERRA contributes to ALT via interacting with the lysine-specific demethylase 1A (LSD1 or KDM1A). We show that TERRA is required for the telomeric localization of LSD1, whose deficiency leads to a loss of ALT signatures.

In addition, LSD1 function in ALT is largely independent of its demethylase activity. Instead, LSD1 promotes TERRA localization to ALT telomeres via its interaction with TERRA. Importantly, chemical dimerization-mediated recruitment of LSD1 to telomeres is sufficient to enrich TERRA on telomeres. Additionally, LSD1 recruitment triggers LSD1 phase separation at telomeres, which appears to depend on LSD1's TERRA binding capacity. In vitro reconstitution studies show that TERRA indeed promotes LSD1 phase separation in a manner that is dependent upon TERRA length and its G-quadruplex structure. Importantly, the formation of TERRA-LSD1 condensates enriches the R-loop stimulating protein Rad51AP1 and increases TERRA-containing R-loops at telomeres, suggesting that phase separation may concentrate R-loop regulatory molecules to stimulate DNA synthesis on ALT telomeres. Our findings reveal that mutual enrichment of LSD1 and TERRA at telomeres can function to promote ALT telomere maintenance, suggesting a mechanism for how biophysical properties of histone modification enzyme-RNA interactions impact chromatin function.

Workshop 38: Neuronal Circuitry in Health and Diseases (Room 3A)

Chairs: Hongjun Song, Hailan Hu

W38-1: Brain-wide Neuronal Circuit Connectome of Human Glioblastoma

Hongjun Song[1]
[1]*Department of Neuroscience and Mahoney Institute for Neurosciences, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.*

Glioblastoma (GBM), a deadly brain cancer, infiltrates the brain and can be synaptically innervated by neurons, which drives tumor progression. Synaptic inputs onto GBM cells identified so far are largely short-range and glutamatergic. The extent of integration of GBM cells into the brain-wide neuronal circuitry is not well understood. We applied rabies virus-mediated retrograde monosynaptically-restricted transsynaptic tracing and herpes simplex virus-mediated anterograde tracing approaches to systematically investigate circuit integration of human GBM organoids transplanted into adult mice. We found that GBM cells from multiple patients rapidly integrated into brain-wide neuronal circuits and exhibited diverse local and long-range connectivity. Beyond glutamatergic inputs, we identified a variety of neuromodulatory inputs across the brain, including cholinergic inputs from the basal forebrain. Acute acetylcholine stimulation induced sustained calcium oscillations and long-lasting transcriptional reprogramming of GBM cells into a more invasive state via the metabotropic CHRM3 receptor. CHRM3 activation promoted GBM cell invasion, whereas CHRM3 downregulation suppressed GBM cell invasion, proliferation, and survival in vitro and in vivo. Together, these results reveal the capacity of human GBM cells to rapidly and robustly integrate into anatomically and molecularly diverse neuronal circuitry in the adult brain and support a model wherein rapid synapse formation onto GBM cells and transient activation of upstream neurons may lead to a long-lasting increase in tumor fitness to promote infiltration and progression.

W38-2: Neuron-Astrocyte Crosstalk in Lateral Habenula Mediates Depression-like Behavior

Hailan Hu[1]
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Major depressive disorder (MDD) represents one of the most prevalent and debilitating mental illnesses. Elevated expression of Kir4.1 in the lateral habenula (LHb), recognized as the brain's anti-reward center, has been linked to depression. As a core component of astrocytic Kir channels, Kir4.1 tightly "wraps" around LHb neuronal soma and predominantly contributes to the regulation of their resting membrane potential (RMP). Genetic downregulation or functional suppression of the astroglial Kir4.1 in LHb ameliorates depressive-like behaviors in mice. However, the mechanisms underlying elevated Kir4.1 in the LHb under depressive states remained unclear. Here, we discovered an evolutionarily conserved secretory protein that mediates the upregulation of Kir4.1. We found this protein is released from the somata of lateral habenular neurons in response to burst firing and acts on receptors in astrocytes to regulate Kir4.1 expression. Bidirectional manipulation of this pathway respectively induced and ameliorated depression-like phenotypes in mice. Meanwhile, pharmacological inhibition of this pathway exhibited potent antidepressant efficacy. Overall, we identify a vicious cycle mediated by neuron-astrocyte cross-talk in the lateral habenula to perpetuate the depression-like state, and uncover novel antidepressant targets for depression treatment.

W38-3: Circuit Regulation of Dentate Gyrus along the Dorsoventral Axis in Health and Disease

Juan Song[1]
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The dentate gyrus, a “gate” that controls the information flow into the hippocampus, is critical for episodic memory, stress response, and mood regulation. The DG is further divided into dorsal (dDG) and ventral (vDG) compartments in rodents or anterior and posterior compartments in primates. It was thought that dDG preferentially contributes to cognitive function, while vDG preferentially contributes to affective function. However, the neural circuit mechanisms regulating dDG- and vDG-mediated distinct functions remain elusive. In my talk, I will first present the data on the differential regulation of dDG and vDG functions by molecularly and functionally distinct populations of neurons in the hypothalamic supramammillary nucleus (SuM). Furthermore, I will extend these findings from healthy mice to mouse models of Alzheimer’s disease.

W38-4: Nanowire Arrays as Artificial Photoreceptors – Rodents, Primates and Human

Jiayi Zhang[1]
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Retinal prostheses could restore image-forming vision in conditions of photoreceptor degeneration. However, contrast sensitivity and visual acuity are often insufficient. In

this talk, we will report the performance, in mice and monkeys with induced photoreceptor degeneration, of subretinally implanted gold-nanoparticle-coated titania nanowire arrays providing a spatial resolution of 77.5 μm and a temporal resolution of 3.92 Hz in ex vivo retinas (as determined by patch-clamp recording of retinal ganglion cells). In monkeys, the arrays were stable for 54 weeks, allowed for the detection of a 10 μW mm–2 beam of light (0.5° in beam angle) in visually guided saccade experiments, and induced plastic changes in the primary visual cortex, as indicated by long-term in vivo calcium imaging. We also demonstrated that nanomaterials as artificial photoreceptors ameliorate visual deficits in patients with photoreceptor degeneration.

W38-5: The Higher-order Auditory Thalamic Complex: Connectivity, Function and AI Application

Kexin Yuan[1]
[1]*School of Biomedical Engineering, IDG/McGovern Institute for Brain Research, Tsinghua Laboratory of Brain and Intelligence, Tsinghua University, Beijing, China*

Central auditory information processing is crucial for detecting environmental threats and understanding the meaning of sounds. The auditory thalamus is a key structure for integrating and processing auditory information. While the function of the primary auditory thalamus in auditory feature perception is well established, the function and working mechanism of the higher-order auditory thalamic complex have long been unclear. We have been focusing on this mysterious thalamic region in the past few years, and for the first time, discovered its critical role in auditory and visual threat perception, revealed the connective architecture for visual-assisted auditory information processing and its applications in artificial intelligence, and developed tissue clearing technique that is based on novel chemical principles to provide accurate connectivity data for the aforementioned achievements. We are currently revealing the heterogeneity in the synaptic input-axonal output architecture of individual higher-order thalamic neurons by using the unique tissue-clearing technique we developed, which would further facilitate our functional probing of this thalamic complex, hopefully shedding new light on the treatment of sensory processing disorder and inspiring AI model capable of dealing with more challenging perception tasks.

Workshop 39: Biology of Plant Diseases and Insect Pests, and Their Management (Room 3B)

Chairs: Jian Wu, Lian-Sheng Zang

W39-1: The Mystery of Genetic Diversity in Rice

Bin Han, Institute of Plant Physiology and Ecology, CAS

W39-2: Introduction of Exotic Hymenopteran Parasitoids for Biological Control of Invasive Pests

Qiao Wang Frsnz
State Key Laboratory of Green Pesticides, Guizhou University, Guiyang, China

With the substantial increase of international trade in recent decades, many insect species have become established outside their natural range of distribution with the potential for causing economic and environmental damage in these novel habitats. Therefore, the intentional introduction of exotic biological control agents for control of invasive pests, still remains important today, if not more. New Zealand has introduced more than 100 species of parasitic wasps in the past century, most of which are not working. Globally, only around 10% of introduced parasitoids have successfully suppressed the invaded pests to acceptable levels. Reasons for the low success rates are diverse but the following are important: introduced parasitoids have low degree of density-dependence and high degree of microhabitat-specificity, and undertake hyperparasitic male production. We often introduce wrong parasitic wasps from overseas in biological control programs. Using examples from the biological control program for two invasive insect pests, *Phoracantha semipunctata* and *P. recurva*, killing a large number of eucalypt trees in the USA and other continents, I discuss the success and failure of their biological control and share my personal experience and lessons in the lengthy adventures of parasitoid searching, field investigation and introduction and re-introduction. I discovered and introduced six parasitic wasps (four larval parasitoids and two strains of an egg parasitoid) from Australian forests to control these pests. The egg parasitoids have successfully controlled the pests while all larval parasitoids have failed.

W39-3: Immune Mechanism of Ethylicin-Induced Rice Resistance to *Xanthomonas oryzae* pv. *oryzae*

Runjiang Song[1*], Hongxia Lu[1], Baoan Song[1]
1 State Key Laboratory of Green Pesticide, Guizhou University, Huaxi District, Guiyang 550025, P. R China

Phytopathogens, encompassing harmful microorganisms such as bacteria, fungi, and viruses, pose a significant threat to global agriculture by causing devastating plant diseases. These infections lead to substantial crop losses, reduce quality, and ultimately jeopardize food security and economic stability. Current challenges in managing phytopathogens include the growing resistance to existing treatments, environmental degradation due to over-reliance on chemical pesticides, and potential health risks associated with pesticide residues. The urgency of discovering new drug targets and developing innovative, effective pesticides is paramount to overcoming these obstacles. This not only ensures sustainable agricultural practices but also safeguards food supplies and promotes a healthier environment.
Rice bacterial leaf blight (BLB), caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most prevalent diseases in regions where rice is a staple food crop. Outbreaks of BLB typically result in a 10–30% reduction in rice yields, with severe cases potentially causing losses exceeding 50%. In light of the ban on bismerthiazol, traditional copper-based bactericides are insufficient for achieving sustainable agricultural practices. Although numerous alternative antimicrobial agents have demonstrated effective inhibitory effects on *Xoo*, their mechanisms of action remain largely unclear. Indeed, the interaction between plants, pathogens, and pesticides forms a highly intricate and dynamic system. As the arms race between these three continues, understanding this complexity is crucial for developing effective and sustainable pest management strategies.

ET is a bionic pesticide derived from garlic, which has been widely documented to have the capacity to prevent and manage damage caused by a broad spectrum of plant pathogenic fungi and bacteria. When compared to the current BLB control agents, ET offers the benefits of superior control efficiency, effortless degradation, and environmental compatibility. We intensively explore the antibacterial mechanism of ET, revealing that ET inhibits *Xoo* by increasing the levels of defense enzymes and chlorophyll in rice. Proteomic analysis provides insights into ET's effects on the abscisic acid signaling pathway in rice, particularly the activation of calcium-dependent protein kinase 24 (OsCPK24). Consequently, OsCPK24 has been identified as a key mediator in rice resistance to *Xoo*, paving the way for the development of novel bactericides leveraging OsCPK24.

W39-4: Olfaction based Pest Management and Food Security

Jing-Jiang Zhou[1,2]

1 State Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, People's Republic of China; 2 MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, UK

The interaction between insect pests and crops can cause direct crop damage and yield reduction due to herbivore feeding. On a global scale, it is estimated that insect pests are responsible for approximately 10-25% of crop yield losses and billions of dollars of the economic losses annually. To keep pace with growing demand, global food production needs to increase by an estimated 70% by 2050. Despite a clear increase in pesticide use, crop losses have not significantly decreased during the past 40 years. These highlight the critical importance of effective pest management strategies in agriculture to minimize yield losses and ensure food security. New technologies are becoming important, especially for surveillance and application. Innovation by the industry, together with fundamental and applied research by universities and research institutes create the opportunities for improving crop protection techniques.

The evolution of distinct, sensory-specific molecular signal transduction mechanisms and sensory structures enable insects to locate hosts within an extraordinary diversity of life histories and ecological niches. Plant defense system has been explored to manipulate insect pest behaviors. In "push-pull" system companion plants release chemicals to repel pests and suppress weeds. Crops can be engineered to release alarm pheromone and repel aphids and attract their enemies.

The Insect Chemical Sensory Proteins (ICSP) in insect olfactory systems play a vital role to locate host-crops, mates, and preys. They include odorant binding proteins (OBP) and olfactory receptors (OR). We explore the possibility of OBPs and ORs as novel targets for pesticide discovery. Plant secondary metabolites are collected against OBPs and ORs. Bioactive metabolites are examined against insect pests through structure and function study. Ideally, there would be a selection of chemicals to choose from, each of which would belong to a different class of chemistry and function through a unique mechanism of action within insect pests.

W39-5: Pesticide Informatics Platform (PIP) and its Application in Novel Plant Growth Regulator Discovery

Ge-Fei Hao[1]

1 State Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, People's Republic of China;

As an important part of agriculture, agrochemicals are important weapons to fight against pests and natural disaster of plant. The need for novel pesticides becomes urgent as a result of the emergence of resistance and environmental toxicity. Pesticide informatics has been applied in different phase processes of pesticide target identification, active ingredient design, and impact evaluation. Therefore, we have constructed the first international systematic Pesticide Informatics Platform (PIP) , which includes several original methods, such as ACFIS (fragmented molecular design), RDFL (bioavailability intelligence analysis of molecular fragments), and TDB (pesticide eco-toxicity prediction). PIP provides information tools for activity, resistance, bioavailability, toxicity, metabolism, and biosafety analysis1-5. The platform has assisted in the discovery of several new pesticides and has been used by nearly 8,000 users in more than 100 countries. The presentation will introduce the construction of a pesticide informatics platform and the recent applications.

W39-6: Asymmetric Organocatalysis for Chiral Agrochemical Development

Xingxing Wu, Kunpeng Teng and Yonggui Robin Chi
State Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, People's Republic of China

Chiral pesticides, characterized by the presence of enantiomers, are gaining attention due to their distinct biological activities and environmental impacts. Enantiomers of a chiral pesticide can exhibit different levels of efficacy and toxicity, affecting target and non-target organisms variably. Therefore, the development and application of chiral pesticides are of increasing significance in modern crop protection. Generally, only one single enantiomer is notably active against the target while the other paired isomer is less or inactive, that thus offering an ideal approach to reduce the excess use of chemical agents by elimination of the less effective stereoisomer in the agrochemical ingredients.

Despite its importance of chiral pesticides, most of these agents are sold in their racemic form in the market despite of their chiral structures and remarkable difference on their biological activities. One of the major problems that hinder the study and development of chiral pesticides rely on the notable shortage of simple practical methods for preparation of highly optically enriched chiral agrochemicals. To address this challenge, we have developed highly effient catalytic construction methods for optically enriched heterocycles promoted by chiral organocatalysts. Bioassay studies showed distinct antibacterial properties against crop pathogens, such as *Xoo* etc. of the two single enantiomers. The findings underscores the fundamental impact of different absolute configurations of chiral compounds on their biological activities, and shall inspire further explorations of chiral heterocyclic molecules as potential drug candidates to control the plant pathogen diseases.

Day 3, July 28 (Sunday)

Concurrent workshops 40-48, 1:30-3:00pm

Workshop 40: Hypoxia and Metabolism Signaling in Cancer Progression and Metastasis (Room 2A)

Chairs: Qing Zhang, Zhaohui Feng

W40-1: The E3 Ubiqutin Ligase Parkin in Metabolism and Tumor Suppression
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Parkin is an E3 ubiquitin ligase. Parkin mutations cause familial autosomal recessive juvenile Parkinson's disease, a neurodegenerative disease. Parkin expression is frequently downregulated in many types of human cancers. Accumulating evidence has suggested that Parkin is a tumor suppressor, but its underlying mechanism is not well defined. Our recent studies suggest that Parkin regulates metabolism as a critical mechanism in tumor suppression. Hypoxia-inducible factor 1α (HIF-1α), which is frequently accumulated in cancer, plays a critical role in promoting glycolysis and metastasis through transcriptional regulation of its target genes. We idenfied that Parkin is an E3 ubiquitin ligase for HIF-1α. Parkin interacts with HIF-1α and promotes HIF-1α degradation through ubiquitination, which in turn inhibits glycolysis and metastasis of cancer cells. Further, lysine 477 (K477) of HIF-1α is a major ubiquitination site for Parkin; K477R HIF-1α mutation largely abolishes the function of Parkin to ubiquitinate HIF-1α. Phosphoglycerate dehydrogenase (PHGDH), the first rate-limiting enzyme of serine synthesis, is frequently overexpressed in human cancer. PHGDH overexpression activates serine synthesis to promote cancer progression. We found that PHGDH is a substrate for Parkin-mediated ubiquitination and degradation. Parkin interacts with PHGDH and ubiquitinates PHGDH at lysine 330 (K330), leading to PHGDH degradation to suppress serine synthesis in cancer cells. Parkin deficiency in cancer cells stabilizes PHGDH and activates serine synthesis to promote tumorigenesis. In addtion, Parkin expression is inversely correlated with the expression of HIF-1α and PHGDH in human cancer specimens. In summary, our results revealed Parkin ubiquitinates HIF-1α and PHGDH as crucial mechanisms to regulate glycolysis and serine synthesis, respectively, which contributes to the tumor-suppressive function of Parkin. Further, our results also demonstrated that the frequent downregulation of Parkin in cancer is an additional mechanism underlying HIF-1α accumulation and PHGDH overexpression in cancer.

W40-2: Studying the Hypoxia Signaling Pathways in Breast Cancer
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Breast cancer is the most common cancer among women. Hypoxia leads to metabolic reprogramming and advanced tumor progression, and is associated with high-grade breast tumor and poor prognosis of breast cancer patients. Hypoxia-inducible factor (HIF) is a well-established regulator that drives cellular adaptation to hypoxia. However, emerging evidence support that sophisticated interaction networks beyond the HIF pathway contribute to cellular adaptation to hypoxia. Mitochondria, the largest consumers of intracellular oxygen, are first in line to confront the fluctuations in oxygen level and the primary sites for hypoxia-induced metabolic reprogramming. Although mitochondria are well-known as intracellular platforms for protein-protein interactions to initiate cell signaling, how they serve as scaffolds for oxygen sensing signaling and therefore communicate their fitness to the rest of the cell under hypoxic stress is not well understood. Through mitochondrial proteomic profiling, we find that the prolyl hydroxylase EglN1 accumulates on mitochondria under hypoxia. Further, we identify AMP-activated protein kinase alpha (AMPKa) as an EglN1 substrate on mitochondria. The EglN1-AMPKa interaction through EglN1 substrate binding region b2b3 loop is essential for their mutual mitochondrial translocation. Specifically, EglN1 prolyl-hydroxylates AMPKa under normoxia, then they rapidly dissociate following prolyl-hydroxylation, leading to their immediate release from mitochondria. While hypoxia results in constant EglN1-AMPKa interaction and their accumulation on mitochondria, leading to the formation of Ca^{2+}/calmodulin-dependent protein kinase 2 (CaMKK2)-EglN1-AMPKa complex to activate AMPKa phosphorylation, consequently ensuring metabolic homeostasis and breast tumor growth. Our findings demonstrate EglN1 as an oxygen-sensitive metabolic checkpoint signaling hypoxic stress to mitochondria through its b2b3 loop region, revealing a therapeutic target for breast cancer.

W40-3: Proline Hydroxylation in Tumorigenesis

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Proline hydroxylation is one of the most abundant protein post-translational modifications (PTMs). Hydroxylation of proline residue is critical for protein stability, enzyme activity, protein interactions and other physiological conditions as well as in diseases. EGLNs-mediated HIF hydroxylation is involved in the regulation of cellular adaptation to hypoxia. Under hypoxia, EGLNs lose their ability to hydroxylate HIFα, which leads to HIFα stabilization and dimerization with HIF1β (ARNT), thereby activating the transcription of key genes involved in cell proliferation, metabolism and angiogenesis. Now, more and more evidence identified non-HIFα substrates of proline hydroxylation, which suggest that functional prolyl hydroxylation is beyond HIF.

By performing a genome-wide screen, we identified Scm-like with four malignant brain tumor domains 1 (SFMBT1) as a proline hydroxylation target. Integrated analyses of ChIP-seq, RNA-seq, and patient prognosis identified pVHL-SFMBT1-SPHK1 axis serves as a potential therapeutic avenue for clear cell renal cell carcinomas(ccRCC). Besides, we found the existence of a histone PTM in mammalian cells, namely histone H3 with hydroxylation of proline at residue 16 (H3P16oh), which is catalyzed by the proline hydroxylase EGLN2. Further, we demonstrated EGLN2-H3P16oh pathway regulates gene expression and breast carcinoma. Undoubtedly, it is imperative to study the multi-functionality of prolyl

hydroxylation in hypoxia signaling and cancer, which may yield additional therapeutic targets in cancer.

W40-4: Activating PPARα to Restore Lipid Homeostasis in the Liver and Inhibit Cancer Cachexia

Wenwei Hu[1] Xue Yang [1], Jianming Wang [1], Chun-Yuan Chang [1], Fan Zhou [1], Juan Liu [1], Maria Ibrahim [1], Maria Gomez [1], Grace L. Guo [2, 3], Hao Liu [1, 4], Wei-Xing Zong [1, 5], Xiaoyang Su [1, 6], Eileen White [1], and Zhaohui Feng [1]

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Cancer cachexia is a systemic metabolic syndrome characterized by involuntary weight loss, and muscle and adipose tissue wasting. Cachexia occurs frequently in advanced cancer patients with many progressing to death. Mechanisms underlying cachexia remain poorly understood. Cancer cachexia can be in part driven by the competition between tumor and host cells for nutrients. Importantly, there are metabolic and signaling crosstalk between organs, including the brain, liver, gut, muscle and adipose tissues, which contribute to the cachectic state.

Leukemia inhibitory factor (LIF), a multi-functional cytokine, has been suggested as a cachexia-inducing factor. We established a transgenic mouse model with conditional LIF expression to demonstrate that systemic elevation of LIF induces cachexia. We found that LIF overexpression disrupts lipid homeostasis in the liver. Liver-specific LIF receptor knockout attenuates LIF-induced cachexia, suggesting that LIF-induced functional and metabolic changes in the liver contribute to cachexia. Mechanistically, LIF overexpression activates STAT3 to downregulate PPARα, a master regulator of lipid metabolism, leading to the downregulation of a group of PPARα target genes involved in lipid metabolism in the liver. Activating PPARα by fenofibrate, a PPARα agonist, restores lipid homeostasis in the liver and inhibits LIF-induced cachexia. These results provide the insights into cachexia, which may help develop strategies to treat cancer cachexia.

W40-5: EGFL9 Drives TNBC Metastasis through Promoting cMET Signaling and Metabolic Reprogramming

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The molecular mechanisms driving metastatic progression in triple-negative breast cancer (TNBC) patients are poorly understood. In this study, we demonstrate that epidermal growth factor-like 9 (EGFL9) is significantly upregulated in basal-like breast cancer cells and associated with metastatic progression in breast tumor samples.

Functionally, EGFL9 is both necessary and sufficient to enhance cancer cell migration and invasion, as well as distant metastasis. Mechanistically, we demonstrate that EGFL9 binds cMET, activating cMETmediated downstream signaling. EGFL9 and cMET co-localize at both the cell membrane and within the mitochondria. We further identify an interaction between EGFL9 and the cytochrome c oxidase (COX) assembly factor COA3. Consequently, EGFL9 regulates COX activity and modulates cell metabolism, promoting a Warburg-like metabolic phenotype. Finally, we show that combined pharmacological inhibition of cMET and glycolysis reverses EGFL9-driven stemness. Our results identify EGFL9 as a therapeutic target for combating metastatic progression in TNBC.

W40-6: Targeting JAG1 Overcomes Pancreatic Cancer Immunotherapy Resistance by Reinvigorating cDC1 and CD8 T cells

Lan Zhou[1,2], Bingqing Zou[1,2], Qiuyun Chen[2], Shuiliang Yu[2], Ernest Chan[2], Yitian Xu[1], Junjun Zheng[1], Shu-hsia Chen[1], Wei Xin[2,3]

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While immune checkpoint inhibitors have revolutionized treatment of melanoma and lung cancer, immunotherapy has little to no efficacy in PanCan patients. Notch signaling is important for PanCan initiation and progression. However, its role in the regulation of PanCan tumor microenvironment (TME) is unclear. JAG1, a predominant Notch ligand in PanCan, correlates with worse prognoses and inversely correlates with tumor infiltrating CD8 T cells and *BATF3*, a transcription factor for type I conventional dendritic cell (cDC1) development. cDCs play a critical role in promoting T cell antitumor immunity in immunotherapy, but cDCs are present in low numbers and often display functional suppression in PanCan patients. In this study we aimed to decipher the mechanism by which JAG1 promotes PanCan therapy resistance by suppressing cDC1 and CD8 T cell anti-tumor response. Using tumors from KPC mice (*LSL-Kras*$^{G12D/+}$*;LSL-Trp53*$^{R172H/+}$*;Pdx-1-Cre*), we found that JAG1 expression is higher in non-T cell-inflamed and immunotherapy-resistant KPC clones but lower in T cell-inflamed and immunotherapy-sensitive clones. Deletion of JAG1 in T cell-low tumors or blocking JAG1 using neutralizing antibodies induced marked cDC1- and T cell-dependent tumor regression with long-term survival in response to immunotherapy. Notably, JAG1 ablation markedly increased the numbers of tumor-infiltrating cDC1s and CD8 T cells and improved cDC anti-tumor function. Furthermore, JAG1 ablation reshaped the tumor immune landscape by increasing DC interaction with CD8 T cells and NK cells. In contrast, increasing JAG1 expression in T cell-high tumors accelerated tumor progression and decreased the numbers of tumor-infiltrating cDC1s and CD8 T cells. Mechanistically, we revealed that targeting JAG1 improved cDC metabolic fitness and expanded Notch-activated $CD8^+$ cells. In summary, we showed that JAG1 creates a suppressive TME in PanCan by inducing cDC metabolic exhaustion, and targeting JAG1 may be a novel immunomodulatory treatment to reinvigorate the anti-tumor immunity for this deadly disease.

Workshop 41: *Tumor Virus* Biology and Oncogenesis (Room 2B)

Chairs: Pinghui Feng, Zhi-Ming Zheng

W41-1：Epstein-Barr virus-targeted therapies in nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is consistently associated with Epstein-Barr virus (EBV) infection in the endemic regions including Southern China and Southeast Asia. The high mortality rates of NPC patients with advanced and recurrent disease highlight the urgent need for effective treatments. The unique interaction between the EBV infection and host cells in NPC strongly implies that targeting EBV may be an efficient approach to cure this virus-associated cancer. Key features of EBV-associated NPC are the persistence of an episomal EBV genome and the requirement for multiple viral latent gene products to enable malignant transformation. By exploiting these unique features, several pharmaceutical agents and therapeutic strategies that target EBV latent proteins and induce lytic reactivation in NPC. While the promising therapeutic effects of the EBNA1-specific inhibitors have been shown in EBV-positive NPC tumors, we developed a synthetic transcriptional activator that specifically activates endogenous lytic genes and efficiently induces lytic reactivation in EBV-positive cancer cells. A lipid nanoparticle encapsulating nucleoside-modified mRNA which encodes a *BZLF1*-specific transcriptional activator (mTZ3-LNP) is synthesized for EBV-targeted therapy. Compared with conventional chemical inducers, mTZ3-LNP more efficiently activates EBV lytic gene expression in NPC and other EBV-associated epithelial cancers. Using the EBV-positive cell lines and patient-dervied xenograft models, we demonstrated the potency and safety of treatment with mTZ3-LNP to suppress NPC tumor growth. The combination of mTZ3-LNP and ganciclovir yields highly selective cytotoxic effects of mRNA-based lytic induction therapy against EBV-positive tumor cells, indicating the potential of mRNA nanomedicine in the treatment of EBV-associated epithelial cancers.

W41-2：Targeting vulnerability of EBV infection for vaccine development

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Epstein-Barr Virus (EBV) commonly infects human at infancy; its infection is related to various diseases, including nasopharyngeal carcinoma, lymphoma and several autoimmune disorders.

However, neither EBV therapeutic nor prophylactic vaccine is available currently. The glycoprotein gp350 is the widely used EBV vaccine design target, but blocking gp350 has little effect on epithelial infection. On the basis of our studies, which identified crucial receptors for EBV infection of epithelial cells, neutralizing and protecting antibodies, we selected gB, gHgL and gp42 as prophylactic vaccine targets and EBNA1, LMP2 as therapeutic vaccine targets. We developed several vaccine platforms, including VSV-based, self-assemble nanoparticle and mRNA platform. Our vaccines effectively activated both cellular and humoral immunity in mice and showed promising results in suppressing tumor progression and improving

survival time in tumor bearing mice, or protecting humanized mice against EBV induce lymphoproliferative diseases.

W41-3：Telomerase and telomeres in HPV life cycle and carcinogenesis

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The E6 and E7 proteins of the high-risk HPVs are both required for the efficient immortalization of human keratinocytes. Previous studies have shown that E6 activates the hTERT promoter, stabilizes hTERT mRNA and binds to the hTERT protein, thereby facilitating cell immortalization. It is unlikely that the primary function of E6-induced telomerase activity in the viral life cycle is to immortalize cells, since transformed, tumorigenic cells are nonpermissive for HPV replication. One possibility is that E6-induced telomerase activity might mediate the conversion of keratinocytes into a stem-like phenotype such that it can facilitate HPV persistence (or latency) in squamous epithelium. Another additional possibility is that hTERT induction might participate in viral DNA replication. To test the latter possibility, we co-transfected hTERT constructs and HPV LCR reporters into human keratinocytes and found that wt hTERT, as well as telomerase-defective hTERT mutant (D868A) induced a 2-5 fold increase in HPV LCR activity. Mutagenic analyses with the HPV18 LCR demonstrated that the TATA box and AP1 sites (distal and proximal) were required for both basal activity and hTERT mediated induction of the HPV LCR. Interestingly, mutagenic analysis revealed that a small sequence (nt34-53) in the HPV18 LCR was critical for hTERT induction. These data suggest that hTERT regulates HPV LCR activity, thereby modulating expression of the early viral genes and potentially providing a mechanism for regulating the viral life cycle. We also showed that noncanonical functions of telomerase components (hTERT and TERC) play critical role in cell immortalization, transformation and tumorigenesis. The significance of gaining insight into the multifaceted roles that TERT and TERC play in cancer pathogenesis, as well as their involvement in the viral life cycle for designing effective anti-cancer therapy approaches.

W41-4： The long noncoding RNA lnc-FANCI-2 restricts RAS signaling and phosphorylation of Akt and Erk in HPV16-infected cervical cancer

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HPV-positive cervical cancer tissues in general exhibit wild-type p53, wild-type K-RAS, and no overexpression of RAS genes. We recently found high-risk HPV infections dysregulate the expression of 195 long noncoding RNAs (lnc-RNAs) including an increased expression of a long noncoding RNA, lnc-FANCI-2, which coincides with cervical lesion progression from CIN1, CIN2-3 to cervical cancer. Viral E7 of high-risk HPVs and host transcription factor YY1 are two major factors driving lnc-FANCI-2 expression in HPV-infected cells (PNAS 118(3): e2014195118, 2021). To explore possible roles of lnc-FANCI-2 in HPV-induced cervical carcinogenesis, we ablated the expression of *lnc-FANCI-2* in the HPV16-positive cervical cancer cell line, CaSki, by CRISPR-Cas9. Knock-out (KO) single-cell clones were characterized for their growth, expression and secretion of cellular soluble receptors, and gene expression profiles by RNA-seq analysis. The lnc-FANCI-2 KO cells expressed HPV16 oncogenes normally but displayed altered cell morphology. Proteomic profiling of cytosolic and secreted proteins from the parental and KO cells showed altered expression of a subset of cell surface and adhesionrelated proteins, including reduction of MCAM, PODXL2 and ECM1 and increased levels of ADAM8 and TIMP2. RNA-seq analyses revealed that relative to the parental cells, the KO cells exhibited significantly increased RAS signaling but decreased IFN pathways. In the KO cells, phosphorylated Akt and Erk1/2, two important RAS pathway effectors, were increased more than 3-fold, accompanied by an increase of IGFBP3, MCAM, VIM, and CCND2 (cyclin D2) and a decrease of RAC3. We further found that in CaSki cells lnc-FANCI-2 specifically interacts with 32 host proteins, including MAP4K4 of which knockdown led to decreased phosphorylation of Akt and Erk1/2. In conclusion, a key function of lnc-FANCI-2 is to regulate RAS signaling, thereby affecting cervical cancer outcomes.

W41-5:KSHV Infection of Mesenchymal Stem Cells Leads to Various Sarcomas

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The association between Kaposi's sarcoma-associated herpesvirus (KSHV) and Kaposi’s sarcoma (KS) is well established, but the process of KSHV-induced oncogenesis remains poorly understood. A fundamental question in KSHV oncogenesis is the origin of KS cells that are infected by KSHV and give rise to KS. Recent evidence suggests that KS may arise from KSHV-infected mesenchymal stem cells (MSCs) through mesenchymal-to-endothelial transition (MEndT). We investigated KSHV-mediated MEndT and discovered that KSHV infection of MSCs activates the expression of PROX1, a master regulator of lymphatic vessel development and endothelial differentiation. This finding revealed a distinctive bivalent epigenetic signature in the PROX1 gene of MSCs, comprising both the active marker H3K4me3 and the repressive marker H3K27me3, which poises gene expression for timely activation upon differentiation signals. KSHV infection resolves the bivalent chromatin state by decreasing H3K27me3 and increasing H3K4me3 to activate the PROX1 gene. Viral interleukin-6 (vIL-6) signaling increases H3K4me3, while the viral G protein-coupled receptor (vGPCR) and VEGF-A axis reduces

H3K27me3. Through this dual signaling process, KSHV activates PROX1 gene expression, initiating MEndT and rendering MSCs tumorigenic. The KSHV-initiated endothelial lineage differentiation is incomplete, leading to hybrid mesenchymal/endothelial (M/E) state cells characterized by the simultaneous expression of mesenchymal markers and endothelial markers. These hybrid M/E cells exhibit high tumorigenic properties in vitro and have the potential to form KS-like tumors when transplanted under the renal capsules of mice. These results suggest that KSHV-infected MSCs resemble KS cells, where proliferating KS spindle-shaped cells also exhibit the hybrid M/E state.
These findings raise the question of whether KSHV infection of MSCs could lead to other sarcomas beyond KS, given that various human sarcomas are known to originate from MSCs. This notion prompted us to explore different sarcomas potentially associated with viral infection, leading to the identification of a connection between KSHV and osteosarcoma, a cancer of mesenchymal origin. (i) Analysis of a group of osteosarcoma patients from the Xinjiang Uyghur autonomous region revealed a significantly elevated prevalence of KSHV in Uyghur osteosarcoma patients compared to the general Uyghur population, indicating KSHV infection as a potential risk factor for osteosarcoma. (ii) The KSHV genome and viral latent nuclear antigen LANA were detected in most osteosarcoma cells. (iii) Gene expression profiling analysis unveiled KSHV-positive osteosarcoma as a distinct subtype, featuring viral gene-activated signaling pathways crucial for osteosarcoma development. (iv) In a xenograft mouse model with mock- and KSHV-infected MSCs injected near the femur of BALB/c nude mice (n=15 each group), tumors were observed in 9 mice from the KSHV-MSC group, contrasting with none in the mock-infected MSC group. Tumors grew on the bone surface, and induce the characteristic "Codman triangle" periosteal reaction. Histological analyses illustrated features characteristic of high-grade sarcoma, and the tumor cells were positive for STAB2, MDM2, and LANA, distingushing osteosarcoma from other sarcomas. Overall, our studies on KS and OS have opened an avenue to fully understand the oncogenic role for mesenchymal cell infection by viruses in human sarcomas.NCI-2 is to regulate RAS signaling, thereby affecting cervical cancer outcomes.

W41-6: Viral pseudo enzymes: from immune regulation to metabolic reprogramming

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Herpesviruses are ubiquitous opportunistic pathogens in human and provide excellent models to investigate fundamental biological processes. My laboratory is interested in the virus-host interaction underpinning viral immune evasion. To understand herpesvirus evasion of innate immune response, we stumbled on the discovery that herpesviruses deploy protein deamidation to regulate key innate immune components. Specifically, viral pseudo enzymes recruit cellular glutamine amidotransferases to deamidate these proteins, such as pattern recognition receptors and transcription factors. Glutamine amidotransferases are *bona fide* metabolic enzymes that catalyze the synthesis of nucleotides, amino acids, glycoproteins, and an enzyme cofactor (NAD+). The gammaherpesvirus genomes encode up to three pseudo enzymes of the phosphoribosylformylglycinamide synthetase (PFAS) that catalyzes the fourth step of *de novo* purine synthesis. We previously reported that one of the three murine gamma

herpesvirus 68 pseudo enzyme of glutamine amidotransferase (thus named vGAT) encoded by ORF75c recruits PFAS to deamidate and hijack RIG-I to suppress inflammatory response. Subsequently, we identified and characterized herpes simplex virus UL37 that directly deamidates RIG-I and cGAS to prevent innate immune activation, defining a *bona fide* viral deamidase. These results collectively support the conclusion that viruses exploit protein deamidation to evade immune response.
To probe the roles of protein deamidation mediated by glutamine amidotransferases, we performed functional screen using NF- B and IFN reporters with knockdown of individual glutamine amidotransferase. We discovered that carbamoyl phosphate synthetase, aspartate transcarbamoylase and dihydroorotase (CAD) deamidates RelA to shunt RelA from mediating an inflammatory response to aerobic glycolysis, whereas CTP synthetase 1 deamidates IRF3 to negate IFN induction. In *de novo* pyrimidine synthesis pathway, CAD and CTPS1 catalyze the first three steps and a downstream UTP to CTP conversion, respectively, both of which are rate-limiting.
Importantly, cancer cells and viruses evolved mechanisms to hijack protein deamidation to evade innate immune response. Some examples will be discussed. Ongoing experiments are directed to interrogate the roles of viral pseudo enzymes in nucleotide synthesis particularly and metabolism in general. Our findings define new fundamental roles of protein deamidation in communicating between immune response and cellular metabolism.

Workshop 42: Molecular Mechanisms of Aging and Cellular Senescence (Room 2C)

Chairs: Yi Zhu,Weiwei Dang

W42-1：Cellular Senescence: a key therapeutic target in aging and diseases

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Cellular senescence, a hallmark of aging, involves the stable exit from the cell cycle in response to cellular damage and stress. Senescent cells (SnCs) often develop a pathogenic senescence-associated secretory phenotype (SASP) that promotes secondary senescence and disrupts tissue homeostasis, impairing tissue repair and regeneration. Transgenic mouse models with the capability for genetic ablation of SnCs have demonstrated their crucial role in driving aging and related diseases. Consequently, senotherapeutics have been developed to either pharmacologically eliminate SnCs (senolytics) or suppress the SASP and other senescence markers (senomorphics). Extensive preclinical studies and initial clinical trials have shown the potential benefits of senotherapeutics, leading to multiple ongoing clinical trials. In conclusion, targeting cellular senescence presents a promising therapeutic strategy for slowing aging and treating age-related diseases.

W42-2：Cytochrome c mediated tissue-specific regulation of super longevity in *C. elegans*

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The highly conserved insulin/IGF-1 signaling (IIS) and target of rapamycin (TOR) pathway play an important role in aging across species. Previously, we have demonstrated that simultaneous inhibition of IIS and TOR pathways via double mutations in DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase/S6K) lead to a nearly 5-fold, synergistic lifespan extension in *C. elegans*. Functional translatome studies reveal that the *daf-2 rsks-1* mutant has reduced expression of CYC-2.1 (cytochrome C) in the germline, which cell-non-autonomously activates the mitochondrial unfolded protein response (UPR^{mt}) and Adenosine monophosphate-activated protein kinase (AMPK) in the metabolic tissue to ensure the super longevity phenotype. Further transcriptomic studies demonstrate that the epidermal tissue also plays an important role in the *cyc-2.1* deficiency-induced lifespan and healthspan extension. The underlying mechanisms involve a positive feedback regulation between the ATFS-1 transcription factor from the UPR^{mt} pathway and CHE-14 (Dispatched) from the Hedgehog pathway. Taken together, these findings highlight the importance of tissue-specific modulation of aging via highly conserved factors.

W42-3: Discovering somatic mutations in single cells during aging

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Single-cell sequencing for analyzing DNA mutations across the genome in somatic tissues is critically important for studying development, cancer, and aging. However, current procedures are prone to artifacts and a reliable protocol for single-cell somatic mutation analysis remains to be developed. With our newly developed methods in single-cell whole-genome sequencing, we analyzed multiple types of primary cells from humans varying in age and smoking status. The results strongly suggest that spontaneous somatic mutations accumulating with age reach high enough levels to contribute to age-related functional decline, such as the well-documented cell-intrinsic changes. Taken together, our single-cell sequencing method provides a firm foundation for analyzing cellular genetic heterogeneity in normal human tissues.

W42-4: Discovery of Bcl-xL targeting senolytics for clinical translation

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The accumulation of senescent cells has been implicated in a host of age-related diseases, including cancer. Consequently, selective elimination of senescent cells using small molecules known as senolytics has emerged as a promising “anti-aging” strategy, with the potential to prevent and treat various age-related diseases and

extend healthspan. The anti-apoptotic protein Bcl-xL is crucial for the survival of many types of senescent cells. ABT-263, a selective Bcl-2 and Bcl-xL inhibitor, is one of the most potent and broad-spectrum repurposed senolytics. However, inhibiting Bcl-xL presents challenges due to severe thrombocytopenia, as platelets rely on Bcl-xL for their viability. To address this issue, we combined the proteolysis targeting chimera (PROTAC) technology with an innovative concept involving E3 ligase-based tissue-selective induction of target protein degradation. This novel approach enabled us to overcome the dose-limiting thrombocytopenia associated with Bcl-xL inhibition. Over the years, this strategy has led to the discovery of three generations of Bcl-xL-targeting PROTAC senolytics, exhibiting unprecendented potency and selectivity. These advancements hold significant potential for numerous applications in treating age-related diseases.

W42-5: Deciphering Cellular Aging: Insights from the Aging Fly Cell Atlas

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Aging phenotypes have been observed and described for centuries and a number of different aging hypotheses have been proposed. However, several critical questions remain largely unaddressed in complex organisms. For example, do different cell types age at different rates? If yes, which cell types age faster? Here I will present our recent work on the Aging Fly Cell Atlas, the first single-nucleus transcriptomic map of the whole aging Drosophila. We characterize 163 distinct cell types and perform an in-depth analysis of changes in tissue cell composition, gene expression, and cell identities. We further develop aging clock models to predict the fly age. This study provides a fresh insight in organism-level aging rate at cellular resolution. In addition, I will talk about our recent unpublished work on the Alzheimer's Disease Fly Cell Atlas (AD-FCA). We performed whole organism snRNA-seq on two Alzheimer's Disease (AD) fly models and revealed surprising peripheral changes caused by AD.

W42-6: Epigenetic alterations drive stem cell aging

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Functional decline and dysregulation of stem cells contribute to tissue and organismal aging. Significant changes in transcriptome during aging, from altered gene expression to aberrant transcription, have been observed in various tissues and cell types, even at the single-cell level. These changes are linked to decline in stem cell function and loss of stem cell identity. However, the molecular mechanisms that underlie these changes remain poorly understood. In this meeting, I will discuss profound chromatin changes we discovered during mammalian aging and describe how these chromatin changes drive transcriptomic changes observed in aged stem cells and tissues.

Workshop 43: Development and Regeneration (Room 2D)

Chairs: Yi Arial Zeng, C.C.Hui

W43-1:Molecular annotation of cell lineage in embryo development and tissue injury by spatial multiomics

Fuqing Jiang[1], Zhuxia Li[1], Fangfang Qu[2], Guizhong Cui[2] and Guangdun Peng[1]
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Single-cell sequencing has emerged as a transformative tool in the realm of biomedical research, providing unprecedented and unparalleled insights that have shed new light on the intricacies of biological systems. Despite the significant advancements that have been made thus far, current single-cell sequencing methods still face certain limitations due to their lack of spatial information, which is a critical component in understanding the complex interactions that occur within tissues. The innovative field of spatial omics aims to capture the natural cell state in tissues, identify location-specific cell types, and unravel the complex cellular interactions that occur within their microenvironment, thereby enabling researchers to gain a deeper understanding of the intricate relationships between cells and their surroundings. By integrating spatial transcriptomics with single-cell data, researchers can explore cellular diversity across various levels, uncover novel molecular connections between the genome and cellular functions, and gain a more comprehensive understanding of the complex biological processes that underlie human health and disease. This integrated approach promises to yield innovative discoveries by shedding light on tissue organization, particularly during the complex and dynamic processes of embryonic development and tissue injury, when the delicate interplay between cellular components is crucial for the formation of functional tissues and organs.

W43-2：Mapping molecular and cellular interaction with proximity labeling

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Understanding the intricate network of molecular and cellular interactions is essential for unveiling the underlying mechanisms of physiology and pathology. In recent years, proximity labeling (PL) has emerged as a key technology for mapping specific subcellular and molecular neighborhoods in biological systems, offering unprecedented spatial and temporal resolution. In my talk, I will provide an overview of the principle of PL, which involves using enzyme-fused proteins of interest that, upon activation, catalyze the attachment of biotin to spatially proximal molecules. This tagging allows for subsequent enrichment and unbiased identification, thereby providing a snapshot of the molecular neighborhood of interest. I will then showcase examples of how the PL toolbox is adapted and applied, in combination with other molecular tools, to play an instrumental role in uncovering novel interactions in neural development, tissue regeneration, and tumor biology. These examples underscore the technique's utility in reshaping our understanding of the dynamic changes in molecular and cellular interactions during various biological processes.

W43-3： Wnt signaling regulates hepatocyte cell division by a transcriptional repressor cascade

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Cell proliferation is tightly controlled by inhibitors that block cell cycle progression until growth signals relieve this inhibition, allowing cells to divide. In several tissues, including the liver, cell proliferation is inhibited at mitosis by the transcriptional repressors E2F7 and E2F8, leading to formation of polyploid cells. Whether growth factors promote mitosis and cell cycle progression by relieving the E2F7/E2F8-mediated inhibition is unknown. We report here on a mechanism of cell division control in the postnatal liver, in which Wnt/β-catenin signaling maintains active hepatocyte cell division through Tbx3, a Wnt target gene. The TBX3 protein directly represses transcription of E2f7 and E2f8, thereby promoting mitosis. This cascade of sequential transcriptional repressors, initiated by Wnt signals, provides a paradigm for exploring how commonly active developmental signals impact cell cycle completion.

W43-4： Generation of Dorsal Spinal GABAergic Neurons from Human Urine Cells by Direct Reprogramming for treating Central Neuropathic Pain

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Central neuropathic pain(CNP) is a debilitating condition following spinal cord injury (SCI), causing spontaneous hyperalgesia and allodynia, which significantly effects the quality of life for SCI patients. To date, there is no effective cure for CNP. A primary contributing factor to CNP is the loss of dorsal spinal GABAergic inhibitory tone in the injured spinal cord. Given the limited regenerative capacity of the adult spinal cord, stem cell-based therapy has emerged as a promising strategy to compensate for neuronal loss after SCI. While some studies have shown pain relief through grafting forebrain interneuron precursors derived from pluripotent stem cells(hPSCs) in SCI models, these studies have certain limitations that hinder their clinical applicability, including the risk of propagating tumor-prone from hPSCs, heterogeneity of grafted cells, and unmatched graft with recipient spinal tissue. Thus, generating a

homogenous population of dorsal spinal GABAergic neurons from patient somatic cells is crucial to enhance therapeutic efficacy.
To achieve lineage reprogramming, we have identified a combination of key transcription factors (TFs) for dorsal spinal GABAergic specification and demonstrated their sufficiency in directly converting hPSCs into dorsal spinal GABAergic neurons. the reprogrammed cells exhibited robust survival in the injury site, expressing mature GABAergic markers and establishing functional integration with the host in a relatively short period of time. This integration enables the production of GABA transmitters, leading to the effective resolution of CNP. Subsequently, we further successfully reprogrammed human urine cells into dorsal spinal neural progenitors using these TFs with the optimized culture conditions. Moving forward, we will further evaluate their therapeutic potential in the SCI model.

W43-5：Rebuilding the Pathways: Exploring Mechanisms of Axon Regeneration and Nerve Repair

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With the goal to identify novel factors regulating axon regeneration, we have conducted a genetic screen using a C. elegans sensory neuron injury model that resembles mammalian nerve injury at the molecular level. From the screen, we identified genes and pathways regulating axon regeneration. We have found that genes involved in the autophagy pathway are required for axon regrowth and observed injury-induced autophagy activation. We found that axon injury led to increased autophagic vesicles and the injury-induced autophagy activation was age-dependent and correlated with axon regrowth capacity. We further identified that injury triggers autophagy activation through DLK pathway and that autophagy limits Notch signaling to promotes axon regeneration.
Given the emerging evidence supporting the crosstalk between autophagy and ferroptosis, we sought to test whether ferroptosis pathway is involved in axon regeneration. Unexpectedly, we discovered that ferroptosis-inducing signals promoted axonal fusion and functional recovery after injury. Axonal fusion represents an efficient way to recover function after nerve injury, but how axonal fusion is induced and regulated remains largely unknown. We found that ferroptosis signaling-induced lipid peroxidation enhances injury-triggered phosphatidylserine (PS) exposure to promote axonal fusion through PS receptor (PSR-1) and EFF-1 fusogen. Axon injury induces PSR-1 condensate formation at the tip of injured axons and disruption of PSR-1 condensation inhibits axonal fusion, suggesting that PSR-1 condensates on axonal membrane to facilitate fusion. Extending these findings to mammalian nerve repair, we observed that loss of GPX4 promotes functional recovery after sciatic nerve injury. Our study reveals an evolutionarily conserved function of lipid peroxidation in promoting axonal fusion, providing novel insights for developing therapeutic strategies to treat nerve injury.

Workshop 44: Immune Cells in Tumor Microenvironment

(Room 2E)

Chairs: Zuquan Hu, Qifang Zhang

W44-1: Aberrant R-loop-mediated immune evasion, cellular communication, and metabolic reprogramming affect cancer progression

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R-loops are three-stranded nucleic acid structures formed by RNA:DNA hybrids and displaced single-stranded DNA that exists throughout the whole genomes. Dysregulation of R-loop homeostasis is closely related to various human diseases, including cancer. However, the causality of aberrant R-loops in tumor progression remains unclear. In our study, using single-cell RNA-sequencing datasets from lung adenocarcinoma (LUAD), we constructed an R-loop scoring model to characterize the R-loop state according to the identified R-loop regulators related to EGFR mutations, tissue origins, and TNM stage. We then evaluated the relationships of the R-loop score with the tumor microenvironment (TME) and treatment response. Results showed that malignant cells with low R-loop scores displayed glycolysis and epithelial-mesenchymal transition pathway activation and immune escape promotion, thereby hampering the antitumor therapeutic effects. Cell communication analysis suggested that low R-loop scores contributed to T cell exhaustion. We subsequently validated the prognostic value of R-loop scores by using bulk transcriptome datasets across 33 tumor types. The R-loop scoring model well predicted patients' therapeutic response to targeted therapy, chemotherapy, or immunotherapy in 32 independent cohorts. Remarkably, changes in R-loop distribution mediated by FANCI deficiency blocked the activity of Ras signaling pathway, suppressing tumor-cell proliferation and dissemination. These findings revealed the underlying molecular mechanism of metabolic reprogramming and T cell exhaustion under R-loop score patterns, and the changes in R-loops mediated by R-loop regulators resulting in tumor progression. Therefore, incorporating anticancer methods based on R-loop or R-loop regulators into the treatment schemes of precision medicine may be beneficial.

W44-2:YTHDF3 regulates the degradation and stability of m6A-enriched transcripts to facilitate the progression of castration-resistant prostate cancer

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Prostate cancer is characterized by epigenetic alterations such as DNA methylation, protein modifications and RNA methylation. RNA N6-methyladenosine (m6A) is very common RNA methylation, recognized and bound by m6A readers to determine RNA fates. YTHDF3 is one of major m6A readers. Our recent studies reveal that YTHDF3 is elevated in prostate cancer cells, and it promotes cell proliferation, migration and invasion of prostate cancer. Silencing YTHDF3 attenuated the growth and metastasis of prostate cancer cells. Additionally, we found that melatonin can compete with m6A to occupancy m6A-binding aromatic cage of YTHDF3，leading to inhibiting YTHFD3 and its target expressions as well as prostate cancer growth. These findings suggest that YTHDF3 is a promising target for prostate cancer therapy.

In my talk, I will discuss our recent findings on the role of YTHDF3 in prostate cancer progression and its mechanism.

W44-3: Role of MEN1-mediated alternative splicing in ferroptosis execution and lung tumorigenesis

Jin Bangming

Department of Physiology, School of Basic Medical Sciences, Guizhou Medical University, Guiyang, Guizhou, China Lung cancer is the most common cause of cancer-related death worldwide and is classified into non-small-cell lung cancer and smallcell lung cancer. The MEN1 gene, a tumor suppressor gene that encodes the protein menin, is a key genetic event in the occurrence of multiple endocrine neoplasia type 1 (MEN1). Because menin lacks motifs homologous to known proteins, identifying its biochemical function and tumor suppression activity is difficult. Although MEN1-mediated apoptosis, cell senescence, and cell-cycle arrest were widely all regarded as important mechanisms of depression of lung tumorigenesis and progression, evidences from my lab demonstrate that other functions of MEN1, such as DNA damage response regulation, are also crucial for tumor suppression. Interestingly, our recent study demonstrates that MEN1-mediated alternative RNA splicing reprogramming also play an important role for its tumor suppressor activity; this aspect of MEN1 function believed to may be associated with ferroptosis rather than apoptosis. Ferroptosis is a characteristic form of cell death triggered by excessive iron-dependent reactive oxygen species (ROS) and plays an important role in suppressing tumor development. MEN1 was found to facilitate lipid ROS generation and sensitizes lung cancer cells to ferroptosis by depressing alternative CD44 pre-mRNA splicing. Loss of Men1 profoundly accelerates the progression of Kras-mutant driven lung adenocarcinoma (LUAD), which is associated with the accumulation of CD44 variant isoforms found in a KrasG12D;Men1-specific deficient mouse model. Furthermore, CD44v6-interfering peptides effectively abrogate the growth and metastasis of established Kras-mutant LUAD and MEN1-deficient tumors by activating ferroptosis. Our findings verify that disruption of MEN1-CD44 variant axis attenuates the efficacy of lung cancer therapy via depressing ferroptosis, indicating that an intensive investigation of these variants may offer attractive targets and regimens for ferroptosis-related cancer therapy. In my talk, I will present our recent findings on the biological function and molecular mechanisms played by which MEN1 in regulating alternative splicing and ferroptosis in lung cancer.

W44-4: Immunological Evaluation of the Anti-Cancer Capability of Engineered Phage-Fusion Vaccine and Bispecific Antibodies

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Prostate cancer is one of the most common malignant tumors in men. Prostate-specific antigen (PSA) has a high tissue specificity, and there are a variety of epitopes that can trigger cytotoxic T-cell responses. The intrinsic immunogenicity of phages makes them an attractive candidate for nanovaccine carriers. In our study, passive immunotherapy was developed for improving the tummor microenvionment

(TEM) by inhibiting prostate cancer stem cell antigen (PSCA) and vascular endothelial growth factor (VEGF), while Fascin1 was chosen as a candidate vaccine target due to its overexpression in the surface of mature dendritic cells (DCs). Therefore, anti-PSCA, VEGF, and Fascin1 single-chain variable fragment (scFv) antibodies were respectively screened by antibody gene library and phage display technology. The fusion expression vector containing anti-PSCA and anti-VEGF scFv was constructed and the high affinity bispecific antibody (BsAb) was obtained. Simultaneously, the gene of the phage was modified by genetic engineering technology, and the -engineered phage (GE-phage) particles were prepared to display the fusion of anti-Fascin1 scFv and PSA65-73. A mouse model of prostate cancer was established to evaluate the synergistic effect of the passive and active combination immunotherapies. In result, the expression vector of the bispecific antibody was successfully constructed and soluble expression was achieved. The affinity constant KD for PSCA and VEGF antigen was 6.21×10-8 M and 6.72×10-8 M, respectively. More CD3+ and CD8+ T cells were detected in the tumor tissue of mice after the combination treatment, and there was no obvious side effect for organ damage. Thus, the BsAb with functional activity was obtained, and the GE-phage vaccine targeting mDCs was developed. Passive immunity interfering with the immunosuppressive TEM can enhance the therapeutic effect of the vaccine targeting DCs in vivo.

W44-5: CDC42 mediates dendritic cell mechanosensing during migration

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Dendritic cells (DCs), the major antigen-presenting cells bridging the innate and adaptive immune responses, have been hotspots for immunotherapies. Studies have shown that the migration of DCs, which are regulated by the stiffness of their surrounding tissues, strongly correlated with their clinical outcomes. However, the detailed molecular mechanisms by which DCs sense environmental stiffness alterations and accordingly adjust their migration strategies are still unknown. Here we found impaired DC migration capability on stiff substrates, which potentially underlay the increased DC infiltration in fibrotic rat livers. Through single-cell RNA-seq analysis of DCs from livers of cirrhotic patients and experimental validation, we discovered CDC42 played a crucial role in the mechanosensing pathway, which granted CDC42 as a smart target to mediate DC migration in vivo and would provide novel strategies for improving the clinical efficacies of DC-based anti-tumor immunotherapy.

W44-6: CD248-expressing cancer-associated fibroblasts regulating tumor microenvironment promotes NSCLC progression

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Lung cancer (LC) is highly prevalent among all cancers. Its prevalence and mortality rates rank second and first, respectively, among all malignant tumors. Non-small cell LC (NSCLC) constitutes ~80–85% of newly diagnosed LC incidences. Exploring the molecular mechanisms underlying NSCLC progression remains the focus of cancer research. As an essential tumor microenvironment (TME) component, cancer-associated fibroblasts (CAFs) can interact with other stromal or tumor cells and modulate immune evasion, drug resistance, and tumor angiogenesis. Because of fibroblast diversity, CAFs have enhanced phenotypic heterogeneity and distinctive functions per phenotype; consequently, the potential molecular mechanism of CAFs in NSCLC tumor metastasis remains poorly understood. CD248 (endosialin/tumor endothelial marker 1) is a type I transmembranal glycoprotein. The majority of tumor neovascular endothelial cells and CAFs express CD248 and not normal vascular endothelial cells. My lab has extensively studied how CD248+CAFs promotes NSCLC progression. In addition, our recent studies reveal that CD248+CAFs induce NSCLC progression by mediating M2-polarized macrophages. CD248 activates the NF-κBaxis, which, consecutively induces the CAFs-based secretion of IL-8, which promotes NSCLC cisplatin resistance. Meanwhile, we also reveal that CD248+CAFs activate the Hippo pathway, thereby inducing CTGF expression, which in turn facilitates the collagen I milieu of the stromal matrix, which promotes NSCLC metastasis. In my talk, I will discuss our recent findings on the mechanism of CD248+CAFs promotes NSCLC progression.

Workshop 45: Metabolic Dysfunction-Associated Steatotic Liver Disease (Room 2F)

Chairs: Yanqiao Zhang, Chaodong Wu

W45-1: STING and the Pathophysiology of MASLD

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As a mediator of innate immunity, stimulator of interferon genes (STING) functions to promote the proinflammatory activation of macrophages. This led to the validation of the relevance of STING expression in liver non-parenchymal cells (NPCs) to the pathogenesis of human metabolic dysfunction-associated steatotic liver diseases (MASLD). Similar findings from mice with diet-induced MASLD further indicated a detrimental role for STING-stimulated macrophages in promoting the pathogenesis and development of hepatic steatosis and inflammation. Given that excessive fat deposition in hepatocytes plays a causal role in initiating the parthenogenesis of MASLD, a role for how hepatocytes regulate STING expression or activation in liver NPCs as it relates to the pathophysiology of MASLD was investigated using mice upon hepatocyte-specific disruption or overexpression of adenosine kinase (ADK), an enzyme whose amount was increased in livers from human subjects with MASLD. Compared to wild-type control mice, hepatocyte-specific ADK-disrupted mice revealed significant decreases in the severities of high-fat diet (HFD)-induced hepatic steatosis and inflammation. In contrast, on a chow diet, hepatocyte-specific ADK-overexpressing mice revealed significantly increased severities of hepatic steatosis and inflammation, as well as adiposity and systemic insulin resistance. When liver lipid profile was analyzed, mice with hepatocyte-specific ADK overexpression revealed significantly increased hepatic levels of bis-monoacylglycerol phosphate and

lysophosphatidylcholine species and decreased hepatic levels of tetralinoleoyl cardiolipin, indicating mitochondrial stress. The latter was substantiated by increased liver amount of mitochondrial DNA (mtDNA), which was accompanied by increased expression of STING in liver NPCs. Collectively, these findings suggest that, while STING plays a role in promoting MASLD, excessive fat deposition in hepatocytes appears to initiate or exacerbate STING expression in liver NPCs in particular macrophages, thereby contributing to the development of liver inflammation. Accordingly, targeting ADK to alter STING is a viable approach to managing obesity-associated metabolic diseases including MASLD.

W45-2: The Role of Epsins in Regulating Liver Disease

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Rationale. Nonalcoholic steatohepatitis (NASH) is a chronic liver disease that can advance to cirrhosis that has no effective therapy. Epsins are an evolutionarily conserved protein family involved in clathrin-mediated endocytosis. We found that Epsin 1/2 expression in the liver are significantly up regulated by Western Diet (WD) induced NASH in mice as well as in liver biopsies from NASH patients.

Hypothesis. We hypothesize that expression of Epsin1 and Epsin2 in the liver are required for NASH progression.

Approach. We conditionally deleted liver Epsin1/2 in mice (Liver-DKO) and established a model of NASH by feeding both wild-type (WT) and Liver-DKO mice a WD+20% fructose for 52 weeks. We used single cell multiome analyses, combined with molecular approaches, to study molecular mechanisms. We also delivered Epsin1/2 siRNAs encapsulated within nanoparticles (NPs) targeting the liver by intravenous injection to treat diet-induced NASH in mice.

Results. We found WD-fed Liver-DKO mice have significant reductions in hepatic fibrosis and less lipid accumulation when compared to WD-fed WT mice. Mechanistically, we identified more inflammatory Kupffer cells, more active hepatic stellate cells, but fewer proliferating hepatocytes in WD-fed WT mice compared with WD-fed Liver-DKO mice. Therapeutically, we found significant resolution of hepatic fibrosis after Epsin1/2 siRNA NP treatment compared to mice injected with control siRNA NPs.

Conclusion. Liver-specific deletion of Epsin1/2 significantly represses NASH progression through suppression of inflammation to protect hepatocytes from severe chronic injury. Our therapeutic studies suggest specifically targeting Epsin1 and Epsin2 in the liver could be a promising therapeutic strategy for NASH treatment.

W45-3: Intergrated Organelle Stress Resposne in Obesity-associarted Fatty Liver Diease

Ling Yang[1*], Qingwen Qian[1], Mark Li[1], Zeyuan Zhang[1], Shannon Davis[2], Kamal Rahmouni[1], Andrew W. Norris[1], Huojun Cao[3], Wen-xing Ding[4], Gokhan Hotamisligil[5]
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The hepatic immuno-metabolic homeostasis is tightly controlled by hormones released from pituitary endocrine cells that rely on an intact endoplasmic reticulum (ER) function. It is recognized that aberrant pituitary hormone levels are positively correlated with prevalence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in rodents and humans. However, the molecular mechanisms underlying the defective pituitary endocrine output to the liver are incompletely resolved. We found that obesity, the major risk factor for NAFLD, is associated with intrinsic pituitary endocrine defects which is attributed to a blunted inositol-requiring enzyme 1 (IRE1)-mediated unfolded protein response (UPR) to restore ER homeostasis in the anterior pituitary cell population. Furthermore, IRE1 deletion in the anterior pituitary impairs pituitary hormone secretion, augments obesity-associated systemic metabolic abnormalities and promotes NASH progression. Conversely, these endocrine and metabolic defects were improved by restoration of spliced X-box binding protein 1 (sXBP1; an key adaptive UPR regulator) in the anterior pituitary. Intriguingly, disruption of the pituitary UPR resulted in impaired hepatic UPR, which was in part due to a defective thyroid hormone receptor (THR)-mediated activation of *Xbp1*. Functionally, activation of the hepatic THR signaling, by using a liver-specific THR agonist (MGL-3196), significantly improved hepatic steatosis, insulin resistance, and the disrupted hepatic ER homeostasis in obese mice with anterior pituitary-IRE1 deficiency. Together, our study provides the first insight into disruption of pituitary hormone-mediated inter-organ UPR communication promotes pathogenesis of NAFLD. Such knowledge is expected to uncover new targets for developing therapies for NAFLD.

W45-4: Adipocyte activating transcription factor 3 is a gatekeeper in preventing metabolic dysfunction-associated steatohepatitis by blocking fatty acid flux to hepatocytes

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The crosstalk between adipose tissue and the liver is finely controlled to maintain metabolic health. Yet, how adipose tissue controls toxic free fatty acid (FFA) overflow into the liver remains to be fully understood. Here we show that adipocyte activating transcription factor 3 (ATF3) was induced in obesity and by palmitic acid. Genetic inactivation of *Atf3* in adipocytes caused obesity, glucose intolerance, and non-alcoholic steatohepatitis (NASH) in chow diet-, high-fat diet (HFD)-fed, or high fat/high cholesterol (HFHC) diet-fed mice. Mechanistically, adipocyte ATF3 inhibited

lipolysis via adipose triglyceride lipase (ATGL), thus preventing hepatocytic lipogenesis and damage. Adipocyte ATF3 also inhibited adipogenesis and inflammation and induced thermogenesis, hence attenuating obesity and insulin resistance. Our data demonstrate that adipocyte ATF3 is a gatekeeper in counteracting the development of NASH under both physiological and pathological conditions.

W45-5: Novel Mechanism of Bile Acids in MASLD

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Metabolic disorders, such as type 2 diabetes mellitus (T2DM) and metabolic dysfunction-associated steatotic liver disease (MASLD), represent hugely problems concerning the health worldwide. The commensal gut microbiome is regarded as a "metabolic organ" for host, which not only takes part in intestinal nutrients absorption, but also regulates host metabolism by producing microbial metabolites like bile acids (BAs). Emerging evidence has indicated the dynamic alterations in BA profiles throughout T2DM and MASLD progression. Different BAs have divergent effects on regulating host metabolism. Disruption of bile acid signaling due to perturbation of the gut microbiota is closely associated with the pathogenesis and progression of metabolic disorders. It is well known that the prevalence of high calorie diet is the key factor contributing to the development of metabolic disorders. However, how fat and sucrose individually affect host metabolism, gut microbiota, and BA pool profiles are still larged unknown. By fecal microbiota transplantation and *ex vivo* culture, we found HFD had a more dramatic influence on composition and function of gut microbiota and a more severe effect on disrupting glucose homeostasis than HSD, accompanied by systemic reduced level of hyocholic acid (HCA) species. In addition, we also found hyodeoxycholic acid (HDCA), a microbial metabolite, showed a reduced level in MASLD patients. Dietary HDCA supplementation ameliorates diet-induced MASLD in mice in a PPARα dependent mannar. Mechanistically, HDCA hindered the formation of RAN/CRM1/PPARα export heterotrimer by direct binding with RAN protein, leading to the accumulation of nuclear PPARα and activated fatty acid oxidation. Our finding demonstrates that HDCA is a promising therapeutic agent for MASLD and provides a therapeutic strategy for MASLD by targeting the PPARα shuttling mechanism.

W45-6: A Genomic and Proteomic Study of Gynostemma pentaphyllum in Regulation of Lipid Metabolism and Liver Immunity

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Gynostemma pentaphyllum (Gp), a perennial herbaceous vine from the Cucurbitaceae family, is known for its significant therapeutic effects on lipid metabolism and liver immune regulation, primarily due to its active components, Gynostemma saponins (Gps). However, the underlying regulatory mechanisms remain unclear. This study investigates the effects of Gps on lipid metabolic disorders using a diet-induced hyperlipidemia mouse model, with a particular focus on the role of the bile acid synthesis and transport pathways and the involvement of the farnesoid X receptor (FXR). Additionally, the study explores the regulation of liver immunity-related genes

during Gps treatment, providing a foundation for understanding the lipid-lowering and liver immune-regulatory mechanisms of Gps.
Liver transcriptomics data reveal that Gps significantly regulates genome-wide expression, including bile acid systems and drug metabolism-related genes. Specifically, Gps upregulates the expression of Cyp7a1, Nr1h4, Fxr, Slco1a4, Cyp4a14, and Cyp4a31, while downregulating Nr0b2 (Shp) and Slc25a4. RT-PCR results show that Gps upregulates Cyp7a1, Cyp8b1, Fxr, Lrh1, Jnk2, and Erk1/2 gene expression, while downregulating Shp expression. CHIP-qPCR data indicate that FXR binding levels to the regions of Sr-Bi, Ostβ, and Bsep are significantly elevated.
Analysis of liver immune-related genes shows an overall upregulation trend, with 31 immune-related genes such as Il18, Ngf, Cd4, Vnn1, Ephx1, and Mtor significantly regulated by high-fat diet and Gps. Positive correlations are observed between LDL-C and TC, Il18bp and body weight, Cnpy3 and TC, H2-Q10 and LDL-C, Il18 and LDL-C, and Il18 and blood glucose. Additionally, H2-Eb1 and Cfp, Fpr1 and Cfp, Cd74 and H2-Eb1, H2-Aa and H2-Eb1 exhibit strong positive correlations, while Ikbkg and H2-Q10, Ikbkg and Irf5, and Prkar1a and Msrb1 show strong negative correlations. Some highly correlated genes are enriched in the same pathways.

Workshop 46: Circadian Rhythms and Sleep Research (Room 2G)

Chairs: Ying Xu , Eric Erquan Zhang

W46-1: SCN regulates metabolic flexibility and substrate utilization for thermogenesis via ADRB3-S100B

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The suprachiasmatic nucleus (SCN) is essential for synchronizing peripheral tissue functions with the environmental light-dark cycle. However, its role in coordinating whole-body energy homeostasis, especially in balancing energy and temperature regulation, is not well understood. In our study, we found that SCN-lesioned mice exhibit reduced total energy expenditure and a shift in metabolic flow in response to time-restricted feeding (TRF) combined with a subthermoneutral environment (STE). Despite this, they show enhanced thermogenic capacity and reduced aging in brown adipose tissue (BAT). Mechanistically, SCN lesioning maintains adrenergic β 3 receptor (Adrb3) activity and increases S100B secretion in BAT via sympathetic nervous system (SNS) activation. Overexpression and knockdown experiments revealed that S100B mimics the effects of SCN on BAT thermogenesis and aging. These findings underscore the critical role of an intact SCN in mediating TRF-STE-induced lipolysis and highlight the pivotal function of SCN-regulated S100B secretion in the anti-aging and thermogenic processes of brown adipocytes. Understanding these dynamics is crucial for developing safe and effective TRF protocols tailored to diverse populations and environmental conditions. The distinct energy regulation strategies observed in SCN-dysfunctional and SCN-intact mice indicate that the SCN’s role extends beyond circadian regulation to critical decisions regarding energy utilization and thermogenesis in response to environmental pressures.

W46-2: Mechanism of 24-h rhythmic interactions of circadian core oscillators in stress responses

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The circadian clock is a timekeeping mechanism synchronizing self-sustained physiological rhythms to the 24-h environmental cycles. We found the detached shoot and root possess 24-h rhythmic protein-protein interactions between clock core components, in which circadian periodicity exhibits a difference in organs. Compared to wild-type, the period length difference between shoot and root was not remarkable in prr7-3 and prr7-3 prr9-1 mutants. Further, the phase transition curve (PTC) indicated that shoot and root clock respond differently to the resetting cues of ambient temperature. PRR9 and PRR7 compensate circadian period between 22° C and 28° C in shoot, not in root. In addition, we found that a subfamily of zinc finger transcription factors, B-box (BBX)-containing proteins, have a critical role in fine-tuning circadian rhythm. Overexpressing Arabidopsis thaliana BBX19 and BBX18 significantly lengthened the circadian period, and the null mutation of BBX19 accelerated the clock pace. Moreover, BBX19 and BBX18 protein, which are expressed during the day, physically and dynamically interacted with PRR9, PRR7, and PRR5 in the nucleus in precise temporal ordering from dawn to dusk, consistent with the respective protein accumulation pattern of PRRs. Collectively, our findings demonstrate the circadian rhythmicity and tissue specificity of interactions between clock proteins, which determine the regulation of their target genes to perform physiological functions and stress responses at specific times of a day and specific organs.

Key words
Circadian clock, Temperature compensation, Dynamic PPI, PRRs, LCI

W46-3:Temporal orchestration of PSEUDO-RESPONSE REGULATORS confers the circadian clock responses to multiple environmental timing factors

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The five pseudo-response regulators expressed sequentially and overlappingly, PRR9, PRR7, PRR5, PRR3, and TOC1/PRR1, act as transcriptional repressors in the Arabidopsis circadian clock. Circadian rhythm defects in prr single, double, and triple mutants are much more diverse, but the underlying mechanism remains largely unknown. Here, by swapping promoters between PRRs and ChIP-qPCR assay, we found that the protein functions of PRR9, PRR5, and TOC1 are highly similar in determining circadian pace and direct binding to CCA1 promoter. Continuous high accumulation of PRRs for approximately 12-h is required to orchestrate an exact 24-h rhythm. The homodimers and heterodimers formed by PRRs with itself or with their respective neighboring PRRs exhibited diurnal and circadian rhythms, with the peaks of protein-protein interactions sequentially covering an approximately 12-h duration. Moreover, PR, EAR, and CCT functional domains were identified as necessary for the daily dynamic formation of PRR9 homodimers in vivo. The phase response curves

and the transcriptome analysis indicated that each PRR contributed differently to the sensitivity of the circadian clock to light, temperature, nitrogen, and iron (Fe) nutrition cues. Collectively, our data suggest the critical role of sequentially expressed multiple PRR proteins in fine-tuning the circadian pace and its environmental responses.

Key words
Circadian clock, PRRs, 24-h rhythms, Zeitgebers, Environmental responses

W46-4: Seasonal Regulation of Depression-Like Behaviors via Glucocorticoid Signaling

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Our brain adapts to seasonal changes. Mis-adaptations may lead to seasonal patterns in various psychiatric disorders, but we know little regarding the underlying mechanisms. Our previous work identified two variants in human circadian clock gene PERIOD3 (PER3-P415A/H417R) which are associated with winter depression, but whether and how these variants lead to the disorder remain to be characterized. Here we found that mice carrying human PER3-P415A/H417R display winter depression-like behaviors that are caused, surprisingly, by the actions of PER3-P415A/H417R in the adrenal gland. Systemic corticosterone level is down-regulated in adaptation to shortening of day length, while PER3-P415A/H417R eliminates this down-regulation by increasing corticosterone synthesis. The enhanced glucocorticoid signaling represses the transcription of Tryptophan hydroxylase 2 which encodes the rate-limiting enzyme of serotonin synthesis, leading to increased depression-like behaviors. Taken together, our findings unveil a mechanism by which glucocorticoid signaling adapts to seasonal changes of day length to modulate mood.

W46-5: PRMT3-mediated ADMA Dynamics Enhance Metabolic Benefits of Time-Restricted Fasting in Visceral Adipose Tissue

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Recent studies highlight the metabolic benefits of dietary restrictions, particularly time-restricted fasting (TRF), showcasing its potential for weight loss and optimising metabolic health. While TRF is known to affect adipose tissue, the precise cellular and molecular mechanisms remain largely undefined. Here, we demonstrate that protein arginine methylation dynamics, mediated by PRMT3, act as a metabolic sensor for fasting, specifically in visceral adipose tissue, fine-tuning metabolic flexibility. Proteins with asymmetric dimethylarginine (ADMA) increase after meals through insulin-pAKT signalling and decrease with prolonged fasting, notably accumulating in the visceral fat of obese individuals. Significantly, 16:8 TRF effectively restores PRMT3 and ADMA expression levels and responses to fasting. Similarly, inhibition of PRMT3 by SGC707 mimics these metabolic benefits, suggesting a role for glucose utilisation via inhibited mitochondrial citrate transport by SLC25A1, resulting in enhanced glycolysis in adipocytes during TRF. These findings reveal that the glycolytic shifts controlled by the PRMT3-SLC25A1 axis in adipocytes are crucial for enhanced metabolic outcomes during TRF and present a potential therapeutic target for obesity.

W46-6: Prolonged sleep deprivation induces a cytokine storm-like syndrome in mammals

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Sleep is required for most organisms in the animal kingdom, and loss of sleep can have serious patho-physiological consequences. Previous studies investing the function of sleep in mice have not been able to completely deprive the mice of sleep. Here we report a novel paradigm that consistently awakens the mice more than 95% of the time. After four days of this treatment, approximately 80% of mice die exhibiting severe inflammation. Mechanistically, sleep deprivation results in increased prostaglandin (PG) D2 in the brain and its efflux across the blood-brain-barrier (BBB) elevates levels of circulating neutrophils and induces cytokine storm-like symptoms. Thus, sleep deprivation results in pathological immune responses in the periphery that are triggered by efflux of a signaling molecule produced in the brain. Importantly, the circadian regulation on BBB efflux plays a crucial role in mediating sleep deprivation signals to the immune response, as evidenced by clock gene Per1 knockout soothes the phenotype. Together, our results indicate that sleep regulates communication between the central nervous system and the peripheral immune system, and PGD2 is the central molecule in mediating this crosstalk.

Workshop 47: Cancer Stem Cells: Drug Resistance and Cell Death

(Room 3A)

Chairs: Chuxia Deng, Kathy Qian Luo

W47-1: Strategies to identify and overcome cancer drug resistance

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Multidrug resistance (MDR) frequently occurs during cancer therapy and remains a major obstacle for the cure of most cancers. MDR could exist intrinsically or be acquired during or post drug treatment, yet factors that regulate the resistance remain elusive. Combining a whole whole-genome-wide RNA interference screening and an evolutionary drug pressure model with MDA-MB-231 cells, it is found that enhanced protein damage clearance and reduced mitochondrial respiratory activity are responsible for cisplatin resistance. Consistently, cisplatin and bortezomib enhance their therapeutic efficacy and alleviate side effects induced by drug combination treatment. Studying human patient-derived organoids for breast or colon cancers with many anticancer drugs indicates that the MDR can be overcome by co-treatment with proteasome inhibitors. Based on these findings, we have designed strategies to identify and overcome cancer drug resistance by targeting the proteasome.

Financial supports
This study was supported by research grants from the National Key Research and Development Program (File number: 2021YFE0206300), Natural Science Foundation of China (File number: NSFC 82030094) and the Science and Technology Development Fund (FDCT), Macau S.A. R, China (File number: FDCT-0092/2020/AMJ and FDCT-0009/2022/AKP)

W47-2: Novel mechanisms in regulating mitophagy and cell death in response to mitochondrial damage

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Mitophagy is a selective form of autophagy for removal or clearance of damaged mitochondria via the autophagy-lysosome pathway. Understanding the molecular mechanisms in control of mitophagy is important for development of novel interventional strategies against mitophagy-related diseseases such as neurodegeneration and cancer. Among various mitophagy regulatory mechanisms, PINK1, a protein kinase, and Parkin, an E3 ligase, are two critical players in control of mitophagy. In our recent studies, we aimed to investigate the upstream regulatory mechanisms of PINK1. I will present some of our recent unpublished data, from the transcriptional regulation of PINK1 to post-transcriptional regulation of PINK1, such as O-GlcNAcylation and the regulatory effect on mitophagy and cell death in response to acute mitochondrial damage. Our results thus provide a deeper insight into the molecular mechanisms in control of PINK1, the guardian of mitochondria and lay foundation for development of novel interventional strategies in PINK1 and mitophagy-related human diseases such as neurodegeneration and cancer.

W47-3: hESC-derived MSCs for clinical trials, developmental study, and cancer research

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Mesenchymal stem cells (MSCs) differentiated from human embryonic stem cells (hESC) (namely EMSCs) are advantageous to somatic tissue-derived MSCs for their unlimited source, stable quality, and remarkable efficacy in a variety of disease models. We have advanced EMSCs to a clinical trial on multiple sclerosis in U.S. and clinical studies on diabetic foot ulcer, graft-versus-host disease, and knee osteoarthritis in China. Recently, EMSCs were found to chimerize with the mouse blastocyst which allowed us to test the developmental potential of human MSCs in the mouse embryo. EMSCs remarkably contribute to cartilages, dermis and even the placenta. They also partially ameliorate skeletal defects caused by *Sox9* mutation. We also demonstrate that EMSCs can be used to study how mesenchymal stromal cells increase the NK resistance of circulatory tumor cells, which facilitates their metastasis.

W47-4: Determining how triple negative breast cancer cells gain malignancy after interacting with macrophages_

Kathy Qian Luo[1,2] and Meng Hao[1]
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[2]*Ministry of Education Frontiers Science Center for Precision Oncology, University of Macau, Taipa, Macao, China*

Triple-negative breast cancer (TNBC) has higher mortality than non-TNBC because of its stronger metastatic capacity. Increasing studies reported that TNBC tumors had more macrophage infiltration than non-TNBC tumors, which promoted the metastasis of TNBC cells. However, how TNBC cells become more malignant after interacting with macrophages is less reported. In this study, we observed that when TNBC cells were co-cultured with macrophages, they displayed higher viability and stronger metastatic ability than non-TNBC cells. Mechanistic studies revealed that TNBC cells acquired these abilities via interactions with macrophages in three phases. First, within 12 h of co-culture with macrophages, some TNBC cells have significantly elevated levels of reactive oxygen species (ROS), which upregulated interleukin 1α (IL1α) expression in ERK1/2-c-Jun- and NF-κB-dependent manners at 24 – 48 h. Second, the secreted IL1α bound to IL1R1 to activate the ERK1/2-ZEB1-VIM pathway which increased metastasis. Third, IL1α/IL1R1 facilitated its own synthesis and induced the expression of IL1β and IL8 at 72-96 h through the MKK4-JNK-c-Jun and NF-κB signaling pathways. Moreover, higher level of IL1α was positively correlate with more macrophage infiltration and shorter overall survival in breast cancer patients. Thus, reducing ROS elevation, downregulating IL1α expression or preventing the interaction between Ilα with its receptor IL1R1 can serve as new strategies to decrease metastasis of TNBC.

Financial supports
This study was supported by research grants from the Science and Technology Development Fund (FDCT), Macau S.A. R, China (File no. 068/2017/A2 and 0147/2020/A3), FDCT Key Project (file no. 0004/2021/AKP) and the Ministry of

Education Frontiers Science Centre for Precision Oncology, University of Macau (SP2021-00001-FSCPO and SP2023-00001-FSCPO).

W47-5: Antibody-mediate immune cell therapy against cancer

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In recent years, immune checkpoint therapy has emerged as a breakthrough strategy to reinvigorate anti-tumor immune responses. Targeting multiple non-small cell lung cancer antigens, we employed phage display antibody library screening techniques to identify several specific monoclonal antibodies. Utilizing yeast display combined with computer-assisted design, these antibodies were recombinantly engineered into high-affinity antibodies, mediating tumor cell killing through the mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC). Additionally, these antibodies were engineered into bispecific antibodies targeting tumor cells and human NK cell markers, capable of engaging NK cells to kill tumors. Furthermore, we constructed chimeric antigen receptor (CAR)-modified T cells targeting non-small cell lung cancer cells. Simultaneously, based on immune cell formulations, we developed nanoparticle drug delivery systems for targeted delivery and controlled release of therapeutics in various in vitro and in vivo models. These data will support further development of therapeutic agents of this class in preclinical and clinical research.

Workshop 48: Sensory Neurobiology (Room 3B)

Chairs: Xinzhong Dong, Qiufu Ma

W48-1: How do we sense the world? Lessons from worms to mice

Shawn Xu[1]
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The environment has a profound impact on animal behavior and physiology. The ability to sense environmental cues is essential for an animal's life. Dr. Xu's lab investigates how animals detect sensory cues — such as temperature, touch, light, sound, and chemicals — through different sensory receptors and channels and the influence these sensory stimuli have on behavior, and on genetic programs affecting health and longevity. To address these questions, Dr. Xu uses both *C. elegans* and mouse models. To survive and adapt to the ever-changing environment, *C. elegans* has evolved a rich repertoire of sensory systems and has emerged as a powerful genetic model to study sensory biology particularly to identify and characterize novel sensory receptors and channels. Because many sensory receptors and channels are evolutionarily conserved, Dr. Xu's lab also investigate their roles in somatosensation and pain in mammals using mouse models. To do so, Dr. Xu takes a multidisciplinary approach, combining molecular genetics, behavioral analysis, functional imaging and electrophysiology. In this presentation, Dr. Xu will discuss his recent work on dissecting how animals sense temperature through novel temperature sensors.

W48-2: Forcing PIEZO Channels to Open

Bailong Xiao[1]

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PIEZO1 and PIEZO2 have been identified by Patapoutian and colleagues as bona fide mechanoreceptors, which mediate the sense of gentle touch, proprioception, blood pressure, tactile pain and regulate the development and functions of cardiovascular, bone and brain. For the landmark discovery of PIEZO2 as touch receptor in mammals, Ardem Patapoutan has shared the 2021 Nobel Prize in Physiology or Medicine with David Julius, who discovered the first temperature receptor TRPV1. Combining cryo-EM structure determination, mutagenesis, electrophysiology, mouse genetics and pharmacology, we have aimed to systematically understand how PIEZOs function as mechanically activated cation channels to effectively convert piconewton-scale forces into selective cation permeation. In this talk, I will present our current understanding of PIEZOs with a particular focus on their unique structural designs, physical principles and gating dynamics that might enable them to serve as versatile and professional mechanosensors.

W48-3: Non-image-forming photoreceptor improves image perception

Tian Xue[1]

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There are mainly three types of photoreceptors in the mammalian retina: rods and cones and intrinsically photosensitive retinal ganglion cells (ipRGCs). It is generally believed that rods and cones mediate image-forming vision, while ipRGCs mediate non-image-forming vision. In recent years, our laboratory discovered the neurophysiological mechanisms of ipRGCs mediated light-at-night induced depression; cortical synaptogenesis promoted by light sensation during infanthood; and even light-regulated glucose metabolism. These works revealed that the non-image-forming vision functions are much more extensive and important than we generally understood.

However, beyond non-image-forming vision, we surprisingly found that ipRGCs' activation also improves orientation selectivity of mouse layer 2/3 neurons in the primary visual cortex (V1) by both increasing preferred-orientation response and narrowing tuning bandwidth. Activation of ipRGCs alone could excite both V1 excitatory pyramidal neurons and inhibitory interneurons. Mechanistically, we found that the tuning properties of V1 excitatory and inhibitory neurons are differentially influenced by ipRGCs' activation, which leads to a reshaping of excitatory/inhibitory balance that enhances visual cortical orientation selectivity. Furthermore, light activation of ipRGCs improves mouse orientation discrimination in visual behavior assay. Importantly, we found that specific activation of ipRGCs in human subjects by visual spectrum manipulation significantly enhances the discriminability of the visual orientation. Our study reveals the so-called "non-image-forming photoreceptor" working together with rods and cones to process the cortical visual features and facilitate image recognition.

W48-4: Neuroproteomics: toward single cell analysis

Junmin Peng[1]

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Dr. Peng has been developing high-throughput mass spectrometry (MS)-based proteomics and systems biology to tackle diverse biomedical challenges, including Alzheimer's disease. His major contributions include establishing target-decoy strategies for MS identification, profiling the ubiquitinated and synaptic proteomes, identifying complex ubiquitin codes, and discovering new proteins and pathways relevant to Alzheimer's disease. Beyond big data analysis, his group also develops functional experiments to validate derived hypotheses in cellular and animal models. Dr. Peng has authored more than 230 peer-reviewed papers and mentored over 90 group members.

In his forthcoming talk, he will present the advances in recent proteomic research, toward single cell resolution. In the case of Alzheimer's disease research, his proteomic studies not only validate amyloid and tau pathways, but also uncover novel components in broad protein networks. Meta-analysis of deep datasets reveals 2,698 differentially expressed (DE) proteins in the landscape of AD brain proteome (n = 12,017 proteins/genes). The hypothesized protective or detrimental roles of selected DE proteins will be discussed, emphasizing top proteins in "amyloidome" (all biomolecules in amyloid plaques) and disease progression. More recently, he has been collaborating with Dr. Xinzhong Dong to explore the application of the latest proteomics platform to the analysis of sensory neurons in the dorsal root ganglia, using laser capture microdissection and mass spectrometry.

W48-5: Neural mechanisms underlying pain-related sensory-motor interaction

Fei Wang[1,2,#], Zhi-Cheng Tian[3,#], Hui Ding[1,#], Xin-Jiang Yang[1], Fu-Dong Wang[1], Lei Xu[4], Zi-Xuan Cao[5], Rou-Gang Xie[1], Sheng-Xi Wu[1,*], **Ceng Luo[1,*]**

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Sensory-motor integration is crucial in the processing of chronic pain. Primary motor cortex (M1) is emerging as a promising target for chronic pain treatment. However, it remains elusive how nociceptive sensory inputs influence M1 activity, and how rectifying M1 defects in turn regulates pain processing at cellular and network level. We show that injury/inflammation leads to hypoactivity of $M1^{Glu}$ pyramidal neurons by excitation-inhibition imbalance between primary somatosensory cortex (S1)→M1. The impaired M1 output further weakens inputs to excitatory parvalbumin neurons of lateral hypothalamus (LH^{PV}) and impairs descending inhibitory system, and hence exacerbating spinal pain sensitivity. When rectifying M1 defects with rTMS, the

imbalance of S1-M1 microcircuitry can be effectively reversed, which aids to restore the ability of M1 to trigger descending inhibitory system, thereby alleviating pain hypersensitivity. Thus, a sensory-motor-sensory loop is identified for pain-related interactions between sensory and motor system and can be potentially exploited toward treating chronic pain.

W48-6: A neuroanatomical basis for electroacupuncture to modulate inflammation and pain

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Acupuncture at specific body regions can distantly modulate body physiology. Since the 1970s, researchers from Japan, Germany, and China have discovered that this long-distant acupuncture effect partially operates through somatosensory-autonomic reflexes. For example, we and others found that low-intensity electroacupuncture (EA) at limb-region acupoints, such as "Zusanli", could drive the vagal-adrenal reflexes and attenuate systemic inflammation induced by bacterial endotoxins. We then identified a group of sensory neurons necessary for EA to drive this anti-inflammatory axis. Based on the projections of these sensory nerves to tissues, we can predict effective and non-effective body regions. Most recently, we found that high-intensity EA is needed to attenuate post-surgery pain, likely via driving a different somatosensory-autonomic pathway. These findings offer neuroanatomical support for EA to modulate inflammation and pain.

Day 3, July 28 (Sunday)

Concurrent workshops 49-57, 3:15-4:45pm

Workshop 49: 3D Genome Architecture in Development and Diseases (Room 2A)

Chairs: Qiang Wu, Cheng Li

W49-1: CTCF Coordinates Cell Fate Specification via Orchestrating Regulatory Hubs with Pioneer Transcription Factors
Cheng Li, Peking University

W49-2: CRISPR-Based Tools for Studyingand Engineering the Three-Dimensional Genome
Haifeng Wang, Tsinghua University

W49-3: Study on HPV Integration and Pathogenesis of Cervical Cancer by Gene 3D Genomics
Guoliang Li, Huazhong Agricultural University

W49-4: Multi-Partite Chromatin Loops Converge Diverse Cis-Regulatory Elements to Co-Regulate and Fine-Tune Gene Expression
Chunhui Hou, Kunming Institute of Zoology, Chinese Academy of Sciences

W49-5: Exploring the Role of Higher Order Chromatin Structure in Regulating DNA Replication
Jiazhi Hu, Peking University

W49-6: 3D Architectural Protein ZNF143 in CTCF/Cohesin Geometry and TAD Boundary Formation
Qiang Wu, Shanghai Jiao Tong University

Workshop 50: Cellular and Molecular Regulation of the Immune System (Room 2B)

Chairs: Chenqi Xu, Li-Fan Lu

W50-1: Regulatory T cell differentiation and functions beyond immune suppression

Zhi Liu[1]
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Since the discovery of Foxp3 as the master regulator for regulatory T cells in 2003, significant progress has been made in understanding the transcriptional, posttranscriptional and epigenetical regulation of how Treg development, differentiation, and immune suppressive function over the last two decades. Moreover, growing evidence suggests Treg cells can also play a pivotal role in non-immunological processes, including tissue maintenance, repair and regeneration, challenging the traditional view of Treg cells as merely a specialized immune suppressive T cell subtype. However, the mechanism by which Foxp3 regulates Treg differentiation through three dimentional (3D) chromatin architecture reorganization, and how Foxp3 collaborates with its partners to control Treg's non-immune functions in non-lymphoid tissues, remain largely unexplored. In my talk, I will briefly introduce two recent studies that address these asepects. The first research explores how Foxp3 orchestrates reorganization of Treg's 3D chromatin architecture to establish its identity. The second study examines how glucocorticoid signaling and Foxp3 cooperate to initiate Treg and tissue stem cell crosstalk, leading to new hair growth upon skin injury. These studies will bring new insights into our understanding of Treg cell biology .

W50-2: Neutrophils: The Power of Many

Lai Guan Ng[1]
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Neutrophils are specialized cells of the early innate immune response. A long-standing question in the field of neutrophil research is whether a distinct subset of these cells truly exists, or different populations are merely a manifestation of the neutrophil maturation/polarization state. Lineage tracing techniques have been used to distinguish different subsets of myeloid cell types; however, more needs to be done with neutrophils. This talk will discuss how in-depth analysis of physiological and pathological granulopoiesis by multiomics and multiparametric technologies can contribute to better understanding neutrophil subsets and discover new functions, with a specific focus on tumor-associated neutrophils.

W50-3: Triggering mechanisms of T cell immunity

Chenqi Xu[1]
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T cells recognize non-self antigens and mediate adaptive immunity to eliminate pathogens and cancer cells. The triggering process of T cell immunity is tightly regulated by a panel of immunoreceptors, including antigen receptor TCR, co-stimulatory receptors and co-inhibitory receptors. How these immunoreceptors integrate complicated environmental cues to induce robust yet safe immune response remains elusive. At the membrane-proximal region, immunoreceptors form a sophisticated electrostatic network with effector proteins, lipids and ions to orchestrate T cell signaling. Introducing a TCR electrostatic element into Chimeric

Antigen Receptor (CAR) improves long-term persistence and antigen sensitivity of CAR-T cells, proving the rationale of charge-based design of next-generation immunotherapy.

W50-4: Bi-directional neuro-immune communication regulates host defense against helminth infection

Yinsheng Wang[1*], Xiaoyu Zhang[2*], Heping Xu[1#], **Coco Chu[2#]**
[1]School of Medicine, Westlake University, China;
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Emerging studies reveal that neuropeptides play critical role in regulating anti-helminth immune responses, hinting at the potential of intrinsic enteric neurons (iENs) in orchestrating intestinal immunity. However, whether and how the iENs getactivated during infection, and whether they engage in a bi-directional communication with the immune cells, remain poorly defined. In our new study, we show that iENs became activated in response to helminth infection, and optimal neuronal activation required participation of group 2 innate lymphoid cells (ILC2s). Single-nucleus RNA sequencing of the iENs revealed significant alterations in gene expression in intrinsic primary afferent neurons (IPANs) during infection. Moreover, we generated new genetic mouse models and revealed a previously unrecognized bi-directional neuro-immune crosstalk in the intestine.

W50-5: Serine enrichment in tumor promotes regulatory T cell accumulation through sphinganine

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$CD4^+$ regulatory T (T_{reg}) cells accumulate in the tumor microenvironment (TME) and cause immunosuppression. We measured 630 metabolites in the TME and found that substrates for the sphingolipid synthesis, serine and palmitic acid, were enriched. A serine-free diet or a deficiency in Sptlc2, the rate-limiting enzyme catalyzing sphingolipid synthesis, suppressed T_{reg} cell accumulation and inhibited tumor growth. Sphinganine, an intermediate metabolite in sphingolipid synthesis, physically interacted with the transcription factor c-Fos, enhanced the genome-wide recruitment of c-Fos to regions near the transcription start sites of target genes including *Pdcd1* (encoding PD-1), promoted *Pdcd1* transcription, and increased T_{reg} cell differentiation in a PD-1-dependent manner. Thus, Sptlc2-mediated sphingolipid synthesis translates the extracellular information of metabolite availability into nuclear signals for T_{reg} cell differentiation and limits anti-tumor immunity.

W50-6: Revitalizing exhausted T Cells with IL-10: a journey from lab discovery to clinical application for enhanced cancer immunotherapy

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Our immune system interacts with many diseases in a multidimensional manner involving substantial biological, chemical, and physical exchanges. Manipulating the disease-immunity interactions may afford novel immunotherapies to better treat diseases such as cancer. My lab aims to develop novel strategies to engineer the multidimensional immunity-disease interactions (or termed 'immunoengineering') to create safe and effective therapies against cancer. We leverage the power of metabolic and cellular bioengineering, synthetic chemistry and material engineering, and mechanical engineering to achieve controllable modulation of immune responses. In this talk, I will share our recent discovery of IL-10 as a metabolic reprogramming agent that reinvigorates the terminally exhausted CD8+ tumor infiltrating lymphocytes. This strategy has been extended to develop metabolically armored CAR-T cells with IL-10 secretion to counter exhaustion-associated dysfunction in the tumor microenvironment for enhanced anticancer immunity. This new CAR-T cell therapy, i.e. IL-10-secreting CAR-T, has shown promise in several on-going IIT clinical trials (ClinicalTrials.gov ID: NCT05715606, NCT05747157, NCT06120166) in the treatment of refractory/relapsed CD19+ B cell leukemia and lymphoma.

Workshop 51: Development, Disease and Aging (Room 2C)

Chairs: Danny Chan, Bo Gao

W51-1: Yap-mediated mechanotransduction in disease and development

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Identifying the cellular and molecular mechanisms underlying genetic diseases provides invaluable insights into normal functions of genes and signaling pathways. By understanding genetic forms of heterotopic ossification (HO), which is pathological bone formation in soft tissues, we identified a self-amplifying, self-propagating loop of Yes- associated protein (YAP)–Sonic hedgehog (SHH) as a core molecular mechanism underlying diverse forms of HO. This self-propagating positive feedback loop was both necessary and sufficient for HO expansion and could act independently of Gnas in fibrodysplasia ossificans progressiva (FOP), another genetic HO, and nonhereditary HO mouse models. Genetic or pharmacological inhibition of YAP or SHH abolished HO without affecting normal bone homeostasis, providing a previously unrecognized therapeutic rationale to prevent, reduce, and shrink HO. We then went on to show that in the developing neural tube (NT), Yap, a key mechanosensor and mechanotransducer, is both necessary and sufficient in biochemical signaling activation during formation of notochord and floor plate, the ventral signaling centers that pattern the dorsal-ventral axis of NT and the surrounding tissues. We showed that Yap activation by a gradient of mechanical stress and tissue stiffness in the notochord and ventral NT induces FoxA2 and Shh expression. Mechanotransduction via Yap activation acts in feedforward mechanisms to induce FoxA2 expression for notochord formation and activate Shh expression for floor plate induction by synergistically interacting with FoxA2.

W51-2: Suppression of CDON-associated apoptosis impairs phalangeal joint formation in the pathogenesis of brachydactyly type A1

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Apoptosis occurs in development when a separation of tissues is needed. Synovial joint formation is initiated at the presumptive site (interzone) within a cartilage anlagen, progresses with in cell differentiation follow by cavitation leading to tissue separation. Apoptosis has been detected in phalangeal joints formation, but its role and regulation have not been defined. Using a mouse model of brachydactyly type A1 (BDA1) with an Ihh^{E95K} mutation, we showed that a missing middle phalangeal bone is due to the failure of the developing joint to cavitate, associated with reduced apoptosis, and a joint is not formed. We identified an intricate relationship in the interzone between IHH and interacting partners, CDON and GAS1, that regulates apoptosis. We propose a model in which CDON/GAS1 may act as dependence receptors in this context. Normally, the IHH level is low at the centre of the interzone, enabling the "ligand-free" CDON/GAS1 to activate cell death for cavitation. In BDA1, there is a high concentration of IHH in the interzone suppressing apoptosis. Our findings provided new insights into the role of IHH and CDON in joint formation, with relevance to hedgehog signalling in developmental biology and diseases.

W51-3: The mechanisms of MTOC maturation in human and mouse oocytes

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The microtubule organization center (MTOC) of mammalian oocytes is essential for meiotic spindle assembly and for ensuring precise chromosome segregations. However, the detailed dynamic processes behind the organization of the MTOC and the underlying regulating mechanisms for the processes remain unclear. Here we explored the dynamics of MTOC maturation in human and mouse GV oocytes and found that MTOC maturation is a conserved process, consisting of two tightly coupled processes referred to as MTOC activation and migration. We found that CKAP5 and TACC3 play key roles in MTOC activation in oocytes. The activation of the MTOC is a prerequisite for migration initiation, and the migration of the MTOC is facilitated by dynein/dynactin in oocytes. Importantly, disruption of MTOC maturation plays a previously unrecognized role in the physiological aging of oocytes and in pathological female infertility characterized by abnormal oocyte maturation.

W51-4: Active transport mechanisms underlie motile cilia integrity

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Mucociliary clearance, the combined actions of beating cilia and mucus to remove pathogens and particulates from the mucosal barrier, represents the primary and foremost host defense mechanism. Studies from human genetics and model organisms on motile ciliopathies have led to the identification of gene modules important for cilia motility. A structural component present in most motile cilia essential for cilia motility is the central apparatus (CA), which contains the central pair microtubule singlets and is connected to the outer nine pairs of microtubule doublets through peripheral proteins. Mutations in CA components impair cilia beating and result in primary ciliary dyskinesia (PCD). While the organization of CA in purified cilia and

flagella have been well documented, the molecular events and pathways regulating CA biogenesis and coordinating cilia motility remain largely uncharacterized. In the talk, we demonstrate that the mammalian KIF27, a kinesin-4 family member and a motile cilia-specific kinesin, forms an evolutionarily conserved protein complex to promote the biogenesis of central pair apparatus of motile cilia by controlling the integrity and stability of the ciliary transition zone. Given the essential function of KIF27 in regulating motile cilia formation, our data suggest that KIF27 may be a candidate gene for human motile ciliopathies.

W51-5: Deciphering the genetic and molecular mechanisms of scoliosis

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Adolescent idiopathic scoliosis (AIS) is the most common form of spinal deformity, affecting ~2% (>20 million) adolescents worldwide, but it lacks a defined theory of etiopathogenesis. Without timely intervention and treatment, AIS can lead to pain, cosmetic disfigurement, functional distress, cardiopulmonary compromise, and disability. Treatment of AIS is limited to bracing and/or invasive surgery after spinal deformity becomes apparent. Preonset diagnosis or preventive treatment remains unavailable. We performed a genetic analysis of a large multicenter AIS cohort (>1700 patients) and identified many disease-causing and predisposing variants of *SLC6A9* in multigeneration families, trios, and sporadic patients. *SLC6A9,* encoding glycine transporter 1 (GLYT1), plays a crucial role in regulating synaptic glycine levels. Glycine is a major inhibitory neurotransmitter in the spinal cord. The identified *SLC6A9* variants reduced the glycine-uptake activity, leading to increased extracellular glycine levels and aberrant glycinergic neurotransmission. *Slc6a9* mutant zebrafish exhibited discoordination of spinal neural activities and pronounced lateral spinal curvature, a phenotype resembling human patients. The penetrance and severity of curvature were sensitive to the dosage of functional glyt1. Administration of a glycine receptor antagonist or a clinically used glycine neutralizer (sodium benzoate) partially rescued the phenotype. Our results indicate a neuropathic origin for "idiopathic" scoliosis, involving the dysfunction of synaptic neurotransmission and central pattern generators (CPGs). CPGs are neural circuits in the spinal cord and are responsible for generating rhythmic and coordinated movements. Defect in CPGs is potentially a common cause of AIS. Our work further suggests avenues for early diagnosis and intervention of AIS in preadolescents.

W51-6: Engineering longevity – computationally guided reprogramming of cell aging

Nan Hao, University of California San Diego

Workshop 52: Antiviral New Development (Room 2D)

Chairs: Jun Wang, Shibo Jiang

W52-1: Development of Broad-spectrum Antiviral Drugs

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In the past twenty years, viral diseases have had a significant impact on human society. By constructing a multidimensional, multi-target, structurally diverse antiviral compounds library, some drug candidates were obtained. At the onset of COVID-19, based on the structure of SARS-CoV M^{pro}, **FB2001** was designed and synthesized, which exhibited broad-spectrum, potent antiviral activity. **FB2001** does not need co-administration with ritonavir and showed potent activity against various clinically mutant strains. Injectable and nebulized forms of **FB2001** are undergoing international multicenter phase II/III clinical trials, with related work published in *Science* (*Science*, **2020**, *368*: 1331-1335) as a cover paper. Additionally, some inhibitors also were designed to target various stages of the coronavirus life cycle. Dual-target inhibitors **14a** and **14b** targeting host cathepsin L and calpain-1 showed pan-coronavirus inhibitory activity and excellent anti-inflammatory activity *in vitro* and *in vivo*. An oral drug candidate **DC406068** was designed to simultaneously inhibit coronaviral M^{pro} and host proteases (cathepsin L and calpain-1), which also exhibited broad-spectrum antiviral activity against coronaviruses and the Ebola virus, and excellent anti-inflammatory activity *in vitro* and *in vivo*. Based on the structures of 3C/$3CL^{pro}$, **DC402209** and **DC402267** were obtained which exhibited potent activity against enteroviruses, noroviruses, and coronaviruses. N protein inhibitors **A43** and **DC056204** displayed potent anticoronaviral effects.

W52-2: Development of virus inactivator-based antivirals

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Currently, most of the antiviral drugs approved for clinical use are "passive defenders", rather than "active attackers". For example, a viral entry inhibitor must wait on the surface of the host cell to act when or after the virus binds to the receptor on the host cell. A viral replication inhibitor, on the other hand, must wait within the host cell to work after virus has entered the host cell for replication. Viral inactivators can actively bind and inactivate the cell-free virions before they attach to the host cells. A protein-based inactivator generally consists of two parts, a soluble virus' receptor (or a functional part of the recptor) and a viral fusion or entry inhibitor. For example, 2DLT, a protein-based HIV inactivator, consists of the first two domains of the HIV's receptor CD4 (D1D2) and an HIV fusion inhibitor T1144. 2DLT first, through its D1D2 region, recognize and interact with gp120 on the surface of HIV to trigger the conformational changes of gp120 and gp41, resulting in exposure of the NHR-trimer in gp41. 2DLT then, through its T1144 region, bind with the NHR-trimer in gp41, resulting in inactivation of the cell-free HIV particles. This report highlights recent advances in the research and development of protein-based HIV and coronavirus inactivators.

W52-3: A new generation Mpro inhibitor with potent activity against SARS-CoV-2 wide type and variants

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Emerging SARS-CoV-2 variants, particularly the Omicron variant and its sublineages, continually threaten the global public health. Small molecule antivirals are an effective treatment strategy to fight against the virus. However, the first-generation antivirals either show limited clinical efficacy and/or have some defects in pharmacokinetic (PK) properties. Moreover, with increased use of these drugs across the globe, they face great pressure of drug resistance. We herein present the discovery and characterization of a new generation antiviral drug candidate (SY110), which is a potent and selective inhibitor of SARS-CoV-2 main protease (M^{pro}). This compound displayed potent *in vitro* antiviral activity against not only the predominant SARS-CoV-2 Omicron sublineage BA.5, but also other highly pathogenic human coronaviruses including SARS-CoV-1 and MERS-CoV. In the Omicron-infected K18-hACE2 mouse model, oral treatment with SY110 significantly lowered the viral burdens in both turbinate and lung and alleviated the virus-induced pathology. Importantly, SY110 possesses favorable PK properties with high oral drug exposure and oral bioavailability, and also an outstanding safety profile. Furthermore, SY110 exhibited sensitivity to several drug-resistance M^{pro} mutations. Collectively, this investigation provides a promising new drug candidate against Omicron and other variants of SARS-CoV-2.

W52-4: Molecular mechanisms of SARS-CoV-2 resistance to nirmatrelvir and the countermeasures

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Since 2019, SARS-CoV-2 has evolved rapidly and gained resistance to multiple therapeutics targeting the virus. Whether SARS-CoV-2 will develop resistance to nirmatrelvir, an approved antiviral to treat COVID-19, is a concern. Here we combined biochemical and structural methods to demonstrate that mutations at the substrate binding pocket of the Mpro can allow SARS-CoV-2 to develop resistance to nirmatrelvir in two distinct ways. Mutations at the S1 and S4 subsites significantly decreased inhibitor binding, while mutations at the S2 and S4' subsites unexpectedly increased protease activity. Hence, we turn to development of host-directed antivirals, which may offer broad spectrum intervention as SARS-CoV-2 evolves rapidly. We identified compounds that show potent inhibition of host proteases involved in viral entry and determined their complex structures with their respective targets. Our findings offer application for broad spectrum treatment of viral pathogenic infections with similar entry pathways.

W52-5: Structural biology enabled rapid discovery of SARS-CoV-2 M^{pro} inhibitors with robust potency and anti-drug resistance profiles

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The ongoing global spread of SARS-CoV-2 and its variants underscores the urgent need for the development of effective broad-spectrum anti-drug resistance agents.[1-3] In our study, a structure biology-guided drug design strategy was employed. This approach integrated multi-site binding and covalent modification strategies to synthesize a series of 1,2,4-tri-substituted piperazine inhibitors targeting SARS-CoV-2 M^{pro}. Notably, **GC-14** (IC_{50} = 0.40 μM, EC_{50} = 1.0 μM) and **GD-9** (IC_{50} = 0.18 μM, EC_{50} = 2.27 μM) demonstrated significant anti-SARS-CoV-2 activity. Crystallography studies revealed that **GC-14** and **GD-9** bound to M^{pro} non-covalently and covalently, respectively. Through further modifications, we discovered that **GC-78-HCl** exhibited enhanced potency and antiviral efficacy (IC_{50} = 0.19 μM, EC_{50} = 0.40 μM), which demonstrated comparable potency in Vero E6 cells to nirmatrelvir (EC_{50} = 0.38 μM). Additionally, it displayed strong antiviral activities against various SARS-CoV-2 variants, HCoV-OC43 and HCoV-229E. In conclusion, these findings substantiate **GC-78-HCl** shows promise for further development.

Besides, we integrated modular reaction and structural biology to rapidly discover potent M^{pro} inhibitors. Using this approach, focused compound libraries were synthesized in 96-well plates utilizing click chemistry. Through direct biological

screening, **C5N17B** demonstrated sub-micromolar M^{pro} inhibitory potency (IC_{50} = 0.12 μM) and excellent antiviral activity in Calu-3 cells (EC_{50} = 78 nM, CC_{50} > 100 μM), which showed superior potency to nirmatrelvir (EC_{50} = 1.95 μM) and similar efficacy to ensitrelvir (EC_{50} = 0.11 μM). Importantly, **C5N17B** displayed high activities against several SARS-CoV-2 variants (EC_{50} = 0.13 - 0.26 μM) and HCoV-OC43. **C5N17B** retained its antiviral activity against nirmatrelvir-resistant strains (T21I/E166V and L50F/E166V). Futhermore, **C5N17B** displayed favorable pharmacokinetic properties. Structural biology studies revealed a unique non-covalent binding mode. In conclusion, this study presents a paradigm for rapid enzyme inhibitor identification and provides a potential drug candidate for clinical therapy.

W52-6: Design of a SARS-CoV-2 papain-like protease inhibitor with antiviral efficacy in a mouse model

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The emergence of SARS-CoV-2 variants and drug-resistant mutants calls for additional oral antivirals. The SARS-CoV-2 papain-like protease (PL^{pro}) is a promising but challenging drug target. PL^{pro} cleaves viral polyproteins and is also involved in antagonizing host immune response. In this study, we discovered a new drug binding site, $Val70^{Ub}$, and subsequently designed and synthesized a library of noncovalent PL^{pro} inhibitors that bind to the $Val70^{Ub}$ and the known BL2 groove pocket near the S4 subsite. Optimized leads inhibited PL^{pro} with inhibitory constant, K_i, values from 13.2 to 88.2 nM. The co-crystal structures of PL^{pro} with eight leads revealed their interaction modes. The *in vivo* lead **Jun12682** inhibited SARS-CoV-2 and its variants, including nirmatrelvir-resistant strains with EC_{50} from 0.44 to 2.02 μM. Oral treatment with **Jun12682** significantly improved survival and reduced lung viral loads and lesions in a SARS-CoV-2 infection mouse model, suggesting PL^{pro} inhibitors are promising oral SARS-CoV-2 antiviral candidates. Collectively, our study revealed **Jun12682** as the first drug-like PL^{pro} inhibitor with in vivo antiviral efficacy in a SARS-CoV-2 infection mouse model, further validating PL^{pro} as a viable antiviral drug target.

Workshop 53: RNA Modifications in Normal and Malignant Hematopoiesis (Room 2E)

Chairs: Jianjun Chen, Haojian Zhang

W53-1: Understanding and targeting RNA m^{6}A modification in acute myeloid leukemia

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Hematopoietic homeostasis is maintained by hematopoietic stem cells (HSCs), and it is tightly controlled at multiple levels to sustain the self-renewal capacity and differentiation potential of HSCs. Dysregulation of self-renewal and differentiation of HSCs leads to the development of hematologic diseases, including acute myeloid leukemia (AML). Thus, understanding the underlying mechanisms of HSC maintenance and the development of hematologic malignancies is one of the fundamental scientific endeavors in stem cell biology. N^6-methyladenosine (m^{6}A) is a common modification in mammalian mRNAs and plays important roles in various biological processes. In this study, we performed a comparative analysis of the dynamics of the RNA m^{6}A methylome of hematopoietic stem progenitor cells (HSPCs) and leukemia-initiating cells (LICs) in AML. We found that RNA m^{6}A modification regulates the transformation of long-term HSCs into short-term HSCs and determines the lineage commitment of HSCs. Interestingly, m^{6}A modification leads to reprogramming that promotes cellular transformation during AML development, and LIC-specific m^{6}A targets are recognized by different m^{6}A readers. Moreover, the very long chain fatty acid transporter ATP-binding cassette subfamily D member 2 (ABCD2) is a key factor that promotes AML development, and deletion of ABCD2 damages clonogenetic ability, inhibits proliferation, and promotes apoptosis of human leukemia cells. This study provides a comprehensive understanding of the role of m^{6}A in regulating cell state transition in normal hematopoiesis and leukemogenesis, and identifies ABCD2 as a key factor in AML development.

W53-2: The role of RNA-dependent CTCF chromatin boundary in normal and malignant hematopoiesis

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The role of CTCF in mammalian genome regulation is dependent on CTCF's ability to homodimerize with itself or heterodimerize with other proteins including cohesin complex. However, it remains unknown how CTCF boundary activities and CTCF-mediated TAD formation are regulated across the genome to participate in cell

type or disease-specific fashion. In light of the discoveries that RNA binding domain of CTCF is responsible for CTCF-mediated long-range interactions and *HOTTIP* lncRNA coordinates CTCF mediated TAD formation in AML cells, we explored the role of *HOTTIP* lncRNA in CTCF boundary function, TAD formation, gene regulation. We show that *HOTTIP* directly interacts and regulates a fraction of CTCF binding sites (CBSs) of key WNT/β-catenin target loci in the AML genome to define the WNT/ -catenin TADs and gene transcription in AML leukemogenesis. Mechanistically, *HOTTIP* lncRNA regulates the CTCF boundary and TAD structure of β-catenin and its target loci by binding to complementary sequences and form R-loop structure in CTCF TAD boundaries that drive leukemogenic transcription. Thus, *HOTTIP*-mediated R-loop formation reinforces CTCF chromatin boundary activity and TAD integrity to drive oncogene transcription and AML progression.

W53-3: RNA modifications in acute leukemia

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Acute leukemia, including acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), is an aggressive form of hematologic malignancy with unfavourable outcomes. Thus, there is a critical unmet medical need to identify promising novel therapeutic targets and thereby develop improved novel therapeutics. Leukemia stem cells (LSCs) are recognized as root cause of leukemia initiation, relapse, and drug resistance. Meanwhile, cancer metabolic reprogramming has been recognized as an emerging hallmark of cancers that plays a critical role in cancer development and drug resistance. Thus, it is essential to identify new therapeutic targets that play crucial roles in leukemogenesis, LSC self-renewal, drug resistance and leukemia metabolic reprograming, which may lead to the development of improved therapeutics for acute leukemia treatment. RNA modifications such as N^6-methyladenosine (m^6A) and 5-methylcytosine (m^5C), have been reported to regulate mRNA fate and gene expression at the post-transcriptional level. Dysregulation of RNA modifications leads to various types of cancers, including acute leukemia. Our recently studies have demonstrated that the m^6A regulators (including writers, erasers and readers) are frequently dysregulated in acute leukemia and play essential roles in acute leukemia development, maintenance, metabolism, drug resistance and immune evasion, as well as LSC self-renewal. Overall, our results highlight the functional importance of RNA modifications in acute leukemia and suggest that targeting dysregulated RNA modification machineries holds great potential for acute leukemia therapy.

W53-4: RNA Modification Reprograms Cell Metabolism in AML

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Acute myeloid leukemia (AML) is a common and aggressive cancer of the hematopoietic system, with a five-year survival rate of only around 30%. Reprogramming of cell metabolism is a key feature of AML. Notably, amino acids serve as primary sources of nitrogen and carbon donors and are required at high levels in AML cells, particularly in leukemic stem/initiation cells (LSCs/LICs). Therefore, studying the regulatory mechanisms of amino acids metabolism may provide insights for targeting this metabolic vulnerability for AML therapy. RNA epigenetics is closely linked to the dynamic regulation of amino acid metabolism. Our research group has reported that METTL3/METTL14-mediated mRNA N^6-methyladenosine (m^6A) modification plays a key role in reprogramming glutamine metabolism in AML cells and LSCs/LICs. One of the m^6A reader proteins, insulin like growth factor 2 mRNA binding protein 2 (IGF2BP2), recognizes m^6A modification in the mRNA transcripts of the glutamine transporter SLC1A5 (solute carrier family 1 member 5) and the glutamate pyruvate transaminase 2 (GPT2) genes to promote their mRNA stability, thereby enhancing glutamine uptake and metabolism to meet the extremely high energy demand of AML cells. Furthermore, we identified an IGF2BP2 inhibitor, namely CWI1-2, and showed that pharmacological inhibition of IGF2BP2 phenocopies knockout or knockdown of IGF2BP2 in suppressing AML cell survival and self-renewal maintenance of LSCs/LICs by interfering with the glutamine metabolism pathway. Our findings provide proof-of-concept evidence of targeting the m^6A reading process to block AML cell metabolism and inhibit leukemogenesis. In addition to m^6A, other RNA modifications are being studied to uncover their roles and underlying mechanisms in AML metabolism, in the hope of providing new therapeutic targets and approaches for AML therapy.

W53-5: Phase separation-competent FBL promotes early pre-rRNA processing and translation in acute myeloid leukemia

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RNA binding proteins (RBPs) are pivotal in acute myeloid leukemia (AML), a lethal disease. While specific phase separation (PS)-competent RBPs are recognized in AML, the impact of their condensate formation on AML leukemogenesis, and the therapeutic potential of PS inhibition are underexplored. In our *in vivo* CRISPR RBP screen, Fibrillarin (FBL) emerges as a crucial nucleolar protein regulating AML cell survival, primarily through its PS domains rather than methyltransferase or acetylation domains. These PS domains, with specific features, coordinately drive nucleoli formation and early processing of pre-rRNA (including efflux, cleavage, and methylation), eventually enhancing the translation of oncogenes like MYC. Targeting FBL's PS capability with CGX-635 leads to elimination of AML cells, suggesting an additional mechanism of action for CGX-635 that complements its established therapeutic effects. Our study underscores the potential of PS modulation of critical proteins as a possible therapeutic strategy for AML.

Workshop 54: Liver Metabolism, Cancer and Immunotherapy

(Room 2F)

Chairs: Aiming Yu, Shi-Mei Zhuang

W54-1:Identification of CD133$^+$ intercellsome for cell-cell communication in liver regeneration and cancer development

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CD133 (prominin 1) is widely viewed as a biomarker for stem/progenitor cells in various tissues and also for cancer stem cells (CSCs), although its function and mechanism are unclear. Further, the expression of this cell surface protien with five transmembrane domains is not restricted to the stem/progenitor cell populations in either healthy or tumor tissues. CD133-negative cancer cells were also shown to contribute to cancer initiation, progression and recurrence. Nonetheless, many reports have documented a significant correlation between CD133 expression and prognosis of cancer patients. One urgent issue is to elucidate CD133 function in cancer progression and drug resistance.

We used hepatocyte-specific Shp2 and Met knockout mouse lines, and dissected the molecular and cellular mechanisms that drive hepatocyte prolferation after partial hepatectomy or CCl_4 injury. We also examined the molecular mechanisms of cell prolferation in various cancer cells after pharmaceutical suppression of the Ras-Erk pathway.

Herein we found that under impaired RTK-Shp2-Ras-Erk signaling, heterogenous hepatocytes formed clusters that divide actively during liver regeneration. These tightly contacting hepatocytes were featured by upregulated CD133 while negative for other progenitor cell markers. The CD133 expression was transiently induced during the liver regeneration process and disappeared after completion, suggesting a physiological role rather than an oncogenic transformation. Pharmaceutical inhibition of proliferative signaling also induced CD133 expression in various normal and cancer cell lines, suggesting an inherent and common mechanism of stress response. Super-resolution and electron microscopy localized CD133 on intracellular vesicles that apparently migrated between cells, which we named “intercellsome”. Isolated CD133$^+$ intercellsomes were enriched with mRNAs rather than miRNAs. Single-cell RNA sequencing revealed lower intracellular diversity (entropy) of mitogenic mRNAs in Shp2-deficient cells, which may be remedied by intercellular mRNA exchanges between CD133$^+$ cells. CD133-deficient cells were more sensitive to proliferative signal inhibition.

We propose that the CD133$^+$ vesicles mediate direct cell-cell communications to compensate intracellular signal deficit, which represents a previously unrecognized stress-responsive mechanism. Activaing this intercellular exchange mechanim may

allow cells to proliferate under insufficient proliferative signaling, likely involved in development of anti-caner drug resistance.
Acknowledgement: This work was supported by NIH grant R01DK128320.

W54-2:Cellular and Metabolic Basis of the Role of CYP1B1 in Liver Fibrosis
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Activation of hepatic stellate cells (HSCs) is central to the pathogenesis of liver fibrosis. The cytochrome P450 1B1 (CYP1B1) was initially recognized as a xenobiotic enzyme that metabolizes xenobiotics. CYP1B1 is better known for its extrahepatic functions, whereas the role of CYP1B1 in the liver fibrosis has not been reported. The goal of this study is to test our hypothesis that CYP1B1 has a cell-type specific role in liver fibrosis. We hypothesize that CYP1B1 has a cell-type specific role in HSC activation and liver fibrosis. Specifically, we hypothesize that HSC and/or HEP ablation or pharmacological inhibition of Cyp1b1 inhibits HSC activation and liver fibrosis. Mechanistically, inhibition of Cyp1b1 attenuates HSC activation by suppressing the intestinal trehalase, leading to the accumulation of trehalose, a non-reducing disaccharide that inhibits HSC activation and liver fibrosis. Our study has uncovered a previously unrecognized endobiotic function of CYP1B1 in liver fibrosis mediated by the liver-intestine organ crosstalk and the trehalose metabolite. Pharmacological inhibition of Cyp1b1 and/or the use of trehalose represent novel strategies for the clinical management of liver fibrosis.

W54-3:The Metabolic Remodeling of Endothelial cells and Its Regulatory Role in Angiogenesis of Hepatocellular Carcinoma
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Hepatocellular Carcinoma (HCC) is a highly prevalent malignancy worldwide, characterized by active angiogenesis, high metastasis and mortality. The liver is the metabolic center of the human body. We aim to investigate the regulatory roles of liver-enriched metabolites, including fructose and acetate, on the metabolic remodeling of endothelial cells and HCC angiogenesis. We reveals that the metabolism pathways of both fructose and acetate are upregulated in the endothelial cells of HCC. And fructose and acetate promote HCC angiogenesis and tumor metastasis. Furthermore, fructose metabolism activates AMPK, enhances mitochondrial respiratory capacity, and generates more energy to promote the migration and proliferation of endothelial cells. On the other hand, acetate increases histone acetylation in endothelial cells, thereby upregulating the expression of pro-angiogenic genes and enhancing endothelial cell migration. These findings not only elucidate the relationship between metabolites in the microenvironment as well as metabolic remodeling of endothelial cells and HCC angiogenesis, but also suggest that limiting dietary intake of fructose and acetate, or interfering with their metabolic processes may be a potential strategy for anti-cancer treatment.

W54-4:Bioengineering novel RNAs to modulate HCC cell metabolism and immunity

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Carcinoma cells are reprogrammed and addicted to continuous supply and metabolism of nutrients to drive the synthesis of biologic molecules for proliferation and growth, as well as evade immune cell surveillance. While noncoding microRNAs (miRNA or miR) have been revealed to control posttranscriptional regulation of many genes underlying HCC cell metabolism and immunity, current research is limited to the use of chemo-engineered miRNA mimics bearing extensive degrees and various types of artificial modifications. We hypothesized that bioengineered RNA (BioRNA) molecules shall better recapitulate the structures and biological actions of natural RNAs as both are produced and folded in living cells. Our efforts have led to a novel RNA bioengineering technology to offer high-quality, unparalleled BioRNAs for research and development. We have demonstrated the mechanistic actions of several bioengineered miRNAs, such as miR-124-3p and let-7-5p isoforms, in the modulation of HCC cell metabolism and immunity through posttranscription gene regulation of respective nutrient transporters, metabolic enzymes, and/or immune checkpoint proteins. Functional differences between let-7 isoforms in the regulation of the same target gene are also revealed. These findings demonstrate the utility of BioRNAs for research and the important roles of miRNAs in cancer cell metabolism and immunotherapy.

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W54-5:Impairing the metabolism of antidepressant duloxetine aggravates its hepatoxicity

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Duloxetine (DLX), a dual serotonin and norepinephrine reuptake inhibitor used for the treatment of major depressive disorder, is primarily metabolized by CYP1A2, CYP2D6, and UGTs in humans. Cases of serious liver injury associated with DLX have been reported. It is well appreciated that drug metabolism play crucial roles in drug toxicities. We investigated the roles of DLX metabolism in its hepatotoxicity both *in vitro* and *in vivo*. Our findings indicate that both DLX and its major metabolite, 4-hydroxy-DLX, are toxic to primary human hepatocytes (PHH), while

the phase II metabolite, 4-hydroxy-DLX-β-D-glucuronide, is nontoxic. Specific inhibitors of CYP1A2, CYP2D6, or UGTs significantly enhance DLX toxicity to PHH. DLX is less toxic to CYP1A2- or CYP2D6-overexpressed HepG2 cells compared to their counterpart HepG2 cells transduced with empty vector. All the data indicate that imparing DLX metabolism increase DLX cytotoxicity. Our animal studies that co-administration of DLX (50 mg/kg) and CYP2D6 inhibitor propranolol in both wild-type and Cyp1a2-null mice led to liver injury. Additionally, DLX caused severe liver injury in Por-null mice. Quercetin, an inhibitor of UGTs, decreased the formation of 4-hydroxy-DLX-β-D-glucuronide, and liver toxicity was observed in mice co-treated with DLX and quercetin. However, yhe CYP1A2 inhibitor and Cyp1a2-null mice did not increase DLX hepatotoxicity, indicating that Cyp1a2 is not a major contributor to DLX detoxification at least in mice. In summary, our findings suggest that blocking the phase I or phase II metabolism of DLX exacerbates its hepatotoxicity. This information is valuable for improving the clinical safety of DLX by avoiding toxicity triggered by drug-drug interactions. Clinical studies are warranted to determine whether inhibiting DLX metabolism increases the risk of hepatotoxicity.

This work was supported by the NIDDK (R01DK121970) and NICHD (R61/33HD099995) to Dr. Feng Li.

Workshop 55: Basic Research on Clinical Diseases (Room 2G)

Chairs: Jishi Wang, Wenfeng Yu

W55-1:Human bone marrow mesenchymal stem cells transplanted into stroke brain sequester inflammatory responses via increasing Tregs and mitochondrial transfer

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Inflammatory responses after ischemia stroke produce various cell death cascades that lead to further brain damage and poor prognosis. Stem cell therapy for neurological disorders is associated with modest survival of grafted cells within the local brain tissue. Interestingly, our recent study reported that human bone marrow mesenchymal stromal cells (hBMSCs) grafted directly into the rat stroke brain migrated peripherally to the spleen. However, the effects of intracerebrally grafted hBMSC that migrated to the spleen on peripheral inflammatory responses remain unclear. The present study explored whether grafted hBMSCs abrogated peripheral inflammatory factors by increasing the proportion of regulatory T cells (Tregs) in spleen, thereby reducing both central and peripheral inflammatory responses. Additionally, we assessed whether hBMSCs transferred healthy mitochondria to damaged neurons in reducing inflammation-mediated cell death, altogether improving stroke prognosis.Our data revealed that surviving hBMSCs were detected in brain and spleen of hBMSC-grafted rats, but not in sham or PBS-infused stroke animals. In spleen and brain, the proportions of total CD4+ and CD8+ cells were significantly less, and the proportion of CD4+/CD25+/FoxP3+ Treg cells were significantly higher, among the isolated splenocytes derived from hBMSC-grafted rats compared to PBS-infused rats. This

brain-to-spleen migration of hBMSCs likely contributed to dampened central and peripheral inflammation as evidenced by reduced T cells and increased proportion of Tregs in the spleen. Immunomodulation of the splenic response, as well as mitochondrial repair, via targeted migration of hBMSCs may aid in brain repair, providing a platform to develop novel therapies for stroke or other CNS injuries associated with aberrant inflammation.

W55-2:Heme oxygenase-1 (HO-1) : a potential therapeutic target for hematological malignancies

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Heme oxygenase-1 (HO-1) is a key enzyme in antioxidant metabolism, which is activated in oxidative stress response and is closely related to cell proliferation and anti-apoptosis, and plays an indispensable role in the occurrence, development and drug resistance of various hematological malignancies. Our study suggests that HO-1 significantly enhances AKT phosphorylation through CXCL12/CXCR4 interactions and regulates autophagy to protect against CML chemotherapy damage. HO-1 decreased the expression of phospho-histone deacetylase-3 protein and activated histone acetylation of P27Kip1 promoter to enhance resistance of diffuse large B-cell lymphoma. HO-1 activated JNK/ C-Jun mediated chemotherapy resistance of AML tumor cells. HO-1 provoked the transcription and activation of EZH2 through pRb-E2F to promote the transformation of MDS into AML. PI3K/AKT pathway mediated overexpression of HO-1 in bone marrow mesenchymal stem cells, inducing insensitivity of B-ALL to vincristine. Both recurrence and aGVHD appear to be related to the relative expression of HO-1, which can be used as a predictor of recurrence after acute leukemia transplantation and a diagnostic factor of aGVHD. Our recent study showed that HO-1 specifically down-regulates the expression of the ligand CD48 of the NK-cell-activated receptor 2B4, reducing the cytotoxic effect of NK cells mediating AML immune escape, suggesting that high HO-1 expression may serve as a marker for the use of immune checkpoint inhibitors in AML. In addition, the high expression of HO-1 promoted the secretion of exosomes by AML cells, resulting in changes in the bone marrow microenvironment and bone marrow stromal cell phenotype conducive to tumor cell growth. These findings suggest that HO-1 is a promising therapeutic target for cancer. In my study, I will discuss the impact of HO-1 expression on the occurrence and development of various hematologic malignancies, drug resistance mechanisms, risk stratification of such diseases, immune regulation, and aGVHD, as well as potential new strategies for HO-1 as a new target to improve current treatment challenges.

W55-3:Molecular mechanisms of endocrine resistance and drug intervention

Yan Chen

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Tamoxifen (TAM) is a first-line therapeutic drug for oestrogen-receptor positive breast cancer; however, like other therapeutics, its clinical use is limited by acquired resistance, and the mechanisms of underlying tamoxifen-resistance remain unclear. We demonstrated that G-protein coupled oestrogen receptor (GPER) was involved in the upregulation of aerobic glycolysis via induction of hypoxia-inducible factor-1α (HIF-1α) expression and transcriptional activity in tamoxifen-resistant cells. Additionally, GPER stabilized HIF-1α through inhibiting its hydroxylation and ubiquitin-mediated degradation, which were associated with upregulation of C-terminal hydrolase-L1 (UCH-L1), downregulation of prolyl hydroxylase 2 (PHD2) and von Hippel-Lindau tumour suppressor protein (pVHL), induction of HIF-1α/UCH-L1 interaction, and suppression of HIF-1α/PHD2-pVHL association. The GPER/HIF-1α axis was functionally responsible for regulating tamoxifen sensitivity. Due to the HIF-1α activation, dysregulated mitochondrial dynamics were involved in TAM resistance by protecting against mitochondrial apoptosis, thus preventing the release of mitochondrial cytochrome c to the cytoplasm following TAM treatment. Furthermore, we found that baicalein suppressed the E2-mediated activation of two key regulatory pathways ERα and GPER. Thus, baicalein reduces the expression and transcriptional activity of HIF-1α. As a result, baicalein downregulated aerobic glycolysis, and interferes with HIF-1α inhibition of mitochondrial biosynthesis, which increased mitochondrial DNA content and mitochondrial numbers, restored the generation of reactive oxygen species in mitochondria, and thus enhanced the TAM-induced mitochondrial apoptotic pathway. Baicalein is a promising candidate to help overcome TAM resistance by sensitizing resistant cells to TAM.

W55-4:Roles of post-transcriptional and post-translational modifications involve in neuroinflammation in ischemia reperfusion injury

Wenfeng Yu

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Ischemia reperfusion (I/R) injury is a great challenge in clinical therapy of acute ischemic stroke (AIS). Neuroinflammation had been defined exerts crucial roles in regulating occurrence and development of I/R injury. Multiple RNAs and proteins undergo changes in stability or activity after being modified differently, thus participate in the regulation of I/R induced neuroinflammation. In our recently lab work, according to the sequencing data and online public databases, we unearthed a number of differentially expressed genes (DEGs) and confirmed that these DEGs are involved in the inflammatory response induced by I/R. The main proteins encoded by these genes are enzymes, including FTO, USP36, RNF8, these enzymes can be involved in regulating the stability of downstream target proteins through a variety of modifications. For example, FTO reduced the m6A modification of TRIM25 and COX2 thus against MCAO/R induced neuroinflammation; USP36 facilitated the SUMOylation of NLRP3 consequently contribute to activation of NLRP3 inflammasome, thereby aggravating neuroinflammation in I/R; RNF8 promoted the ubiquitination of HDAC2 then through regulating the Reelin/GSK3β axis alleviated I/R injury. In conclusion, exploring the types and regulatory mechanisms of these RNA or protein modifications can deep our understanding of biological processes of

neuroinflammation in I/R. The precise regulation of modifications also helps to the drug development and preparation, providing a means to treat AIS.

W55-5:Uric acid-lowering components of Caragana sinica root and the anti-hyperuricemia effect of its major constituent α-viniferin

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Overproduction of uric acid (UA) by xanthine oxidase (XO) and/or insufficient excretion of UA by renal transporters are two main factors responsible for hyperuricemia (HUA). Long-lasting HUA would probably cause gout. Clinically available urate-lowering agents are quite effective for the treatment of HUA and gout, however, more or less side effects or safety concerns limited their long-term use. Caragana sinica roots (CSR) are a folk herbal drug with good efficacy and high safety for the treatment of gout. Our investigation showed that the water or ethanol extract of CSR had a good safety profile and significantly decreased the serum UA level and protein expression of XOD in liver and markedly downregulated URAT1 expression in the kidney. Among the 14 isolated compounds showing XO inhibitory activity better than or comparable to that of the positive drug allopurinol, trimeric resveratrol stilbene (+)-α-viniferin was chosen for in vivo evaluation against HUA. The results showed that α-viniferin treatment significantly reduced serum UA levels and markedly mitigated HUA-induced kidney injury in the HUA mice. Besides, α-viniferin did not show any obvious toxicity in mice. Mechanism study showed that α-viniferin not only significantly inhibited the production of UA through down-regulation of XOD in HUA mice, but also markedly down-regulated the expressions of URAT1 and GLUT9 and up-regulated the expressions of ABCG2 and OAT1 to promote the excretion of UA. Transcriptome analysis shoed that α-viniferin could prevent HUA mice from renal damage by regulating the IL-17, chemokine, and PI3K-AKT signaling pathways. Collectively, α-viniferin was a promising anti-HUA agent with desirable safety profile.

W55-6:Revolutionizing Medical Imaging: Advanced Artificial Intelligence Techniques for Image Segmentation and Predictive Diagnostics

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The rapid advancement in artificial intelligence (AI) is reshaping the landscape of medical imaging, offering unprecedented tools for image segmentation and predictive diagnostics. As medical practice shifts towards precision medicine, the demand for accurate and detailed medical imaging analysis has grown significantly. In this presentation, we explore the integration of AI, machine learning, and image segmentation in enhancing the precision of medical diagnostics across various domains such as liver, heart, musculoskeletal disorders, and quantitative fat

assessment, etc.. Utilizing methodologies ranging from traditional statistical models like logistic and Cox regression to deep learning, we also delve into the potential clinical application of radiomics and radiobioinformatics. These technologies facilitate refined image segmentation, enabling precise delineation of pathological features which are critical for accurate diagnostics and tailored treatment strategies. Our research underscores the pivotal role of AI in not only augmenting the capabilities of radiologists but also in providing substantial clinical value, thereby significantly improving patient outcomes and addressing human health challenges comprehensively. This talk will highlight our latest findings and demonstrate how AI-driven tools can serve as vital assets in clinical settings for clinical decision-making, ultimately benefiting patient care through enhanced diagnostic accuracy and predictive insights.

Workshop 56: RNA and Cytosolic DNA in Genome Maintenance (Room 3A)

Chairs: Zefeng Wang, Xin Li

W56-1:A Conserved Set of Ultra-Stable mRNA in Mammalian Sperm

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Despite transcription ceasing weeks before sperm fertilize oocytes, sperm still deliver a variety of RNAs to the zygote. Existing knowledge of the sperm transcriptome, particularly regarding long RNAs, has been hindered by a lack of sensitive, high-throughput techniques capable of distinguishing intact RNAs from the fragmented RNAs prevalent in sperm. In this study, we employed a combination of single-molecule long-read sequencing and short-read sequencing to detect sperm intact RNAs (spiRNAs). We identified 3,440 spiRNA species in mice and 4,100 in humans. The spiRNA profile, consisting of both mRNAs and long non-coding RNAs, shows evolutionary conservation between mice and humans and is notably enriched in mRNAs encoding ribosomal proteins. Overall, our research delineates the landscape of intact long RNAs in sperm and sets the stage for further investigations into their roles in transgenerational inheritance and their mechanisms of degradation resistance.

W56-2:Therapeutic Potential of mRNA-based Interventions for Cardiac Rare Genetic Disease

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease characterized by irregular heart rhythms and dilated right ventricle. Here we identified a novel *DSC2* mutation in a family in China which caused manifestation of ARVC in several members at their mid-age. Decreased DSC2 expression was also observed in patients with non-inherited dilated cardiomyopathy as well as in mice

with pressure overload-induced heart failure. *Dsc2* loss-of-function mice developed phenotypes of right ventricular dilation and dysfunction which recapitulated the disease symptoms in human. Transcriptomic analysis revealed that Myl7 was the most downregulated gene in the right ventricles of DSC2 deficient mice and restoration of its expression by AAV rescued heart function. Delivery of *Dsc2* mRNA with or without lipid nanoparticle encapsulation into cardiomyocytes through direct cardiac injection or simulated coronary artery injection normalized heart size and function in DSC2 deficient mice. Therapeutic effects elicited by a single dose of mRNA in mouse lasted for two months before relapse.

W56-3:Boosting Mucosal Anti-Tumor Immune Responses for Treating Colorectal Cancer

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Messenger RNA (mRNA) cancer vaccines are a new class of immunotherapies that can activate the immune system to recognize and destroy cancer cells. However, their effectiveness in treating colorectal cancer located on the mucosal surface of the gut is limited due to the insufficient activation of mucosal immune response and inadequate infiltration of cytotoxic T cells into tumors. To address this issue, a new mRNA cancer vaccine is developed that can stimulate mucosal immune responses in the gut by co-delivering all-trans-retinoic acid (ATRA) and mRNA using lipid nanoparticle (LNP). The incorporation of ATRA has not only improved the mRNA transfection efficiency of LNP but also induced high expression of gut-homing receptors on vaccine-activated T cells. Additionally, the use of LNP improves the aqueous solubility of ATRA, eliminating the need for toxic solvents to administer ATRA. Upon intramuscular injections, ATRA-adjuvanted mRNA-LNP significantly increase the infiltration of antigen-specific, cytotoxic T cells in the lamina propria of the intestine, mesenteric lymph nodes, and orthotopic colorectal tumors, resulting in significantly improved tumor inhibition and prolonged animal survival compared to conventional mRNA-LNP without ATRA. Overall, this study provides a promising approach for improving the therapeutic efficacy of mRNA cancer vaccines against colorectal cancer.

W56-4:Application of a novel antisense oligonucleotide technology to treat neuromuscular diseases

Yimin Hua

founder of ASOcura Pharmaceuiticals

RNA targeted ASO technologies have potential to treat various diseases. However, the ASO efficacies in the present technologies are generally mediocre, limiting their therapeutic application. Therefore, new technologies with higher ASO efficiencies are in urgent need. Here, we developed a novel ASO-mediated exon skipping technology,

which has 10-fold efficiency compared to conventional ASO technologies based on studies in cultured cells and animal models. Several ASOs designed using this technology hold great promise for treatment of Duchenne muscular dystrophy and spinocerebellar ataxia.

W56-5:Rational design of microRNA-responsive switch for programmable translational control in mammalian cells

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Artificial RNA translation modulation usually relies on multiple components, such as RNA binding proteins (RBPs) or microRNAs (miRNAs) for off-switches and double-inverter cascades for on-switches. Recently, translational circular RNAs (circRNAs) were developed as promising alternatives for linear messenger RNAs (mRNAs). However, circRNAs still lack straightforward and programmable translation control strategies. Here, we rationally designed a programmable miRNA-responsive internal ribosome entry site (IRES) translation activation and repression (PROMITAR) platform capable of implementing miRNA-based translation upregulation and downregulation in a single RNA construct. Based on the PROMITAR platform, we constructed logic gates and cell-type classifier circRNAs and successfully identified desired mammalian cell types. We also demonstrated the potential therapeutic application of our platform for targeted cancer cell killing by encoding a cytotoxic protein in our engineered circRNAs. We expect our platform to expand the toolbox for RNA synthetic biology and provide an innovative approach for potential biomedical applications in the future.

W56-6:A novel L-RNA aptamer to regulate the pUG fold RNA-induced gene expression *in vivo*

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G-quadruplex (G4) is a guanine-rich secondary structure found in DNA and RNA involved in various biological roles. Recently, a non-canonical RNA G-quadruplex (rG4) known as poly(UG) (pUG) fold, was discovered in *C. elegans*. This unique structure was found to induce RNA interference (RNAi) upon recruitment of RNA-dependent RNA polymerase (RdRP), resulting in trans-generational gene silencing. Herein, we develop a novel L-RNA aptamer, L-apt3.1, that binds to the pUG fold. We uncover that L-apt3.1 consists of a parallel rG4 structural motif, and

mutagenesis analysis illustrates that the rG4 motif in L-apt3.1 is essential for pUG fold recognition. We show that L-apt3.1 interacts strongly with pUG fold, and notably it is the first reported aptamer that can bind to pUG fold *in vitro*. Furthermore, we report that L-apt3.1 can interact with pUG fold *in vivo*, and with a better performance than the G4 ligand, *N*-methyl mesoporphyrin (NMM), to inhibit gene silencing in *Caenorhabditis elegans*. Overall, we demonstrate the first use of L-RNA aptamer for *in vivo* G4-mediated gene regulation, making a significant breakthrough in the field of RNA-based gene control. We envision L-apt3.1 as a potential tool for studying pUG fold structure within living systems including humans. Further development and optimization of L-RNA aptamers can likely unlock their capabilities for diverse biological and biomedical applications.

Workshop 57: Exploring New Horizons: Innovations at the Intersection of Basic and Translational Research in Obesity and Diabetes (Room 3B)

Chairs: Feng Liu, Li Qiang

W57-1: Perivascular Adipose Tissue and Smooth Muscle Cell Cross-Talk in Atherosclerosis and Hypertension

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Perivascular adipose tissue (PVAT) surrounds most blood vessels, from the aorta to the tiny vessels in the skin, and consists of various cells like fat cells, fibroblasts, stem cells, mast cells, and nerve cells. Despite its presence, its role has been largely ignored. PVAT was once thought to only support blood vessels and cushion them, but recent research shows that it actively regulates blood vessel tone through substances it releases. PVAT also contributes to atherosclerosis by secreting bioactive substances such as adipokines and cytokines. Thus, PVAT affects blood vessel functions and interacts with the vessel layers, including vascular smooth muscle and endothelial cells. Recognizing its crucial role in maintaining vascular structure and function, current studies are focusing on how PVAT is involved in conditions like hypertension and atherosclerosis. Future research should explore how changes in PVAT metabolism influence cardiovascular disease and uncover the mechanisms of communication between PVAT and the blood vessel cells.

W57-2: Erythrocyte Hypoxia Sensor: a critical regulator of metabolism in aging, disease, and longevity

Yang Xia
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Aging is frequently associated with chronic hypoxia and is a risk factor for almost every organ functional decline. However, longevity individuals have a lower susceptibility to chronic hypoxia, inflammation, oxidative stress, and aging-related diseases. It has long been speculated that "rejuvenation molecules" exist in the blood of longevity individuals to promote their extended lifespan. Using both human and mouse genetic coupled with a high throughput metabolomics and isotopically labelled glucose tracing analyses, we recently identified that the erythrocyte contains non-HIF-dependent but glucose and adenosine-dependent hypoxic sensor including ENT1-ADORA2B-S1P-PP2A-AMPK axis and TG2 orchestrating its hypoxic metabolic reprogramming and promoting O2 delivery to combat tissue hypoxia (Cell Metabolism 2021， Circulation Research 2020). Additionally, we demonstrated that "erythrocyte hypoxic sensor" leads to severe tissue microenvironment hypoxia, renal carnitine loss, impaired bioenergetics, and chronic kidney disease (CKD) progression, while carnitine treatments slow down its progression (Cell Metabolism 2021 and JASN 2023). Recently, we found that most elderly individuals (70-89 yrs) show a reduced oxygen release and aging-related metabolic profiling in their erythrocytes. In contrast, we unexpectedly discovered that longevity individuals (>90 yrs) exhibit a distinct rejuvenated erythrocyte oxygen release function and glucose and glutamine metabolic reprogramming which are similar to that seen in young individuals. Mechanistically, we revealed that "erythrocyte hypoxic sensor"-mediated metabolic reprogramming plays a critical role for rejuvenated erythrocytes to allow longevity individuals to better counteract peripheral tissue hypoxia, inflammation, and oxidative stress and thus maintain healthspan.

W57-3: Investigation of the multiple-hit pathogenesis of non-alcoholic fatty liver

Junli Liu
Shanghai Jiaotong University

Obstructive sleep apnea syndrome (OSAS), characterized by chronic intermittent hypoxia (CIH), is an independent risk factor for aggravating non-alcoholic steatohepatitis (NASH). The prevailing mouse model employed in CIH research is inadequate for the comprehensive exploration of the impact of CIH on NASH development due to reduced food intake observed in CIH-exposed mice, which deviates from human responses. To address this issue, we conducted a pair-feeding investigation with CIH-exposed and normoxia-exposed mice. We revealed that CIH exposure aggravated DNA damage, leading to hepatic fibrosis and inflammation. Our analysis of genome-wide association study (GWAS) data also disclosed the association between Eepd1, a DNA repair enzyme, and OSAS. Furthermore, we revealed that CIH triggered selective autophagy, leading to the autophagic degradation of Eepd1, thereby exacerbating DNA damage in hepatocytes. Notably, Eepd1 liver-specific knockout mice exhibited aggravated hepatic DNA damage and further progression of NASH. To identify a therapeutic approach for CIH-induced NASH, we conducted a drug screening and found that Retigabine dihydrochloride suppressed CIH-mediated Eepd1 degradation, leading to alleviated DNA damage in

hepatocytes. These findings imply that targeting CIH-mediated Eepd1 degradation could be an adjunctive approach in the treatment of NASH exacerbated by OSAS.

W57-4:Review and strategic thinking on innovative diabetic drug upon its crossing the death-valley.

Qinghua Wang
Founder and CEO, Innogen Pharmaceuticals Inc.

The 21st century marks a significant shift in medicine, transitioning from chemical drugs to biologicals. This lecture will delve into the initial stages of drug target discovery, verification, DNA vector construction, and engineered cell development. Unlike the traditional R&D process for chemical drugs—which involves compound screening, target identification, hit identification, validation, and lead optimization and selection, often taking many years and incurring high costs—biological drugs offer a more targeted approach. These biologics, often derived from endogenous peptide hormones using advanced proteomics and metabolomics techniques, are more specific and effective. However, the development of biological molecules presents unique challenges. Their large and complex structures demand rigorous processes to ensure consistency and stability in production. Additionally, the potential for immunogenic responses necessitates extensive modifications and testing. Scaling up manufacturing while maintaining product quality and purity is another significant hurdle. Meeting stringent regulatory requirements for safety, efficacy, and quality involves comprehensive and costly testing and documentation. This presentation will summarize the R&D journey of an antidiabetic biomedicine, highlighting the early stages of CMC, preclinical studies, and clinical trials, exemplified by Supaglutide. Supaglutide, a long-lasting, weekly or bi-weekly dosing humanized GLP-1 receptor agonist, has completed its preclinical and pivotal clinical studies in patients with type 2 diabetes in China. Its BLA application was submitted to the CDE last September for review and approval. Clinical data demonstrate Supaglutide's superior effectiveness in reducing HbA1c with rapid hypoglycemic action and good tolerance in T2D patients. The presentation will also discuss the commercialization challenges of bringing a proven drug to clinical application, targeting large patient populations who can benefit from innovative therapies that are affordable and ensure good clinical compliance.

W57-5:Hormones in Health and Disease

Yiguo WANG
School of Life Sciences, Tsinghua University

Metabolic homeostasis is the cornerstone of overall bodily health, with disruptions in this equilibrium potentially leading to a range of metabolic diseases. Hormones play a pivotal role in orchestrating dynamic metabolism across various tissues and organs, serving as vital therapeutic targets in clinical disease management. Therefore, the identification of new hormones and elucidation of their roles in maintaining metabolic homeostasis carry significant scientific importance for both basic research and clinical

applications. Over the past decade, our laboratory has identified several novel hormones involved in glucose and lipid metabolism through rigorous screening and validation processes. In this presentation, we will highlight the discovery of Cholesin, a gut-derived hormone, and its role in cholesterol metabolism.

W57-6: Fibroblast Growth Factors-based Pharmacotherapy for Obesity-related Metabolic Complications

Aimin Xu
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The fibroblast growth factor (FGF) family, which comprises 22 structurally related proteins, plays diverse roles in cell proliferation, differentiation, development, and metabolism. Among them, two classical members (FGF1 and FGF4) and two endocrine members (FGF19 and FGF21) are important regulators of whole-body energy homeostasis, glucose/lipid metabolism, and insulin sensitivity. Preclinical studies have consistently demonstrated the therapeutic benefits of these FGFs for the treatment of obesity, diabetes, dyslipidemia, and metabolic dysfunction-associated fatty liver disease. Several genetically engineered FGF19 and FGF21 analogues with improved pharmacodynamic and pharmacokinetic properties have been developed and progressed into various stages of clinical trials. These FGF analogues are effective in alleviating hepatic steatosis, steatohepatitis, and liver fibrosis in biopsy-confirmed patients with metabolic steatohepatitis (MASH), whereas their antidiabetic and anti-obese effects are mild and vary greatly in different clinical trials. In this presentation, I will discuss recent advances in biopharmaceutical development of FGF-based therapies against obesity-related metabolic complications, highlight major challenges in clinical implementation, and propose possible strategies to overcome these hurdles.

Day 4, July 29 (Monday)

Concurrent workshops 58-67, 10:30am-12:00pm

Workshop 58: Infectious Agents (Room2A)

Chairs: Jade Lee-Lee Teng, Man Lung Yeung

W58-1:Mycolyltransferase as virulence factor for Tsukamurella keratitis, an emerging ophthalmological emergency

Jade Lee-Lee Teng[1], Ying Tang[1], Man Lung Yeung[2], Susanna Kar-Pui Lau[2], and Patrick Chiu-Yat Woo[2]

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Tsukamurella, a group of multi-drug resistant, Gram-positive, aerobic and partially acid-fast bacteria, are emerging causes of bacterial conjunctivitis and keratitis. However, the pathogenesis of *Tsukamurella* keratitis is largely unknown. To address this, we used New Zealand White rabbits to develop the first eye infection model and conducted *in vitro* tests to study the pathogenesis mechanisms of *Tsukamurella*. There is increasing evidence that biofilms play a significant role in ocular infections, leading us to hypothesize that biofilm formation is crucial for effective *Tsukamurella* infection. In order to look for potential candidate genes which are important in biofilm formation and *Tsukamurella* keratitis. We performed genome sequencing of two ocular isolates, *T. pulmonis*-PW1004 and *T. tyrosinosolvens*-PW899, to identify potential virulence factors. Through *in vitro* and *in vivo* studies, we characterized their biological roles in mediating *Tsukamurella* keratitis. Our findings confirmed that *Tsukamurella* is an ocular pathogen by fulfilling the Koch's postulates, and using genome sequence data, we identified *tmytC,* encoding a mycolyltransferase, as a crucial gene in biofilm formation and causing *Tsukamurella* keratitis in the rabbit model. This is the first report demonstrating the novel role of mycolyltransferase in causing ocular infections. Overall, our findings contribute to a better understanding of *Tsukamurella* pathogenesis and provide a potential target for treatment. Specific inhibitors targeting TmytC could serve as an effective treatment option for *Tsukamurella* infections.

W58-2:Insights from surface plasmon resonance (SPR) experiment on the Dynamics of Enterovirus Infection

Man Lung Yeung[1] and Liang Jie[1]

[1]Department of Microbiology, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China;

This study presents a comprehensive kinetic analysis of the interaction between EVA71 and EVD68 VP proteins with human tryptophanyl-tRNA synthetase (hWARS) and lysosomal integral mem-brane protein-2 (SCARB2) under various conditions. The binding kinetics were evaluated at different temperatures (25°C, 33°C, and 37°C)

and pH levels (neutral and acidic) using surface plasmon resonance (SPR) technology. Our findings reveal distinct binding affinities and kinetic parameters for the interactions between the viral proteins and the host receptors. Specifically, we observed differences in KD values, Rmax, and Chi2/Rmax across different temperature and pH conditions, indicating temperature and pH-dependent variations in the binding kinetics. Furthermore, the binding kinetics were fitted using both 1:1 binding and two-state reaction models, providing insights into the molecular mechanisms underlying the interactions between the viral proteins and the host receptors. This study paves the way for our further research into exploring the intricacies of these interactions and provides crucial SPR experimental strategies for investigations across various fields.

W58-3:Broad-spectrum antiviral peptide studies against multiple respiratory viruses

Hanjun Zhao[1, 2], Chuyuan Zhang[1], and Xinjie Meng[1,2]
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The high rates of mutation in RNA viruses pose a significant challenge to the effectiveness of antiviral drugs. Throughout the last century, an extensive range of variants of respiratory viruses such as influenza, SARS-CoV-2, parainfluenza virus and rhinovirus have continued to cause outbreaks of pandemics, epidemics, or endemics in human populations. Specific antiviral drugs have palied important roles in controlling the viral infectious diseases, while antivirals with new mechanism are urgently needed to combact with new emerging viruese and drug-resistance mutants. In our studies, we are systemicly studing broad-spectrum antiviral peptides derived from human, mice and frog to investigate the broad-specturm antriviral activities against respiratory viruses including influenza virus, SARS-CoV-1, SARS-CoV-2, MERS-CoV, rhinovirus and parainfluenza virus in vitro and in vivo. The results provide that engineered-branch peptides could show potent and broad-spctrum antiriviral activities against multip respiratory virses with a high barrier to drug resistance.

W58-4:Current small animal models for studying enterovirus infection

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Enterovirus (EV) is a rapidly emerging infectious virus responsible for over 1 billion enteroviral infections annually worldwide. Several EV strains, such as EV-A71 and EV-D68, pose a significant threat due to their ability to cause severe diseases. Efforts have been made to develop antiviral drugs and vaccines for treating enteroviral infections; however, a major research challenge in the field is the absence of an appropriate animal model for enterovirus infection. This limitation hinders the

evaluation of in vivo effects of potential treatment options. In this study, we will provide a comprehensive review of the currently tested small animal models, discussing their importance and limitations in addressing disease pathogenesis. Furthermore, we will introduce a novel in-house small animal model that we have recently developed. We will discuss its utility in testing the virulence of different enterovirus species, as well as its potential applications in drug testing and vaccine development.

W58-5:Induction of proinflammatory response in bystander macrophages by extracellular vesicles-delivered SARS-CoV-2 accessory protein ORF3a

Sin-Yee Fung[1], Kam-Leung Siu[1], Man Lung Yeung[2], Judy Wai Ping Yam[3], and Dong-Yan Jin[1]

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Severe respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19. In severe COVID-19, acute respiratory distress syndrome and thrombosis are often related to exacerbated release of proinflammatory cytokines interleukin (IL)-1β and IL-6. SARS-CoV-2 ORF3a is capable of inducing inflammasome activation and IL-1β secretion. Macrophages are thought to be major producers of proinflammatory cytokines. However, it remained debated if macrophages are susceptible to infection with SARS-CoV-2. The relevance of proinflammatory cytokine release from macrophages to the life cycle of SARS-CoV-2 remains to established. We hypothesized that certain secretory viral factors might be responsible for proinflammatory response in macrophages. In this study, we identified secretory ORF3a as a key player in proinflammatory cytokine release in macrophages through extracellular vesicles (EVs) transfer. In overexpression and infection system, we detected the existence of secretory ORF3a in culture medium. We further identified the presence of ORF3a in EVs by Western blotting and immunoelectron microscopy. ORF3a containing EVs are capable of transferring to neighboring cells. Feeding phorbol-12-myristate-13-acetate-differentiated THP-1 cells with ORF3a EVs trigger proinflammatory cytokine release. Taken together, our demonstration of ORF3a EVs-mediated proinflammatory cytokine release lent support to the notion that SARS-CoV-2 could modulate host EV biogenesis to facilitate pathogenic inflammation. Our findings also have implications in the development of novel therapeutic strategies to mitigate cytokine storm in severe COVID-19. Supported by RGC (C7142-20GF and T11-709/21-N) and HMRF (22211042).

Workshop 59: Extracellular Vesicles (Room 2B)

Chairs: Shizhen Emily Wang, Tiebang Kang

W59-1: Extracellular vesicles in cancer's systemic effect

Shizhen Emily Wang[1]

[1]*Department of Cancer Biology, University of California San Diego, La Jolla, California, USA*

Our knowledge of short- and long-range intercellular communication had an exponential growth in the past decade partially owing to the discovery of the diverse functions of extracellular vesicles (EVs). EVs are a variety of membrane-enclosed nanosized particles that carry and transfer between cells functional cargoes including RNA, DNA, proteins, and lipids. Secretion of EVs is a fundamental and evolutionarily conserved biological process broadly found from bacteria to humans and in all cell types in a higher organism. Due to their bulk loading nature, EVs play a critical and versatile role in the intercellular communication perhaps in all physiological and pathological processes, including the cancer-host crosstalk. Research from our group and others has shown that cancer cell-secreted EVs partake in vascular remodeling, immunomodulation, and formation of pre-metastatic niches. Circulating EV-based biomarkers are being exploited for risk prediction, early diagnosis and prognosis of human diseases such as cancer. I will showcase recent studies from my group elucidating how EVs secreted by breast cancer cells influence a variety of non-cancer cell types in the brain, lungs, liver, muscle, endocrine tissues, and blood vessels to impair many aspects of normal physiological functions and facilitate tumor growth and metastasis. Research in my lab accentuates a holistic understanding of human diseases such as breast cancer, with a focus on EV-mediated cancer-host crosstalk.

W59-2: Deciphering the mechanism of Rafeesome formation

Tiebang Kang[1]

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Rafeesomes, recently identified MVB-like organelles, originate from the fusion of RAB22A-mediated ER-derived noncanonical autophagosomes with RAB22A-positive early endosomes. However, the mechanism underlying the formation of Rafeesomes remains unknown. Herein, we report that RAB22A/TMEM33/RTN4 assembly is the driving force for the biogenesis of Rafeesomes. RAB22A interacts with the tubular ER membrane protein TMEM33, which binds the TM2 domain of the ER-shaping protein RTN4 to promote RTN4 homo-oligomerization, generating RTN4-enriched microdomains and thereby increasing local tension on the ER membrane. Consequently, the RTN4 microdomain buds off the ER membrane to form RTN4 vesicles, which are transported by ATG9A to develop into an isolation membrane (IM), probably through homotypic fusion. Then, this IM is anchored with LC3-II, a process catalyzed by the ATG12-ATG5-ATG16L1 complex, enabling the growth and elongation of the IM to form a sealed RTN4 noncanonical autophagosome. By assembling R-SNARE VAMP7 with Q-SNAREs STX18, BNIP1 and USE1, the RTN4 noncanonical autophagosome fuses with RAB22A-positive early endosomes to generate Rafeesomes, which eventually fuse with the plasma membrane to produce RAB22A-induced extracellular vesicles (R-EVs). Our findings reveal a new model for specific tubular ER-derived autophagosome formation and TMEM33 as a marker for R-EVs

W59-3: Extracellular vesicles, linking metabolic disorders to lung inflammation in sepsis

Wei Yan[1]

[1]*Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan, Hubei, China.*

Sepsis is systemic inflammation with high mortality, accompanied by multi-organ failure including acute respiratory distress syndrome (ARDS). Extracellular vesicles (EVs) encapsulating bioactive cargoes, mediate organ-organ communication in vivo. However, whether and how host responds to bacterial infection through EVs in sepsis is poorly understood. Here, we identify macrophages secrete exosomal G6PD protein upon bacterial infection, due to more nuclear NRF2 translocation and elevated Rab11b expression. Circulating G6PD recruits deubiquitinase USP39 to stabilize enzyme IMPDH2 and enhances guanine accumulation in lung, where guanine suppresses de novo EZH2 synthesis to reduce H3K27 trimethylation. Genes including Nos2, Ccl6 and Il6, screened by chip-seq, are tightly regulated by G6PD-mediated guanine accumulation. In summary, our finding reveals circulating G6PD promotes lung inflammation through rewiring the nucleotide metabolism and remodeling the epigenetic profile in sepsis, shedding light on the critical role of EV as a proinflammatory signal and targeting G6PD for future sepsis diagnosis and treatment.

W59-4: Exploring the mechanisms and physiological functions of vesicle-mediated organelles export

Hongyun Tang[1]

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Mitochondrial export into the extracellular space is emerging as a key process in a variety of physiological activities. While some studies have begun to illuminate the processes for discarding damaged mitochondria, the mechanisms of mitochondrial export, as well as the physiological implications of such release, remain largely enigmatic. In particular, the existence of a mitochondria-specific export mechanism remains underexplored. Our lab has recently discovered 'mitopherogenesis', a previously unknown process that specifically secretes mitochondria from cells via a unique extracellular vesicle, termed 'mitopher'. In this presentation, I will share our latest findings on the general and conserved role of mitophers. Furthermore, the question of whether other organelles can also be exported through the generation of extracellular vesicles remains open, and I will also share our insights on this topic. Our research may identify vesicle-mediated organelle export as a fundamental mechanism for controlling organelle quality and quantity, potentially playing crucial roles in cell development and the maintenance of homeostasis.

W59-5: Extracellular vesicles for pulmonary regeneration and drug delivery

Ke Cheng

Columbia University

Workshop 60: Advances in Psychiatry: from Basic Research to Clinical Treatments (Room2C)

Chairs: Xiao-Ming Li, Ti-Fei Yuan

W60-1: Reverse-translational study of neuropsychopharmacology

Ji Hu[1]

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1 in every 8 people, or 970 million people around the world were living with a psychiatric disorder. However, medication for psychiatric disorders has not been updated for 50 years, partly because of a lack of knowledge about the etiology of mental illness, which makes translational research inefficient. We are applying a reverse-translational approach to gain insight into the neurobiological mechanisms underlying human psychiatric disorders and the effects of pharmacological treatments. We have found that atypical antipsychotics antagonize GABAA receptors in the ventral tegmental area GABA neurons to relieve psychotic behaviors. Also, we reported that propofol actively and directly binds to the dopamine transporter (DAT), but not the serotonin transporter, which contributes to the rapid relief of anhedonia.

W60-2: A molecularly defined amygdala-independent pathway for odor-driven innate fear and anxiety

Hao Wang[1], Xiao-Ming Li[2]

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Fear-related disorders (for example, phobias and anxiety) cause a substantial public health problem. To date, studies of the neural basis of fear have mostly focused on the amygdala. Here we identify a molecularly defined amygdala-independent tetra-synaptic pathway for olfaction-evoked innate fear and anxiety in male mice. This pathway starts with inputs from the olfactory bulb mitral and tufted cells to pyramidal neurons in the dorsal peduncular cortex that in turn connect to cholecystokinin-expressing (*Cck*$^{+}$) neurons in the superior part of lateral parabrachial nucleus, which project to tachykinin 1-expressing (*Tac1*$^{+}$) neurons in the parasubthalamic nucleus. Notably, the identified pathway is specifically involved in odor-driven innate fear. Selective activation of this pathway induces innate fear, while its inhibition suppresses odor-driven innate fear. In addition, the pathway is both necessary and sufficient for stress-induced anxiety-like behaviors. These findings reveal a forebrain-to-hindbrain neural substrate for sensory-triggered fear and anxiety that bypasses the amygdala.

W60-3: Structural and functional plasticity during electroconvulsive therapy-induced depression remission: a longitudinal study

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[2] *Department of Psychology and Sleep, Second Affiliated Hospital of Anhui Medical University, 230601, Hefei, China*

Electroconvulsive therapy (ECT) is significantly effective against depression. However, neuroplastic changes during the ECT course have not been completely

investigated. Here, we examined the dynamic nature of brain changes during ECT-induced depression remission. Patients completed at least eight ECT sessions and underwent clinical assessment and magnetic resonance imaging at baseline and after each ECT session. We investigated ECT-induced structural and functional plasticity changes and their association with depression remission using voxel-based morphometry and functional connectivity analysis. We found that the participants already exhibited depression remission during the early phase of ECT. Decreased HAM-D scores after the first ECT were associated with changes in the functional connectivity of the right hippocampus. The GMV of the left Rolandic operculum, left temporal pole, bilateral insula, and bilateral hippocampus increased during ECT. The GMV of these regions increased significantly after the fifth ECT. Decreased HAM-D scores were associated with increased GMV in the right hippocampus throughout ECT. The ECT-induced antidepressant effects are associated to initial functional changes and late structural changes, revealing the neuroplastic basis of ECT-induced depression remission.

W60-4: To explore the cross discipline development of psychiatry from the influence of anesthesia on the brain

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[2]*Mental Health Center affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200030, China.*

The clinical objective of anesthesia and surgery is to be carried out safely and stably. However, there still exist short-term and long-term effects on central nervous system which should not be neglected, such as cognitive dysfunction and conversion to AD. We have focused on the mechanism of neuro-psychiatric disorders due to anesthesia and surgery in fragile brain, conducted series researches, revealed the mechanism that how anesthesia and surgery could increase the risk of AD, autism and other diseases and has initially proved effective intervention strategies targeting peripheral pathways. Meanwhile, anesthetics also have the potential to treat mental disorders, such as ketamine, which has been developed as a new antidepressant with greatest potential. In this way, integration with other discipline will be a breakthrough of pathological mechanism research on mental disorders, and will also provide more possibility to develop new drugs in mental disorders.

W60-5: Serotonin modulation of transition to compulsive cocaine intake

Yue Li[1]
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Compulsive drug use despite adverse consequences defines addiction. While mesolimbic dopamine signaling is sufficient to drive compulsion, psychostimulants such as cocaine also boost extracellular serotonin (5-HT) by inhibiting reuptake. We used SERT Met172 knockin (SertKI) mice carrying a transporter that no longer binds cocaine to abolish 5-HT transients during drug self-administration. SertKI mice showed an enhanced transition to compulsion. Conversely, pharmacologically elevating 5-HT reversed the inherently high rate of compulsion transition with optogenetic dopamine self-stimulation. The bidirectional effect on behavior is explained by presynaptic depression of orbitofrontal cortex–to–dorsal striatum

synapses induced by 5-HT via 5-HT1B receptors. Consequently, in projection-specific 5-HT1B receptor knockout mice, the fraction of individuals compulsively self-administering cocaine was elevated. In addition, we observed aberrant excitability of dorsal raphe 5-HT neurons in mice compulsively self-administering cocaine, indicating a dysfunction of serotonin system in addiction.

W60-6: Progress in clinical evidence-based research on depression and gut microbiota

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Depression is a kind of serious mental illness that poses a threat to human health. The gut microbiome, as the "second genome" of humans, plays an important regulatory role in the onset and treatment of depression. Screening out potential key functional strains of depression and clarifying their regulation of the gut brain axis mechanism is of great significance for promoting the prevention and treatment of depression. We screened key functional strains of depression by constructing a microbiome database, and utilized techniques such as photogenetics, neurophysiology, single-cell sequencing, and spatial transcriptomics to reveal the potential gut brain axis mechanisms by which key gut microbiota regulate depression.The enrichment of pro-inflammatory bacteria in the intestine and the depletion of anti-inflammatory bacteria are key microbial changes in depression. The transmission of intestinal inflammatory signals to the brain leads to neuroinflammation, and microglia in the brain are the key. Primates have biological characteristics that are highly similar to humans, possess advanced cognitive behavior and a humanoid social structure, and are excellent experimental carriers for studying brain diseases such as depression

Workshop 62: Lung Biology and Diseases (Room 2E)

Chairs: Yuehai Ke

W62-1: Adeno-to-squamous transition drives resistance to KRAS inhibition in LKB1 mutant lung cancer

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$KRAS^{G12C}$ inhibitors (adagrasib and sotorasib) have shown clinical promise in targeting *$KRAS^{G12C}$*-mutated lung cancers; however, most patients eventually develop resistance. In lung patients with adenocarcinoma with *$KRAS^{G12C}$* and *STK11/LKB1* co-mutations, we find an enrichment of the squamous cell carcinoma gene signature in pre-treatment biopsies correlates with a poor response to adagrasib. Studies of *Lkb1*-deficient *$KRAS^{G12C}$* and *$Kras^{G12D}$* lung cancer mouse models and organoids treated with KRAS inhibitors reveal tumors invoke a lineage plasticity program, adeno-to-squamous transition (AST), that enables resistance to KRAS inhibition. Transcriptomic and epigenomic analyses reveal ΔNp63 drives AST and modulates response to KRAS inhibition. We identify an intermediate high-plastic cell state marked by expression of an AST plasticity

signature and *Krt6a*. Notably, expression of the AST plasticity signature and *KRT6A* at baseline correlates with poor adagrasib responses. These data indicate the role of AST in KRAS inhibitor resistance and provide predictive biomarkers for KRAS-targeted therapies in lung cancer.

W62-2: AI Optimizes Structural Analysis of Key Signal Proteins in Pulmonary Fibrosis or Lung Cancer

Ying Chi, Zhejiang University

W62-3： Environmental carcinogenesis and therapeutic vulnerability of lung cancer

Guang-Biao Zhou[1]
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Tobacco smoke and air pollution (or haze) account for > 90% of the annual 1.8 million lung cancer deaths worldwide, demonstrating the importance of the related carcinogenesis in lung cancer. We showed that long-term exposure to air pollution caused somatic mutations in human genomes and perturbed the profiles of microRNA and long non-coding RNA to facilitate lung carcinogenesis. Tobacco smoke and haze (hereafter, smohaze) induced the production of chemokine CXCL13 and the expression of PD-L1 in lung epithelial cells exposed to smohaze carcinogen benzo(a)pyrene (BaP). Both CXCL13 and PD-L1 were target genes of transcription factor AhR. Knockdown of CXCL13 and AhR significantly inhibited BaP-induced lung cancer, indicating the critical roles of these molecules in smohaze-induced tumorigenesis. Smohaze carcinogens upregulated CDC34 to stabilize EGFR and promote cell proliferation, and induced IDO1 expression to enhance tryptophan metabolism and immune suppression. Our results indicated that the environmental carcinogenesis play a central role in the initiation and development of lung cancer, and therapeutic approaches targeting this process hold promise for conquering this deadly disease.

W62-4：Mesenchymal-Specific Knockout of Fstl1 Causes Pulmonary Bullae

Wen Ning[1]
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Alveologenesis, formation of alveoli by a septation process, allow sufficient gas exchange area for breathing. Disruption of alveologenesis results in simplification of alveoli, as is seen in premature infants diagnosed with bronchopulmonary dysplasia (BPD), a prevalent lung disease that is often associated with lifelong breathing deficiencies. Although a critical role has been proposed for alveolar myofibroblasts (aMYF) to drive the septation process, their characterization and regulation are poorly understood. We previously reported the role of matricellular protein FSTL1 in lung development. Here, we generated *Tbx4-rtTA; TetO-Cre; Fstl1*$^{f/f}$ mouse line to specific delete *Fstl1* from lung mesenchymal cells and observed the abnormal bullae of alveolar simplification. scRNA-seq analysis of the mutant lung cells identified a PDGFRa+ ITGA+ aMYF subpopulation that are associated with the septation.

Mesenchymal deletion of Fstl1 decreased the population size and impaired the elastin fiber assembly function of aMYFs via TGF- 1 signaling, resulting the abnormal a-SMA-aMYF-elastin network and subsequently alveolar simplification. Functional increase of FSTL1 by i.p. addition of recombinant protein and TGF- 1 agonist, as well as i.t. addition of UCMSC EVs, rescued hyperoxia-induced BPD in mice and rats. Therefore, we provide in vivo and in vitro evidence to demonstrate that FSTL1 modulates alveologenesis and aMYF behavior, in part, through TGF- 1/elastin assembly signaling axis.

W62-5：Multi-omics investigation of lung cancer development

Jian Liu[1]
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Lung cancer is the leading cause of cancer-related death worldwide. Targeted therapies have been developed by targeting these driver mutations in lung adenocarcinoma, such as *EGFR*. However, there is a lack of targeted therapy specifically for treating lung squamous cell carcinoma (LUSC). We have shown that the single ablation of *Lkb1* (*Stk11*) led to the spontaneous development of LUSC. Kinase array analysis of the pre-tumor or tumor stages of the *Lkb1*-deletion-driven LUSC identified the p-JNK1/2 significantly downregulated. The deletion of JNK1/2 promoted *Lkb1*-deletion-driven LUSC development. To explore the LUSC development mechanisms, we conducted the RNA-Seq, whole exon sequence (WES), single-cell RNA-Seq of *Lkb1*-deletion-driven LUSC.
Moreover, we integrated 11 types of omics, including Hi-C, bulk RNA-seq, ATAC-seq, ChIP-seq, DNA methylation, DNA mutation, copy number variations, single-cell RNA-seq, and ATAC-seq. We established and constructed a web-based database, CancerOmics3D (http://www.CancerOmics3D.net), by developing three integration strategies to effectively identify the functional genomic regions among seven types of cancer, including lung cancer, with the offering of four online analysis tools.
In summary, we aim to investigate the mechanisms of lung cancer development, especially LUSC, by integrating multi-omics and genetic mouse models to identify more targets to aid the therapy development.

W62-6：Machine learning unveils metabolic post-translational modifications of signal transducer in lung fibrosis

Yuehai Ke[1]
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Clinical evidence has highlighted a strong correlation between dysregulated glycolipid metabolism and the progression of lung fibrosis. However, metabolic signal events in controlling of fibrotic effects remain largely unclear. In recent years, Artificial Intelligence (AI) has provided powerful tools for investigating the spatiotemporal effects of cellular metabolism. Here, we utilized AI-based machine learning-empowered single-cell spatial omics to functionally dissect signal transduction in the regulation of lung fibrosis. Our data indicated that glycolipid

metabolic defects are prevalent in the lungs and play a significant role in cellular physiological activities. Furthermore, metabolic post-translational modifications (PTMs) such as palmitoylation, lactylation, and glycosylation have been closely linked to well-studied fibrotic signals. These non-typical PTMs could serve as early warning signs and targets for intervention, providing potential therapeutic options for lung fibrosis, which is currently untreatable.

Workshop 63: Viral Hepatitis (Room 2F)

Chairs: Guangxiang George Luo, Haitao Guo

W63- 1: Critical Role of HBV Capsid during Entry

Jianming Hu
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Hepatitis B virus (HBV) relies on the core (or capsid) protein (HBc) to gain entry into host cells productively, as defined by the formation of the covalently closed circularized DNA (cccDNA), as well as to carry out almost every step of its lifecycle following cccDNA formation. Multiple copies of HBc form an icosahedral capsid shell that encapsidates the viral pregenomic RNA (pgRNA) and facilitates the reverse transcription of pgRNA to a relaxed circular DNA (rcDNA) within the capsid. During infection, the complete HBV virion, which contains an outer envelope layer in addition to the internal nucleocapsid containing rcDNA, enters human hepatocytes via endocytosis and traffics through the cytoplasm to deliver the input rcDNA to the nucleus to produce cccDNA. In addition, progeny rcDNA, newly formed in cytoplasmic nucleocapsids, is also delivered to the nucleus in the same cell to form more cccDNA in a process called intracellular cccDNA amplification or recycling. Here, we focus on recent evidence demonstrating differential effects of HBc in affecting cccDNA formation during *de novo* infection vs. recycling, obtained using HBc mutations and small molecule inhibitors. These results reveal a critical role of HBc in determining HBV trafficking during entry as well as in nucleocapsid disassembly (uncoating) to release rcDNA, events essential for cccDNA formation. HBc likely functions in these processes via interactions with species-specific host factors, which contributes critically to HBV host tropism. A better understanding of the roles of HBc in HBV entry, cccDNA formation, and host species tropism should accelerate ongoing efforts to target HBc and cccDNA for the development of an HBV cure and facilitate the establishment of convenient animal models for both basic research and drug development.

W63-2:Roles of apolipoprotein E isoforms and apoE-binding receptors in hepatitis B virus infection

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We have previously demonstrated that human apolipoprotein E plays an important role in both hepatitis B (HBV) and C (HCV) viruses infection. Likewise, apoE-binding receptors such as low-density lipoprotein receptor (LDLR) family

proteins (LDLR, very low-density lipoprotein receptor-VLDLR, and LDLR-related proteins-LRPs) and heparan sulfate propteoglycans (HSPGs) serve as host factors promoting HBV/HCV infection. In the present study, we have further determined the importance of apoE isoforms (apoE2, apoE3, and apoE4) in HBV infection. Interestingly, apoE2 and apoE4 enhanced HBV production in the HBV-transgenic mice crossbred with individual apoE2-, apoE3-, and apoE4-knockin mice. More significantly, human apoE3 has the greatest capacity of promoting HBV infection compared to mouse apoE and human apoE4. In contrast, apoE2 appeard not to promote HBV infection. This is consistent with our previous findings that LDLR is important for HBV infection via interaction with the HBV-associated apoE. It is well known that human apoE2 does not bind to LDLR family receptors although it has similar HSPGs-binding activity tp apoE3 and apoE4. We are also examining the importance of human apoE isoforms in HBV infection when ectopically expressed in $HepG2^{NTCP}$ cell line in which endogenous apoE was knocked out. These findins validate the important role of human apoE in the mediation of HBV cell attachment via specific interactions with LDLR family receptors.

W63-3: Highly infectious hepatitis B virus mutants: immune escape and pathogenesis

Jisu Li and Shuping Tong

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Chronic infection with hepatitis B virus (HBV) is a leading cause of liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). It is mostly attributed to perinatal transmission of genotype B or C in East Asia. Although hepatitis B e antigen (HBeAg, secreted version of core protein) and hepatitis B surface antigen (HBsAg, viral envelope proteins secreted as subviral particles) are dispensable for viral lifecycle, they induce immune tolerance to promote chronic HBV infection. The subsequent immune clearance phase often selects for adaptive mutations in the viral genome. In-frame deletion of 15-24nt in the 5' preS1 region is highly prevalent in genotypes B and C, and strongly associated with HCC development. Such deletions shift down the translation initiation site to shorten large (L) envelope protein by 11aa just like wild-type genotype D. Our molecular characterization of such 5' preS1 deletion mutants from a genotype C patient revealed that the 11-aa deletion in L protein markedly enhanced genotype B/C infectivity in cell culture, which was at least partly attributed to higher affinity to sodium taurocholate cotransporting polypeptide (NTCP), the functional HBV receptor. In addition, deletion in L protein helped virus escape of some neutralizing antibodies against N-terminal preS1 domain. The deletions also increased genome replication and virion production. Furthermore, the deletion mutant was associated with more profound endoplasmic reticulum (ER) stress than wild-type virus. A retrospective study of liver biopsy samples from the patient harboring such 5' preS1 deletion mutants revealed liver fibrosis despite normal ALT level. These observations raise an important question as to whether earlier anti-viral therapy is need to prevent liver fibrosis for patients infected with such 5' preS1 deletion mutants, especially if combined with other mutations that further increase genome replication (such as core promoter mutations).

W63- 4:Progress in basic research and clinical application of serum HBV RNA

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HBV is an enveloped DNA virus that replicates its DNA genome *via* reverse transcription of a pregenomic (pg) RNA intermediate in hepatocytes. Interestingly, HBV RNA can be detected in virus-like particles in chronic hepatitis B (CHB) patient serum and has been utilized as a biomarker for intrahepatic cccDNA activity in treated patients. However, the biogenesis of serum HBV RNA and its clinical significance remain to be further defined. In our study, we demonstrated that the cell culture supernatant contains a large amount of pgRNA-containing nonenveloped capsids and a minor population of pgRNA-containing virions, the pgRNA-virion utilizes the multivesicular body (MVB) pathway for egress, whereas the release of pgRNA-containing nonenveloped capsids is independent of it. In addition, screening of NEDD4 E3 ubiquitin ligase family members revealed that AIP4 stimulates the release of nonenveloped capsids irrespective of their HBV genome contents, which relies on AIP4 protein integrity and E3 ligase activity. To further explore the potential clinical significance of serum HBV RNA, we retrospectively monitored the emergence and reversion of the rtM204I/V mutant, a signature lamivudine resistance (LAM^R) mutation in biopsy cccDNA and longitudinal serum HBV RNA from two clinical trials, we found that cccDNA turnover occurs relatively rapidly (several months), offering a possibility of HBV cure with finite therapy through completely blocking cccDNA replenishment. Moreover, through comprehensive analysis of the association between serum HBV RNA and HCC development in 1374 patients from two prospective CHB cohorts, we found that serum HBV RNA declines at year 1 and 2 were significantly associated with on-treatment HCC risk. In summary, our study not only shed light on the molecular biology of serum HBV RNA in HBV life cycle, but also aid the development of serum HBV RNA as a novel biomarker for CHB treatment prognosis.

W63- 5:Hepatitis B virus-related hepatocellular carcinoma exhibits distinct intra-tumoral microbiota and immune microenvironment signatures

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Emerging evidence supports a high prevalence of cancer type-specific microbiota residing within tumor tissues. The intra-tumoral microbiome in hepatocellular carcinoma (HCC), especially in viral (HBV/HCV) HCC, has not been well characterized for their existence, composition, distribution, and biological functions. We report herein a finding of specific microbial signature in viral HCC as compared to non-HBV/non-HCV (NBNC) HCC. However, the significantly diverse tumor microbiome was only observed in HBV-related HCC, and Cutibacterium was identified as the representative taxa biomarker. Biological function of the unique tumor microbiota in modulating tumor microenvironment (TME) was characterized by using formalin-fixed paraffin-embedded (FFPE) tissue-based multiplex immunofluorescence histochemistry (mIFH) allowing simultaneous in situ detection of the liver cancer cells surrounded with high/low density of microbiota, and the

infiltrating immune cells. In HBV_HCC, the intra-tumoral microbiota are positively associated with increased tumor infiltrating CD8+ T lymphocytes, but not the CD56+ NK cells. Two subtypes of myeloid-derived suppressor cells (MDSCs): monocytic MDSCs and polymorphonuclear MDSCs, were also found to be positively correlated with the intra-tumoral microbiota in HBV_HCC, indicating an inhibitory role of these microbial species in antitumor immunity and the contribution to the liver TME in combination of chronic viral hepatitis during HCC development.

W63- 6:HEV secreted pORF2 and antigen tests

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Hepatitis E virus (HEV) is one of the most important pathogens of viral hepatitis worldwide. In recent years, we found that the HEV orf2 gene, which encodes the viral capsid protein, also encodes a secreted protein pORF2S during the replication. pORF2S is released in large quantities into the extracellular of the liver cells and enters the blood circulation. The concentration of pORF2S in the blood is nearly 1000 times that of viral capsid particles. The blood HEV pORF2 antigen detection method showed excellent performance for diagnosing current HEV infection in the cohort specimens. Further studies on the metabolism of pORF2S showed that the protein enriches at renal proximal tubule cells through a specific pathway, and is transported across tubule cells into urine and becomes ~20 kDa urine antigen named pORF2U. This enrichment process makes urine pORF2 antigen levels more than 80 times higher than those in serum. Clinical cohort studies showed that urinary antigen has the best sensitivity and specificity in a single diagnostic index. The detection of pORF2 antigen in blood and urine is of great significance for the clinical diagnosis, treatment and management of HEV.

Workshop 64: Epigenetics, Chromatin and Transcription (Room 2G)

Chairs: Guohong Li

W64-1: Protein-Nucleic Acids interaction during active retrotransposition of LINE-1

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LINE-1 (or L1) is the sole retrotransposon known to be still active in humans today. It continues to drive the evolution of the human genome, influences embryonic development and aging, and contributes to the development of diseases. Proper comprehension of the L1 functions demands a deep understanding of the molecular

mechanisms of ORF2p, which is an L1-encoded protein being both an endonuclease (EN) and a reverse transcriptase (RT), including those that govern the choice of template mRNAs and the determination of genomic loci to integrate retrotranscribed cDNAs. We have purified human ORF2p from baculovirus-infected insect cells and transient-transfected HEK293 cells, and found that it forms tight complexes with nucleic acids from host cells. Nuclease digestion revealed that the majority of bound nucleic acids are host DNA. Structural analyses by cryo-EM show a forked DNA is tightly bound to the highly positively charged surface of ORF2p, with the single-stranded end facing the EN domain. A DNA-RNA hybrid occupies the RT active site channel. DNA and RNA sequencing, in vitro DNA cleavage assays and cellular retrotransposition analyses support the structural findings, and reveal that ORF2p binds a variety of abundant mRNAs, and preferentially targets genomic loci where introns and transposons reside. These results advance our understandings of the retrotransposition mechanism of L1, and provide important insights into the physiopathological functions of L1 in a setting with an elevated level of L1 expression.

Key Words: Retrotansposon, LINE-1, DNA-RNA hybrid, Cryo-EM, endonuclease, reverse transcriptase

W64-2: Histone Lysine Acylations: Enzymes and Roles in Transcription, Physiological and Pathological Processes

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Acylations refer to a series of short chain fatty acid modifications that occur in lysine, including classic acetylation and recently discovered modifications such as propionylation, butyrylation, crotonylation, succinylation, etc. Different types of histone acylations theoretically have differential transcriptional regulation potential due to their differences in the acyl chain length, hydrophobicity, and electrical properties. Like acetylation, all acylation modifications rely on corresponding acyl-CoAs such as propionyl CoA, butyryl CoA, crotonyl CoA, and succinyl CoA, which are metabolic intermediates of glucose, lipids and amino acids, highlighting the intimate and complicated relationships between metabolic status and histone acylations. Although acetylation is one of the classic and best characterized protein modifications, currently there is limited understanding of the enzymes that orchestrate various other types of histone acylations. Furthermore, there is also limited understanding on the roles and mechanisms by which various histone acylations play in transcription and chromatin organization. In this talk, I will present our recent study on histone acylations, especially the identification and characterization of novel histone syuccinyltransferases and their roles in transcriptional regulation, physiological and pathological processes.

Key Words: Histone Acylations; Histone Succinylation; Transcription; Phase Separation; Tumorigenesis.

W64-3: Unveiling the role of histone lactylation in breast cancer metastasis

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Histone lactylation, which is derived from lactate produced during energy metabolism, serves as a link between metabolic reprogramming and epigenetic regulation1. The regulation of gene expression through histone lactylation plays a crucial role in various biological processes, such as macrophage polarization, cell reprogramming and tumorigenesis. However, it remains unclear whether histone lactylaiton can be recognized and interpreted by readers. In this study, we have identified AF9 as the reader for histone lactylation. The high expression level of AF9 is linked to the development of luminal breast cancer due to its activation of multiple tumor-associated pathways, including the TGFβ pathway, which connects epigenetics and signaling pathway. Besides, AF9 could regulate key enzymes of metabolic pathways, such as glycolysis and the serine pathway, both crucial to tumorigenesis. Interestingly, the recognition of AF9 and histone lactylation upregulates KLF family proteins, with KLF2 serving as the transcription factor for AF9, creating a positive feedback pathway that enhances the downstream effects of epigenetic recognition. In summary, our findings demonstrate that AF9 is the primary reader for histone lactylation and emphasize the connection between metabolism-epigenetics-signaling pathways in advancing luminal breast cancer tumorigenesis.
Key Words: Histone Lactylation, Breast Cancer, Epigenetics

W64-4: Spatiotemporally controlled function of a chromatin remodeler distinguishes pancreatic malignancy from tissue regeneration

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease, and the average survival rate of stage IV PDAC patients 5 years after diagnosis is less than 1%. The cell of origin for PDAC is adult acinar cells. Acute pancreatitis induces a transient progenitor-like cell fate switch called acinar-to-ductal metaplasia (ADM), which allows pancreatic tissue regeneration or chronic pancreatitis in the presence or mutant Kras, which then can progress into PDAC upon loss of tumor suppressor genes. Tumor cell intrinsic and environmental cues both have recently been demonstrated to induce the series of neoplastic reprogram and the accompanied epigenetic remodeling. In addition, the Kras signaling is activated in both wildtype or mutant Kras setting, and its activity has been paradoxically shown to be required for both normal tissue regeneration (with wile type Kras) and tumorigenesis (mutant Kras). Thus, an intervention that specifically reverses the mutant Kras activity without affecting

normal Kras signaling would be a therapeutically preferred. Although many studies have tried to identify epigenetic determinants of early chromatin alterations during pancreatitis, factors such as BRD4 and BRG1 ablation not only stops cancer formation, but also causes tissue atrophy . Thus, the effects of general chromatin factors such as Brd4 inhibitors are less than satisfying. An optimal intervention of cancer formation would be such measure that only prevent malignant transformation without affecting the ability of normal adult tissue to regenerate.
In this study, we set out to map the series chromatin switches from the earlies time point during acute pancreatitis to tumorigenesis, aiming to reveal a mechanism that can differentiate malignancy and tissue regeneration.
Key Words: Chromatin remodeler, pancreatic cancer, transcription factor

W64-5: A Mediator switch tunes transcription pausing to drive erythropoiesis

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The Mediator complex regulates various aspects of transcription and development, but how its compositional dynamics contribute to functional diversity remains unclear. Our study uncovers a Mediator shift towards dominance by MED26, reconfiguring chromatin for global transcription pausing and repression. MED26 steers erythroid fate determination during the hematopoietic stem and progenitor (HSPC) stage, while its dominant abundance in later stages distinctively facilitates transcription pausing. In the HSPC stage, over half of MED26 occupancy sites did not co-localize with MED1, a representative Mediator subunit. Notably, MED26-enriched loci were associated with RNA polymerase II pausing. MED26 manifested a markedly preferential recruitment of pausing-related factors, leading to an increase in Pol II pausing critical for genome-wide transcription repression in erythropoiesis. Moreover, MED26 exhibited pronounced condensate-forming capability, crucial for its function in promoting erythropoiesis and recruiting pausing-related factors. Collectively, this study unveils the mechanism of Mediator in transcription pausing, and highlights a single-subunit-dominated mechanism of the Mediator that transforms the transcription landscape for lineage commitment and differentiation.
Key Words: Mediator, transcription, erythropoiesis

W64-6: H3.3 Ser31 phosphorylation facilitates the facultative-to-constitutive heterochromatin consolidation during X chromosome inactivation

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Heterochromatin is crucial for silencing repetitive elements and restraining gene expression during development. Alterations of heterochromatin closely associate with the pathogenesis of various human diseases. Increasing evidences show that the histone variant H3.3 plays key roles in heterochromatin formation at repetitive genomic loci including retrotransposons and pericentromeric regions. However, the exact mechanism underlying the H3.3-primed heterochromatin establishment and/or maintenance remains largely unknown. Here, we demonstrate that the phosphorylation of H3.3 unique residue Ser31, which is absent from canonical histones H3.1/H3.2, is essential to heterochromatin instatement during two key developmental processes, retrotransposon silencing and X chromosome inactivation. Ser31 phosphorylation of H3.3 promotes H3K27me3 recognition of the Polycomb protein CBX2/7, which further recruits KAP1-SETDB1, enabling the formation of H3K9me-associated heterochromatin across the genome. Expression of the nonphosphorylatable H3.3 (H3.3S31A) or the CBX7 mutant that is defective in H3.3 pSer31 recognition (CBX7R22A) leads to global reduction of H3K9me3, along with the derepression of multiple types of retrotransposons. Importantly, these mutants also lead to defective H3K9me2/3 marks setup at inactivated X chromosome in a female mouse cell line, implying the significance of H3.3 Ser31 in the transition from facultative to constitutive heterochromatin during X chromosome inactivation. Taken together, our results reveal a mechanism by which H3.3 and its phosphorylation promotes the H3K27me3-to-H3K9me3 heterochromatin consolidation during X chromosome inactivation.
Key words: Histone variant H3.3, CBX7, KAP1, heterochromatin, consolidation, X chromosome inactivation, retrotransposon

Workshop 65: Neuroimmunology: Neuroimmune Interactions in Pain, Anesthesia, Cognition, Host Defense, and Cancer (Room 2H)

Chairs: Ru-Rong Ji, Zhihua Gao

W65-1:Immunotherapy for pain and dementia through neuroimmune modulation

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Pain typically begins with the activation of primary sensory neurons in the peripheral nervous system (PNS). Chronic pain, including conditions like arthritic pain, low-back pain, neuropathic pain, and cancer pain, affects approximately 10-20% of the global population. This widespread health issue involves complex dysregulations

of various neurocircuits within the central nervous system (CNS). There is increasing evidence indicating that both immune cells, such as macrophages and T cells, and glial cells, like microglia and astrocytes, in the PNS and CNS are crucial in the development, persistence, and resolution of chronic pain. My presentation will demonstrated how microglia in the spinal cord drives neuropathic pain after nerve injury. Additionally, I will discuss how immunotherapies, which have shown efficacy in treating various cancers, also play a role in pain management. Specifically, I will explore how the PD-L1/PD-1 and STING/IFN signaling pathways influence pain through neuroimmune modulation. Intriguingly, these immune pathways not only impact immune and cancer cells but also modulate neuronal signaling in pain, suggesting a broader therapeutic potential. Finally, I will discuss how emerging immunotherapy approaches could be pivotal in treating neurodegenerative disorders such as dementia and Alzheimer's Disease, highlighting the interplay between the immune system and nervous system and potential immunotherapies for neurological disorders.

W65- 2: Neuroimmune interactions in pain and host defense

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Neuroimmune crosstalk is critical for mucosal tissue physiology. However, the mechanisms by which sensory neurons communicate with local immune cells to mediate barrier protection at homeostasis and during inflammation are not well understood. Here, we find in the gut that nociceptor neurons are juxtaposed with and signal to intestinal goblet cells to drive mucus secretion and gut protection. Nociceptor ablation led to decreased mucus thickness and dysbiosis, while chemogenetic nociceptor activation or capsaicin treatment induced mucus growth. Nociceptors signal via the CGRP-Ramp1 pathway to induce rapid goblet cell emptying and mucus secretion. In the absence of nociceptors or epithelial Ramp1, mice showed increased epithelial stress and susceptibility to colitis. In another study we find in the lung that nociceptors regulate myeloid cells to protect host against influenza A virus infection. Taken together, our findings demonstrate a neuron-immune cell axis that orchestrates mucosal barrier protection and host defense.

W65-3: Crosstalk between nociceptive innervation and pancreatic ductal adenocarcinoma

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The emerging field of cancer neuroscience has demonstrated great progress in revealing the crucial role of the nervous system in cancer initiation and progression. Pancreatic ductal adenocarcinoma (PDAC), constituting 90% of pancreatic cancers, manifests as a highly malignant solid tumor characterized by insidious onset, strong invasiveness, and a notable propensity for recurrence or metastasis. Consequently, the 5-year survival rate for PDAC remained dismally low at approximately 10% in 2021. Notably, PDAC is characterized by perineural invasion and modulated by autonomic (sympathetic and parasympathetic) and sensory innervations. In this study, we further demonstrated that within the tumor microenvironment (TME) of PDAC, nociceptor neurons interacted with pancreatic stellate cells (PSCs) through calcitonin gene-related peptide (CGRP) and nerve growth factor (NGF). In PDAC patients, nociceptive innervation in tumor tissue is negatively correlated with the infiltration of NK cells while positively correlated with pain intensity. This association serves as an independent prognostic factor for both overall survival and relapse-free survival for PDAC patients. Our findings demonstrate that the crosstalk between nociceptor neurons and PSCs can modulate NK cell functions, contributing to the development of PDAC. Targeting nociceptor neurons or CGRP signaling may offer a promising therapy for PDAC and cancer pain.

W65- 4: The role of microglia in general anesthesia

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General anesthesia (GA) is an unconscious state produced by anesthetic drugs, which act on neurons to cause overall suppression of neuronal activity in the brain [1]. Recent studies have revealed that GA also substantially enhanced the dynamics of microglia, the primary brain immune cells, with increased processes motility and territory surveillance [2, 3]. However, whether microglia are actively involved in GA modulation remains unknown. Here, we report a previously unrecognized role for microglia engaging in multiple GA processes. We unexpectedly found that microglia ablation reduced the sensitivity of mice to anesthetics and substantially shortened duration of loss of righting reflex (LORR) or unconsciousness induced by multiple anesthetics, thereby promoting earlier emergence from GA. Microglia repopulation restored the regular anesthetic recovery, and chemogenetic activation of microglia prolonged the

duration of LORR. In addition, anesthesia-accompanying analgesia and hypothermia were also attenuated after microglia depletion. Single-cell RNA sequencing analyses showed that anesthesia prominently affected the transcriptional levels of chemotaxis and migration-related genes in microglia. By pharmacologically targeting different motility pathways, we found that blocking of P2Y12 receptor (P2Y12R) reduced the duration of LORR of mice. Moreover, genetic ablation of P2Y12R in microglia also promoted quicker recovery of mice from anesthesia, verifying the importance of microglial P2Y12R in anesthetic regulation. Our work presents the first evidence that microglia actively participate in multiple processes of GA through P2Y12R-mediated signaling and expands the non-immune roles of microglia in the brain.

Workshop 66: RNA Granule and Phase Separation (Room 3A)

Chairs: Yi Lin, Zhi-Ming Zheng

W66-1: Protein phosphatases govern the dynamics of transcriptional control

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RNA polymerase II progression from initiation to elongation is driven in part by a cascade of protein kinases acting on the core transcription machinery. Conversely, the corresponding phosphatases, notably PP2A and PP1—the most abundant serine-threonine phosphatases in cells—are thought to mainly impede polymerase progression, respectively restraining pause release at promoters and polymerase elongation at terminators. Our previous studies established the role of PP2A, mainly in the context of Integrator-PP2A (INTAC) in antagonizing master transcriptional activator P-TEFb kinase to attenuate the release of paused RNA polymerase II. Here we reveal an unexpected role of PP1, within the PNUTS-PP1 complex, in sustaining global transcriptional activation of protein-coding genes. Acute disruption of PNUTS-PP1, the predominant PP1-containing complex associated with active transcription, leads to severe defects in the release of paused polymerase and subsequent downregulation for the majority of transcribed genes. PNUTS-PP1 has a distinct substrate preference from PP2A within INTAC, and exhibits antagonistic functions compared to INTAC phosphatase, which generally inhibits pause release. Our research thus highlights the opposing roles of PP1 and PP2A in modulating genome-wide transcriptional pausing and gene expression.

W66-2: Circadian clocks are modulated by compartmentalized oscillating translation

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Terrestrial organisms developed circadian rhythms for adaptation to Earth's quasi-24-h rotation. Achieving precise rhythms requires diurnal oscillation of fundamental biological processes, such as rhythmic shifts in the cellular translational landscape; however, regulatory mechanisms underlying rhythmic translation remain elusive. Here, we identified mammalian ATXN2 and ATXN2L as cooperating master regulators of rhythmic translation, through oscillating phase separation in the suprachiasmatic nucleus along circadian cycles. The spatiotemporal oscillating condensates facilitate sequential initiation of multiple cycling processes, from mRNA processing to protein translation, for selective genes including core clock genes. Depleting ATXN2 or 2L induces opposite alterations to the circadian period, whereas the absence of both disrupts translational activation cycles and weakens circadian rhythmicity in mice. Such cellular defect can be rescued by wild type, but not phase-separation-defective ATXN2. Together, we revealed that oscillating translation is regulated by spatiotemporal condensation of two master regulators to achieve precise circadian rhythm in mammals.

W66-3: Guiding a transition phase from RNA processing bodies to stress granules by RNA helicase DDX6 and its two partners CNOT1 and 4E-T

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Two prominent cytoplasmic RNA granules, ubiquitous RNA-processing bodies (PB) and inducible stress granules (SG), regulate mRNA translation and are intimately related. We recently discovered that a Kaposi's sarcoma-associated herpesvirus RNA-binding protein, ORF57 or MTA, blocks the formation of both PB and SG during KSHV lytic infection. ORF57 inhibits PB formation by interacting with AGO2 and GW182 and blocks RISC formation in a miRNA-independent manner (NAR 47: 9368-9385, 2019), whereas ORF57 suppresses SG formation by interacting with PKR and PACK and preventing PKR activation and eIF2α phosphorylation (PLoS Pathog 13: e1006677 2017). In this study, we found that arsenite (ARS)-induced SG form in a stepwise process that is topologically and mechanically linked to PB. Two essential PB components, GW182 and DDX6, are repurposed under stress to play direct but

distinguishable roles in SG biogenesis. By providing scaffolding activities, GW182 promotes the aggregation of SG components to form SG bodies, while DEAD-box helicase, DDX6, is essential for the proper assembly and separation of PB from SG. DDX6 deficiency results in the formation of irregularly shaped 'hybrid' PB/SG granules with accumulated components of both PB and SG. Wild-type DDX6, but not its helicase mutant E247A, can rescue the separation of PB from SG in DDX6KO cells, indicating a requirement of DDX6 helicase activity for this process. DDX6 activity in biogenesis of both PB and SG in the cells under stress is further modulated by its interaction with two protein partners, CNOT1 and 4E-T, which knockdown resembles DDX6KO phenotype. Together, these data highlight a new functional paradigm between PB and SG biogenesis during stress. (Nucleic Acids Research 51: 9337-9355, 2023).

W66-4: The NS2B-PP1α-eIF2α Axis: Inhibiting Stress Granule Formation and Boosting Zika Virus Replication

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Stress granules (SGs), formed by untranslated messenger ribonucleoproteins (mRNPs) during cellular stress in eukaryotes, have been linked to flavivirus interference without clear understanding. This study reveals the role of Zika virus (ZIKV) NS2B as a scaffold protein mediating interaction between protein phosphatase 1α (PP1α) and eukaryotic initiation factor 2α (eIF2α). This interaction promotes eIF2α dephosphorylation by PP1α, inhibiting SG formation. The NS2B-PP1α complex exhibits remarkable stability, resisting ubiquitin-induced degradation and amplifying eIF2α dephosphorylation, thus promoting ZIKV replication. In contrast, the $NS2B^{V35A}$ mutant, interacting exclusively with eIF2α, fails to inhibit SG formation, resulting in reduced viral replication and diminished impact on brain organoid growth. These findings reveal PP1α's dual role in ZIKV infection, inducing interferon production as an antiviral factor and suppressing SG formation as a viral promoter. Moreover, we found that NS2B also serves as a versatile mechanism employed by flaviviruses to counter host antiviral defenses, primarily by broadly inhibiting SG formation. This research advances our comprehension of the complex interplay in flavivirus-host interactions, offering potential for innovative therapeutic strategies against flavivirus infections.

W66-5: G3BP1-centric network in stress granule and viral condensate formation

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Liquid-liquid phase separation (LLPS) orchestrated by G3BP1/2 (G3BP) proteins and non-translating mRNA confers the basis of stress granule (SG) assembly under various conditions. The conservation and commonality of this principle in different species have yet to be explored. Our analysis of the phylogenetic evolution of G3BP orthologs from unicellular yeast to mammals reveals the conserved and diverged features of G3BP protein. The protein interaction network facilitated by the NTF2L domain, especially the cooperativity it mediates, is a crucial determinant of their ability to nucleate SG in cells. The evolution of the G3BP1 network coincides with the exploitation of G3BP orthologs by certain viruses, which is reflected by the interaction between viral protein and G3BP orthologs from insects to vertebrates. Thus, by analyzing the G3BP ortholog as a tool, we dissected the importance of the G3BP interaction network in human SG formation and implicated the diverged function of this network during evolution. I will also describe our effort in dissecting a viral-G3BP interaction in viral condensate formation. This work provides further evidence that the G3BP1-centric network plays important roles in both SG formation and viral condensate formation.

W66-6:Potential Pathogenic Functions of Repeat Expansion RNAs

Boxun Lu[1], Yuyin Pan[1], and Shiping Zhang[1]

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The expansion of short tandem nucleotide repeats (e.g. CAG repeats) beyond a threshold can lead to a range of neurodegenerative diseases (Repeat Expansion Disorders, REDs), but the molecular mechanism governing this characteristic threshold remains unresolved. For most REDs, the mutant proteins containing expanded amino acid repeats are considered as the major pathogenic contributors by forming protein aggregates. In this talk, I'm going to discuss some of our recent efforts in elucidating such mechanisms by studying the cellular functions and properties of repeat expansion RNAs, especially the functional impact of cellular RNA condensates (foci) formed by sol-gel phase transitions.

Workshop 67: Cardiovascular and Renal Research (Room 3B)

Chairs: Zhe Han and Xiaolei Xu

W67-1:Precision Disease Modeling for Heart and Renal Diseases

Zhe Han, PhD

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The rapid increase in patient whole-exome sequencing (WES) or whole-genome sequencing (WGS) has led to the identification of large numbers of genetic variants that could be causal for various diseases. However, determining whether a potential disease variant truly causes disease is often challenging, due to the low number of patients with the same variant or the lack of knowledge about the affected gene and

variant. Functional validation for candidate disease variants in an established model system is in great demand for establishing disease causal association. Furthermore, a precision disease model with the exact patient variant for studying disease mechanisms and developing personalized treatments is also highly desirable.
My lab has developed a tissue-specific gene replacement approach in Drosophila to provide the much-needed functional validation for novel genetic variants identified from patient WES or WGS. I also established the Center for Precision Disease Modeling at the University of Maryland School of Medicine to provide this service to the public (anyone can submit their variant-of-interest online for a free evaluation of precision disease modeling feasibility). In the past decade, we have developed numerous Drosophila platforms to provide functional validation for candidate disease variants from patients with different types of diseases including heart diseases, renal diseases, leukemia and rare genetic diseases. In this talk, I will discuss how we established Drosophila as a model for congenital heart disease, dilated cardiomyopathy, and nephrotic syndrome. I will also present examples of Drosophila precision disease models for these three types of heart and renal diseases, and how to use them to establish genetic variant causal association, discover disease mechanisms, and test mechanism-based treatments.

W67-2:Heart development, regeneration and cardiovascular disease

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It is now recognized that majority of our genome is actively transcribed to produce thousands of non-coding transcripts in many cell types and tissues. Our research focuses on non-coding RNAs and RNA binding proteins (RBPs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs); and how they regulate expression and function of genes in the heart, as well as how dysregulation of their function is related to cardiac diseases.
Using combination of gain- and loss- of function approaches in animal models and molecular dissection, our studies establish the function of miRNAs, and RBPs in heart development, cardiomyocyte proliferation and cardiac regeneration. We will discuss some of our recent studies revealing the function of miRNAs and RBPs in regulating heart development and regeneration. Our results suggest that miRNAs, lncRNAs and RBPs could be novel therapeutic targets for cardiac regeneration.

W67-3:14-3-3 Binding Motif phosphorylation Disrupts Hdac4 Organized Condensates to Stimulate Cardiac Reprogramming

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Cell fate conversion is associated with extensive epigenetic and post translational modifications (PTMs) and architectural changes of sub-organelles and organelles, yet how these events are interconnected remains unknown. We report the identification of

a phosphorylationcode in 14-3-3 binding motifs (PC14-3-3) that greatly stimulates induced cardiomyocyte (iCM) formation from fibroblasts. PC14-3-3 has been identified in pivotal functional proteins for iCM reprogramming, including transcription factors and epigenetic factors. Akt1 kinase and PP2A phosphatase are a key writer and eraser of the PC14-3-3 code, respectively. PC14-3-3 activation induces iCM formation with the presence of only Tbx5. In contrast, PC14-3-3 inhibition by mutagenesis or inhibitor-mediated code removal abolishes reprogramming. We discover that key PC14-3-3 embedded factors, such as Hdac4, Mef2c, Nrip1, and Foxo1, form Hdac4 organized inhibitory nuclear condensates. Notably, PC14-3-3 activation disrupts Hdac4 condensates to promote cardiac gene expression. Our study suggests that sub-organelle dynamics regulated by a post-translational modification code could be a general mechanism for stimulating cell reprogramming and organ regeneration.

W67-4:A Hippo in the Heart

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The sinoatrial node (SAN) known as the natural cardiac pacemaker initiates cardiac electrical activity and maintains regular heartbeats. SAN dysfunction can result in different cardiac arrhythmias or even sudden death, and its incidence significantly increases with aging. Up to date, SAN is one of the most poorly understood cardiac entities with large lack of understanding of its molecular and genetic regulation. The Hippo signaling pathway is a fundamental pathway regulating cardiac development and regeneration, yet its role in the SAN remains largely unknown. This study encompasses a wide range of approaches, including mouse genetics, cell models, physiology studies, imaging, and next-generation sequencing techniques to study Hippo signaling in the adult SAN. We conditionally inactivated the Hippo signaling kinases in SAN pacemaker cells. Deficiency of the Hippo signaling caused cardiac arrhythmias in mice. We discovered that Hippo signaling maintains calcium homeostasis in pacemaker cells in the SAN. We also found that Hippo signaling non-autonomously regulates fibroblasts in the SAN through cell-cell communication and adhesion, partially mediated by Tgfb signaling. We reveal that the Hippo signaling is essential in maintaining SAN homeostasis.

W67-5:Cell migration in heart development and congenital heart disease

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Congenital heart defects (CHDs) are common birth anomalies, with outflow tract (OFT) defects comprising one-third of CHDs, leading to significant morbidity and mortality across all ages. Understanding the behavior of second heart field (SHF) cells and the molecular mechanisms driving OFT development has been challenging. Over the past ten years, studies in my lab have established gene networks governed by key cardiogenic transcription factors, including Tbx5, Gata4, and Osr1 in heart

development. We specifically aim to elucidate crucial molecules and signaling pathways guiding SHF cell migration, essential for OFT formation. We will discuss how we identified Hedgehog (Hh) signaling pathways and chemokine signaling as contributors to SHF cell migration through an integrated analysis of single-cell RNA sequencing (scRNA-seq) and spatial transcriptome data, validated by traditional genetic approaches in both mice and Drosophila models.

W67-6:Novel Mechanisms and Therapies for Chemotherapy-induced Cardiotoxicity

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While chemotherapy such as anthracyclines has been effective in treating cancer patients, the related cardiotoxicity severely limits their use. No mechanism-based therapies are available to treat anthracycline-induced cardiotoxicity (AIC). To decipher genetic basis of AIC, we leveraged adult zebrafish as an efficient vertebrate model. We developed a forward genetic screen-based approach that enables systematic discovery of genetic modifiers. Detailed genetic studies of mtor and rxraa, two salutary modifiers, uncovered spatiotemporally specific mechanisms of their modifying effects, and prompted two mechanism-based therapeutic strategies for anthracycline-induced cardiotoxicity. Moreover, we implement a MMEJ-based genome editing technology for establishing genotype-phenotype relationship in F0 adult zebrafish, enabling rapid discovery of AIC modifiers. From a pilot screen of candidate cardiomyopathy pathways, we identified Erk signaling as a potential therapeutic signaling. In summary, our work established adult zebrafish as a powerful vertebrate model for discovering new genes for chemotherapy-induced cardiotoxicity, accelerating the development of mechanism-based therapies.

Day 4, July 29 (Monday)

Concurrent workshops 68-76, 1:30 – 3:00pm

Workshop 68: SARS-CoV-2 and COVID-19: from Molecular Virology to Vaccine Development (Room 2A)

Chairs: Shan-Lu Liu, Dongyan Jin

W68-1: Evolution and entry mechniasm of SARS-related coronaviruses from wildlife

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In the past 20 years, two different strains of SARS-related coronaviruses (SARSr-CoV), SARS-CoV and SARS-CoV-2, have caused pandemics. Both of them are considered to have a bat origin as the closely-related CoVs were discovered in bats. Furthermore, civets and pangolins were considered as accident hosts of SARS-CoV and SARS-CoV-2 related viruses. Bat SARSr-CoV are carried by different rhinolophus species which are only distributed in the Old World. Our group collected around one thousand SARSr-CoV positive samples from bats in the past 20 years and found the phylogenetically close relatives of SARS-CoV and SARS-CoV-2 in restrict areas of Yunnan province. Phylogenetic analysis of know SARSr-CoV sequences found by us and other teams indicates that bat SARSr-CoVs are divided into at least seven sublineages with different geographic distribution. Bat SARSr-CoVs show similar evolution pattern as SARS-CoV-2, but higher genetic diversity. The major differences of these SARSr-CoV are located in the spike proteins which are responsible for virus entry, induce the neutralization antibody and pathogenicity. SARS-CoV-1 and -2 utilize the same receptor ACE2, while bat SARSr-CoVs can be divided into ACE2-dependent and-independent group. Some ACE2-dependent bat SARSr-CoVs have a high binding affinity to human ACE2 and efficiently infect human ACE2 transgenic mice and wild type hamsters showing different severity of lung damage. These results showed that some bat SARSr-CoVs have potential interspecies transmission. Active surveillance and the countermeasures should be prepared in advance.

Key Words: SARS-related coronavirus, entry receptor, ACE2, pathogenicity

W68-2: Zoonotic and reverse zoonotic transmission potential of SARS-CoV-2 and its related CoVs

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SARS-CoV-2 is believed to have been originated from bat coronavirus (CoV), and both SARS-CoV-2 and its closely related bat CoVs not only infect humans but also various animals, posing zoonotic and reverse zoonotic risks. In this study, we first determined potential host susceptibility of two bat CoVs BANAL-20-52 and 236 among different bat species and animal species, and found both might have extensive host ranges, indicating high zoonotic transmission potential. We also determined the cryo-EM structures of BANAL-20-52 and BANAL-20-236 S proteins, and found that both trimeric S proteins adopt all three receptor binding domains (RBDs) in "closed" conformation and the unique sugar moiety at N370 of bat SC2r-CoVs acts like a "bolt" and crosses over two neighboring subunits, facilitating the S proteins in the "closed" conformation. We further found that the highly conserved sugar moiety at N370 might result from the selective advantages in stability of S prpotein during the fecal-oral transmission and better immune evasion during virus evolution.
As SARS-CoV-2 rapidly evolves, newly emerged omicron variants like BA.2.86 and JN.1 have become dominant globally. We also determined the reverse zoonotic potential of XBB.1.16, EG.5.1, BA.2.86, and JN.1 among different animal species, and found that, similar to WT, the omicron variants also exhibited potential broad host ranges, but JN.1 displayed substantially higher overall reverse zoonotic risk potential than other variants except for EG.5.1. Further mechanistic analysis revealed that L455S mutation in JN.1 S proteins might be responsible for significant decrease in overall receptor binding affinity but substantial increase in overall fusogenecity and infectivity with various animal ACE2s and hACE2. L455S change slightly decreased S protein thermostability, likely resulting in lower the overall energy barrier required for conformational changes of S protein after receptor binding.
Together, our findings aid a better understanding of the molecular basis of CoV entry, selective evolution, and immunogenicity and highlight the importance of surveillance of susceptible hosts of these viruses to prevent potential outbreaks.
Key Words: SARS-CoV-2, reverse zoonotic transmission, ACE2, bat coronavirus,

W68-3: Development of live attenuated SARS-CoV-2 vaccines
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Live attenuated SARS-CoV-2 vaccines stimulate host immunity of all different arms and stages. Similar to a natural infection, they are more efficient in blocking viral transmission when compared to existing vaccines in use. Here we present two

strategies in the development of live attenuated vaccines constructed on a molecular clone of SARS-CoV-2. The first is dual inactivation of NSP16 and ORF3a and the second is trans complementation of a spike (S)-deleted virus with S expression from cultured cells. In the first approach, ORF3a accessory protein was inactivated by inverting its open reading frame, whereas a point mutation was introduced to NSP16 leading to a catalytically dead 2'-O-methytransferase. In the second approach, a single infectious cycle SARS-CoV-2 was constructed by complementing a ΔS virus with S expression in trans in cultured cells. Both viruses appeared to be highly attenuated in hamsters and K18-hACE2 transgenic mice. Intranasal vaccination with these viruses significantly stimulated humoral, cell-mediated and mucosal immune responses, conferring sterilizing protection against SARS-CoV-2 Delta and Omicron variants. A version of recombinant viruses expressing the XBB.1.16 S protein offered better protection to circulating variants. In addition, they were highly efficacious boosters for protection of against the Omicron subvariants, with full blockade of viral transmission. Overall, our work established a platform for generating safe and effective live attenuated vaccines against SARS-CoV-2. Supported by National Key R&D (2021YFC0866100, 2023YFC3041600 and 2023YFE0203400), HMRF (COVID190114, CID-HKU1-9 and 23220712), RGC (C7142-20GF and T11-709/21-N) and ITF (MHP/128/22) grants.

W68-4: Structural basis of broad and potent antibodies that neutralize all SARS-CoV-2 variants

Lingshu Wang[1], John Misasi[1], Quenelle McKim[1], Maryam Musayev[1], Rosemarie Mason, Qiong Zhou[1], Qi Qiu[1], Wei Shi[1], Misook Choe[1], Eun Sung Yang[1], Yi Zhang[1], I-Ting Teng[1], David Van Wazer, Sabrina Bush, Tatsiana Bylund[1], Claudia Jenkins[1], Tyler Stephens[2], Evan Cale, Sijy O'Dell1, Emily Tourtellott-Fogt[1], Sarah Kerscher[1], Chaim Schramm[1], Amy R. Henry[1], Danielle Wagner[1], Baoshan Zhang[1], Yaroslav Tsybovsky[2], Daniel Douek[1], Nancy J Sullivan[1], Peter D Kwong[1], Richard Koup, Theodore Pierson[1], Nicole Doria-Rose[*1], **Tongqing Zhou[*1]**

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The continued evolution of SARS-CoV-2 after the emergence of B.1.1.529 (Omicron) variant has brought resistance to neutralization by vaccinee and convalescent sera. To search for monoclonal antibodies with broad neutralization, we carried out B cell isolation by single-cell sorting with SARS-CoV-2 variant spike protein probes from vaccinees and omicron BA.1 breakthrough subjects. Sorted B cells underwent rapid assembly, transfection and production of immunoglobulin (RATP-Ig). Neutralization assay with isolated antibodies indicated these subjects can develop cross-reactive antibodies against most recent variants including JN.1 sublineages and other sarbecoviruses. Some antibodies even neutralize the current KP.3 variant. Most potent

and broad antibodies identified target class I and IV epitopes on the SARS-CoV-2 RBD. Neutralization performed with antibody cocktails indicated that class I and III antibody pairs neutralize with synergistical effects. Protection and challenge studies in in hamsters showed that class I-IV antibodies are protective against EG.5.1 when given as monotherapy. To elucidate the structural basis of neutralization, we solved the cryo-EM structures of antibodies in complex with spikes of various SARS-CoV-2 variants. Structural data revealed different machanisms by which the class I tyo IV antibodies neutralize SARS-CoV2 viruses and other sarbecoviruses: several class I antibodies eveloved to have cavities on the complementarity determinant region to accommendate variations on RBD interfaces. One antibody, which broadly neutralizes all SARS-CoV-2 variants, SARS-CoV and SARS-CoV-like varius, binds to a class IV epitope with significantly smaller epitope surface area. Sequence analysis indicated that this epitope region is highly conserved between sarbecoviruses. The isolation of broad neutralizing antibodies and structural definintion of highly conserved epitopes on the spike of sarbecoviruses may provide medical counter measurements for emergeing new SARS-CoV-2 variants and guide the design of next generation of vaccine that overcomes extensive spike mutations.
Key Words: SARS-CoV-2, Variant of concern, antibody, Receptor-binding domain

W68-5: Pathogenicity and virological features of emerging SARS-CoV-2 Omicron variants

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SARS-CoV-2 Omicron first appeared in November 2021 has caused substantial waves of outbreak on a global scale. The pathogenicity of Omicron has been concerning due to its unusually high transmissibility and robust immune escape. We revealed that the pathogenicity of the Omicron variant is significantly attenuated and demonstrated that the Omicron spike is less efficient in utilizing transmembrane serine proteases, which reduces its efficiency of facilitating cell surface entry, leading to reduced virus replication and ameliorated damage in the lung. As Omicron sublineages continue to evolve, we reveal that the pathogenicity of the subsequent Omicron subvariants continue to attenuate in the lung and is becoming more robust in their replicaiton fitness in the human nasal epithelial cells, which may contribute to their efficient virus spread. These findings have major impacts on the regional and international measures on COVID-19 prevention and control, and contribute to our understanding on the virus-host interaction and adaption of coronaviruses in the human population.
Key Words: SARS-CoV-2, Omicron, pathogenicity, virological features, virus entry, protease, transmissibility

W68-6: Requirement of Niemann-Pick C1 (NPC1) for SARS-CoV-2 Entry

Ilyas Khan[1], Sunan Li[1], Lihong Tao[1], Chong Wang[1], Bowei Ye[2], Huiyu Li[2], Xiaoyang Liu[1], Iqbal Ahmad[1], Wenqiang Su[1], Gongxun Zhong[1], Zhiyuan Wen[1], Jinliang Wang[1],

Rong-Hong Hua[1], Ao Ma[2], Jie Liang[2], Xiao-Peng Wan[1], Zhi-Gao Bu[1], **Yong-Hui Zheng[3]***
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NPC1 is a multi-pass transmembrane protein on the late-endosomal membrane that redistributes cholesterol from late endosomes to the other cellular membranes. In addition, NPC1 serves as a receptor for filoviruses. Recently, we reported that NPC1 plays an important role in SARS-CoV-2 entry (PMID:38167417). To develop Ebolavirus antivirals, we identified natural compounds Tubeimosides as novel NPC1 inhibitors that block filovirus entry. Notably, Tubeimosides strongly inhibited SARS-CoV-2 infection at IC50 below 100 nM, indicative of a requirement of NPC1 for SARS-CoV-2 entry. Using in-silico, biochemical, and genomic approaches, we demonstrate that NPC1 binds to SARS-CoV-2 spike (S) protein on the receptor-binding domain (RBD) via its luminal loop C (NPC1-C) that also binds to Ebola virus RBD, and this binding is disrupted by Tubeimosides. After knocking out NPC1 in CHO, Vero, A549, Caco2, and Calu3 cell lines, we demonstrate that SARS-CoV-2 infection is strongly inhibited in these knockout cells. These results suggest that NPC1 is required for SARS-CoV-2 fusion in late endosomes during the endocytic entry. We are now investigating the role of NPC1 in cell-cell transmission and its contribution to cell-cell fusion during SARS-CoV-2 infection. We suggest that in addition to the cell surface ACE2, NPC1 is a critical host factor for SARS-CoV-2 entry.
Key Words: SARS-CoV-2; NPC1; Ebola; virus entry; COVID19; endosomes.

Workshop 69: From Molecules to Diseases: Unraveling Endocrine Biology and Metabolic Disorders (Room2B)

Chairs: Shuibing Chen, Sheng Wu

W69-1:Single-cell multiomics guided mechanistic understanding of Fontan-associated liver disease

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The Fontan operation is the current standard of care for single-ventricle congenital heart disease. Individuals with a Fontan circulation (FC) exhibit central venous hypertension and face life-threatening complications of hepatic fibrosis, known as Fontan-associated liver disease (FALD). The fundamental biology and mechanisms of FALD are little understood. We recently generated the first transcriptomic and epigenomic atlas of human FALD at single-cell resolution using multiomic snRNA-ATAC-seq. We discovered profound cell type-specific transcriptomic and epigenomic changes in FC livers. Central hepatocytes (cHep) exhibited the most significant changes, featuring profound metabolic reprogramming. These cHep changes preceded substantial activation of hepatic stellate cells and liver fibrosis, suggesting cHep as a potential first "responder" in the pathogenesis of FALD. We also identified a network of ligand-receptor pairs that transmit signals from cHep to hepatic stellate cells, which may promote their activation and liver fibrosis. Together with metabolomics studies, our single-cell transcriptomic and epigenomic atlas revealed mechanistic insights into the pathogenesis of FALD and may aid identification of potential therapeutic targets.

W69-2:Targeting pancreatic islet beta cells

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Impairment of beta cell function and loss of beta cell mass cause both forms of diabetes, yet existing diabetes medicines only provide symptomatic treatment with no direct benefits on beta cells. Accumulating evidences indicate that phenylbutyrate (PB) may preserve β-cell function and survival. PB is safe and well-tolerated in human and even children. However, its therapeutic potential in diabetes is limited by its short half-life in the circulation, and by the lack of targeting specificity to β-cells in vivo. To unlock the therapeutic potential of PB to enhance beta cell fitness, we are developing a beta cell targeted delivery strategy based on a beta cell targeting motif (BTM) we recently invented. Conjugating PB with BTM leads to BTPB, a beta cell targeted PB. We observed highly efficient, cellular zinc-dependent uptake of BTPB by islet beta cells. We assayed the biological activity of BTPB in MIN6 and INS-1 cell cultures exposed to palmitate or inflammatory cytokines, and confirmed its cytoprotective activity in stressed beta cells. Impressively, compared to the parent drug PB, BTPB's potency in beta cell protection was enhanced by more than two orders of magnitude. BTPB also enhanced insulin secretion in INS1 cells. In a type 1 diabetes mouse model, BTPB protected female NOD mice from developing diabetes with a remarkable efficacy (75% diabetes free with BTPB vs. 25% with vehicle control or equimolar PB, $p<0.01$). The protective effect appeared to be mediated through islet beta cells instead of immune system since we did not detect a change in T-cell infiltration of pancreatic islets. Collectively, through the targeted delivery of PB prodrugs to β-cells, the reported benefits of PB on reducing ER stress and promoting cell survival were augmented, ultimately resulting in the preservation of

β-cell function and restoration of physiological glycaemic control.

W69-3：Androgen Regulates Glucose Homeostasis and Energy Expenditure in Lean Female Mice

Sheng Wu

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Hyperandrogenemia and polycystic ovary syndrome are a result of the imbalance of androgen levels in females. Androgen receptor (Ar) mediates the effect of androgen, and this study examines how neuronal or hepatic Ar mediates metabolism under normal and increased androgen conditions in female mice. A glucose tolerance test revealed impaired glucose tolerance that was partially alleviated in the neuronal or hepatic AR knockout-dihydrotestosterone (DHT) mice compared with Con-DHT mice after 4 months of DHT treatment. Heat production and food intake was higher in Con-DHT mice than in Con-veh mice; these effects were not altered between ARKO-veh and ARKO-DHT mice, indicating that excess androgens may partially alter calorie intake and energy expenditure in females via the neuronal or hepatic Ar. The pAkt/Akt activity was higher in the hypothalamus in Con-DHT mice than in Con-veh mice, and this effect was attenuated in neuronal ARKO-DHT mice. Western blot studies show that markers of inflammation and microglia activation, such as NF-kB p-65 and IBA1, increased in the hypothalamus of Con-DHT mice compared with Con-veh. These studies suggest that neuronal or hepatic Ar mediates the metabolic impacts of androgen excess in females.

W69-4: MeBoCost reveals metabolite-mediated intercellular signaling in obesity by scRNA-seq

Rongbin Zheng[1,2,#], Yang Zhang[3,4,#], Tadataka Tsuji[3,4], Xinlei Gao[1,2], Farnaz Shamsi[3,4], Allon Wagner[5,6,7], Nir Yosef[5,6,7,8], Kui Cui[9,10], Hong Chen[9,10], Michael A. Kiebish[11], Juan J. Aristizabal-Henao[11], Niven R. Narain[11], Lili Zhang[1,2], Yu-Hua Tseng[3,4,*], **Kaifu Chen[1,2,12*]**

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We introduce MEBOCOST, a novel algorithm combining single-cell RNA-seq with flux balance analysis to infer metabolite-mediated cell-cell communications (mCCC). The algorithm identifies mCCC events where metabolites are secreted by sender cells and travel to interact with sensor proteins of receiver cells in a tissue. MEBOCOST includes a manually curated database of metabolite-sensor partners and defines sender and receiver cells based on rates of metabolite efflux and influx, along with expression levels of enzyme and sensor genes, respectively. Comprehensive benchmarking analyses demonstrated the robustness of MEBOCOST. Spatial transcriptomics analysis verified that the mCCC events detected by MEBOCOST more frequently occurred among spatially proximal cell types than distant ones. CRISPR screen data and patient survival analysis confirmed that MEBOCOST uncovered functionally important mCCC events between cancer and NK cells in colorectal tumor samples. In application to mouse brown adipose tissue, MEBOCOST successfully recaptured known and unveiled new mCCC events. When applied to human visceral white adipose tissue, MEBOCOST effectively delineated mCCC dynamics during obesity in samples from individuals with varying body mass. Therefore, MEBOCOST is a valuable tool for researchers investigating mCCC in diverse biological contexts and disease samples. The MEBOCOST software is freely available at https://github.com/zhengrongbin/MEBOCOST.

W69-5：RNA G-quadruplexes in obesity-associated diseases

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RNA G-quadruplex (G4) is a four-stranded structure formed by guanine (G)-rich RNA sequences, which contains two or more layers of hydrogen-bonded G-quartets. RG4s have been shown to regulate diverse fundamental biological processes, including transcription, pre-mRNA splicing, mRNA localization and translation, and participate in a number of human pathologies and diseases, such as cancer, virus infection and neurological diseases, representing potential targets for treatment of these diseases. However, the involvement of RG4s in metabolism and metabolic disorders is poorly understood. Our recent work reports a role of RG4s in metabolism and obesity-associated diseases. We identified a G-rich sequence in pre-miR-26a-1 that can fold into RG4 structure, thereby impairing miR-26a maturation in vitro and in vivo. Using miR-26a knockin or knockout mouse models, we showed that this RG4 affects lipid metabolism and insulin sensitivity. Moreover, we revealed DHX36 as a helicase unfolding this RG4 and thus defined a dynamic DHX36/RG4/miR-26a regulatory axis during obesity. With the emergence of COVID-19, we also explored the involvement and therapeutic potential of RG4s in this disease. We characterized the existence of RG4s in both SARS-CoV-2 genome and several host factors, including ACE2, AXL, FURIN and TMPRSS2, and identified Topotecan and Berbamine, two approved drugs, as novel RG4-stabilizing agents that can repress

RG4-containing host factors and protect cells and mice against SARS-CoV-2 infection. Together, these findings highlight the significance of RG4 in obesity-associated diseases and provide an attractive broad-spectrum therapeutic strategy for human diseases.

W69-6：Evolutionary bifurcating neuroendocrine regulation via the autonomous nervous system

Feng Rao[1]

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How zeitgebers differentially entrain diurnal and nocturnal animals has remained mysteric. In this talk, we describe a nocturnal-specific brain-body axis that responds to morning light or acute light-at-night (LAN) by secreting insulin, which in turn promotes glucose storage while inhibiting lipolysis-driven non-shivering thermogenesis in brown adipose tissue. Chemo-genetic and lesioning studies reveal a photic sensing neurocircuit originating from the melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) and stimulating pancreatic islets via the parasympathetic nervous system (PSNS). Mice with β-cell-specific depletion of 5-diphosphoinositol pentakisphosphate (5-IP7), a cholinergic stimuli-sensing 2nd messenger, resist LAN-induced insulin secretion and cooling. Interestingly, diurnal mammals do not display LAN-induced insulin secretion and thermogenesis inhibition, which correlates with their degenerate parasympathetic control of islets. These findings unveil a light-sensing neuroendocrine axis marking evolutionary bifurcation between nocturnality and diurnality.

Workshop 70: Rising Insights for Prostate Cancer Mechanisms and Therapies (Room 2C)

Chairs: Changsheng Zhao,Rendong Yang

W70-1:Epigenetic determinants of prostate cancer lineage plasticity

Jindan Yu[1]

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Neuroendocrine (NE) transformation of prostate cancer represents a lethal progression of the disease that is a major challenge in clinical management nowadays. This aggressive subtype of prostate cancer, termed NE prostate cancer, exhibits a distinct transcriptional program characterized by a complete loss of the androgen receptor and a gain of NE gene expression. However, the molecular underpinning of NET that leads to this cell identity switch has not been illustrated. In my talk, I will discuss how we use single-cell multiome, Nanopore Long-read sequencing, and Hi-C approaches to trace clonal expansion and transformation and delineate the critical regulators. We

demonstrate that a set of lineage-specific transcription factors cooperate to alter global and regional DNA demethylation, NE enhancer priming and activation, and 3D chromatin re-organization, ultimately resulting in transcriptional reprogramming and cell identity switch.

W70-2：Synthetic lethal combination therapy targeting the ATR/CH1/WEE1 axis in advanced prostate cancer

Wenxiao Zheng, Yuzhou Chen, **Qiming Jane Wang[1]**

[1]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Castration-resistant prostate cancer (CRPC) poses a significant clinical challenge, partly due to its high heterogeneity. There is a pressing need to develop effective treatments against all subtypes of CRPC. Frequent mutations or loss of tumor suppressor genes render CRPC more vulnerable to therapies targeting DNA damage response (DDR) pathways and G2/M cell cycle checkpoint. The ataxia telangiectasia and Rad3-related (ATR), CHEK1 (CHK1), and WEE1 kinases are crucial regulators of DDR and G2/M cell cycle. Although inhibitors of these kinases are under clinical trials for various cancers, their efficacy in prostate cancer, especially CRPC and neuroendocrine prostate cancer (NEPC), has not been thoroughly investigated. We reported aberrant upregulation of WEE1 and CHK1 in CRPC/NEPC cells and tumors. Inhibition of WEE1 by AZD1775 and CHK1 by SRA737 synergized in killing of CRPC/NEPC cells, regardless of their genetic backgrounds. This drug combination also synergized in suppressing tumor growth and metastases, and improving overall survival in a transgenic mouse model of NEPC. In contrast, combining the ATR inhibitor ceralasertib with the WEE1 inhibitor AZD1775 did not show significant synergy in CRPC/NEPC cells. Notably, SRA737 demonstrated significant anti-tumor and anti-metastatic effects as a single agent in vivo, underscoring its potential for treating advanced prostate cancer. Additionally, we identified the lysine demethylase 5D (KDM5D) as a novel regulator of CHK1. Overexpression of KDM5D induced CHK1 and enhanced tumor sensitivity to genotoxic chemotherapy and the CHK1 inhibitor SRA737, demonstrating its potential as a biomarker for CHK1 inhibitors. In conclusion, the synthetic lethal combination of CHK1 and WEE1 inhibition represents a promising treatment strategy for CRPC and NEPC. KDM5D may be useful in stratify patients receiving CHK1 inhibitors or genotoxic chemotherapies.

W70-3：Comprehensive Analysis of DNA Methylation in Prostate Cancer

Changsheng Zhao[1]

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Alterations in DNA methylation patterns, especially shifts in 5mC and 5hmC levels, are intricately linked with the onset and progression of prostate cancer. These modifications hold promise as potential biomarkers for both the detection and prognosis of the disease, as well as targets for epigenetic treatments. The implications

of 6mA methylation in prostate cancer remain less defined, prompting the need for more extensive research to uncover their exact roles and clinical relevance. In my presentation, I will detail how we use different techniques, including MeDIP-seq, nano-hmC-Seal, WGBS, RRBS, Nanopore and DiMeLo-seq to identify 5mc, 5hmc, and 6ma methylations in prostate cancer. Moreover, I will elaborate on how our findings, derived from various analytical pipelines and methodologies, can be invaluable resources for wet-lab researchers.

W70-4：Identify genetic determinants of immunotherapy targeting B7-H3

Wei Shi[1], **Di Zhao[1]**

[1]*Department of Experimental Radiation Oncology; The University of Texas MD Anderson Cancer Center, Houston, TX*

Genetic inactivation of tumor suppressors PTEN and TP53 is common in malignancies, but treatment options for patients carrying these alterations are limited. To identify PTEN- and TP53-associated immune checkpoints, we performed multi-omics analyses of 51 checkpoint molecules in cancer patients and identified B7-H3 as the most abundant immune checkpoints in tumors containing PTEN and TP53 deficiencies. B7-H3, encoded by the CD276 gene, has emerged as a promising therapeutic target; however, much remains to be understood regarding its role in cancer progression, predictive biomarkers for B7-H3 targeted therapy, and combinatorial strategies. In this study, we identified that genetic defects in PTEN and TP53 are key determinants of immunotherapy targeting B7-H3. Our mechanistic study revealed that PTEN-AKT signaling co-opts with the p53 pathway to inhibit B7-H3 expression by suppressing transcriptional factor Sp1. By generating a novel tissue-specific Cd276 deletion genetically engineered mouse model, we demonstrate that B7-H3 is required for promoting cancer progression and immunosuppression of T and NK cells in Pten/p53 deficient tumors. Prostate cancers show modest responses to checkpoint immunotherapy but have frequent PTEN and TP53 deficiencies. In preclinical models, we showed that the monoclonal antibody against B7-H3 exhibited anti-tumor effects by increasing T cell infiltration; however, the enriched regulatory T cells and elevated PD-L1 expression in myeloid cells in the tumor microenvironment hindered its therapeutic efficacy. Finally, we demonstrate that B7-H3 inhibition combined with blockade of PD-L1 or CTLA-4 can achieve durable effects and have curative potential in cancers with PTEN/TP53 deficiency. Given that B7-H3 targeted therapies have been evaluated in early clinical trials, our studies provide new insights into the potential of biomarker-driven combinatorial immunotherapy targeting B7-H3 in malignancies.

W70-5：Uncovering structural variations and splicing variants of androgen receptor inprostate cancer

Rendong Yang[1]

[1]*Department of Urology, Northwestern University Feinberg school of medicine,Chicago, IL*

Prostate cancer (PCa) is the most frequently diagnosed male cancer in the U.S. If primary treatment modalities are not capable of controlling the disease, inhibiting androgen receptor (AR) activity is an effective treatment for advanced prostate cancer patients. However, the duration of patient responses to AR-targeted therapy is variable, and all patients will ultimately develop resistance and progress to a castration-resistant form of the disease associated with morbidity and death. Our previous in vitro studies suggest genomic rearrangements in the AR gene underlie synthesis of variant AR mRNA species encoding COOH-terminally truncated, constitutively active AR proteins that can support androgen-independent growth of prostate cancer cells. Based on this finding, we hypothesize that detection of AR gene rearrangements could help predict drug response. Here, we developed an integrated structural variation (SV) and RNA alternative splicing (AS) detection pipeline which leverages multiple SV and AS signals for studying the spectrum of AR gene rearrangements and splicing with high accuracy and resolution. With our analysis, we have identified diverse AR gene rearrangement events in the tumor genomes and aberrant splicing event in tumor transcriptome from castration-resistant PCa patients. Overall, these data indicate that AR gene rearrangements are frequent, yet non-recurrent, in CRPC patients. This knowledge supports future studies designed to understand the impact of these AR gene rearrangements on AR mRNA and protein expression patterns, as well as patient responses to AR-targeted therapy.

W70-6：The short-chain fatty acid acetate modulates epithelial-to-mesenchymal transition

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Normal tissue and organ morphogenesis requires epithelial cell plasticity and conversion to a mesenchymal phenotype through a tightly regulated process—epithelial-to-mesenchymal transition (EMT). Alterations of EMT go far beyond cell-lineage segregation and contribute to pathologic conditions such as cancer. EMT is subject to intersecting control pathways; however, EMT's metabolic mechanism remains poorly understood. Here, we demonstrate that transforming growth factor β (TGF-β)–induced EMT is accompanied by decreased fatty acid oxidation (FAO) and reduced acetyl-coenzyme A (acetyl-CoA) levels. Acetyl-CoA is a central metabolite and the sole donor of acetyl groups to acetylate key proteins. Further, the short-chain fatty acid acetate increases acetyl-CoA levels–-robustly inhibiting EMT and cancer cell migration. Acetate can restore EMT-associated α-tubulin acetylation levels, increasing microtubule stability. Transcriptome profiling

and flow cytometric analysis show that acetate inhibits the global gene expression program associated with EMT and the EMT-associated G1 cell cycle arrest. Taken together, these results demonstrate that acetate is a potent metabolic regulator of EMT and that therapeutic manipulation of acetate metabolism could provide the basis for treating a wide range of EMT-linked pathological conditions, including cancer.

Workshop 71: Leukemia and Pre-Leukemia: Mechanisms, Risks, and Therapeutic Opportunities (Room 2D)

Chairs: Ruibao Ren, Jun Lu

W71-1: Inflammation and MDS

Yan Liu, Northwestern University

W71-2: Risk Assessment of Clonal Hematopoiesis and Interventions

Ruibao Ren, Shanghai Jiaotong University

W71-3: Transcription Hijack by Mutant NPM1 in Acute Myeloid Leukemia

Xiaotian Zhang, University of Texas Health Center at Houston

W71-4: New Therapeutic Opportunities Targeting Leukemia Stem Cells and Their Niche

Hong Qian, Karolinska Institute

W71-5: Targeting NUP214 Eradicates Leukemia Stem Cells by Inducing Ferroptosis

Yu Hou, Congqing Medical University

Workshop 72: Pharmacology Considerations for Therapies (Room 2E)

Chairs: Wen Liu, Huichang Bi

W72-1： Gut-brain axis in depression: metabolic insights and therapeutic potential

Wei Jia

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Recent studies suggest a complex interplay between gut microbiota, metabolism, and depression. However, the underlying mechanisms of the gut-brain axis remain unclear. To address this, we analyzed a cohort of patients with first-episode depression to

investigate alterations in fecal metagenome and serum metabolome. Our findings revealed a depletion of gut bacterial species, including *Roseburia intestinalis* and *Bifidobacterium longum*, as well as the neurotransmitter homovanillic acid (HVA) in depressed patients and mouse models. Interestingly, *R. intestinalis* does not directly produce HVA, but can enhance the abundance of *B. longum*, which in turn generates HVA, highlighting a synergistic interaction among gut microbiota in regulating intestinal neurotransmitter production. We administered HVA and *B. longum* to mouse models of chronic unpredictable mild stress and corticosterone-induced depression, resulting in significant improvements in depressive symptoms. Mechanistically, HVA inhibited synaptic autophagic death by preventing excessive degradation of LC3 and SQSTM1/P62 proteins, thus protecting hippocampal neurons' presynaptic membrane from damage. Overall, our findings underscore the role of the gut microbiota in modulating synaptic integrity and highlight potential novel treatment strategies for depression. These insights could pave the way for the development of innovative therapies that target the gut-brain axis and provide hope for patients with depression.

W72-2：Ligand identificaiton and micro-environmental pharmacology of GPCRs

Jinpeng Sun

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Since starting my laboratory in 2011, I have focused on G protein coupled receptors, in particular, the ligand identification, physiological functions and the molecular mechanism of biased signaling of GPCRs. Our first main research aspect is the identification of endogenous ligand of GPCRs. We have identified the receptor subfamily to sense the steroid hormones. For instance, membrane receptor GPR97 is able to sense glucocorticoid to mediate its rapid actions, the progesterone and 17-hydroxyprogesterone membrane receptor are GPR126. We also identified DHEA, DHEAS and DOC are endogenous ligands of GPR64 etc (Nature, 2021a, Nat Chem Biol 2022, PNAS 2022b). By collaboration with Prof. Wei Kong, we found Nidogen as the agonist of LGR4 and the COMP is an endogenous allosteric ligand of AT1R (Circulation Research 2022, Cell Research 2021). By collaboration with Prof. Xiao Yu, we identified that Olfr109 could recognized denatured insulin and insulin fragment to control the insulin quality (Cell Metabolism 2022). Our second main research aspect is dissecting the molecular mechanism underlying sensation of force, ordor and itch by GPCRs. We have elucidated the mechanism of receptors' perception of itch, olfactory and force (Cell 2023, Nature 2021b, 2022a, 2022b, 2023a, 2023b). Our third main research aspect is the working mechanism of GPCR. For arrestin mediated biased signaling, we have proposed a "flute model" to explain the arrestin mediated GPCR functions (Nature communications,2015; Molecular Pharmacology,2017; Recommended by Faculty 1000, Nature Chemical Biology 2018). We proposed that the 10 distinct phosphorylation interacting sites along the N-terminal of arrestin is the "phospho-code" reader of the arrestin, which recognized the information passed by GPCR, then translated to more than 1000 distinct functions.

Our discovery can partially explain how limited effector proteins (16 G protein subtype and 4 arrestin subtype) can translate sophisticated information dictated from more than 800 GPCR to numerous cellular functions. We identified a new mechanism in activation of ion channel by GPCRs. A prototype thinking is that the ion channels are activated by GPCRs through either direct G protein interaction or second messengers downstream of G protein activation. We identified that arrestin can mediated AT1R/TRPC3 or M3R/TRPC3 coupling by forming a complex of AT1R/β-arrestin-1/PLCγ/TRPC3 or M3R//β-arrestin-1/TRPC3 (Nature communications, 2017, Nature communications,2018). We also identified that orphan receptor GPR64 forms complex with β-arrestin-1 and CFTR at apical membrane of efferent ductules to regulate the salt/water metabolism (eLife 2018, Faculty 1000 recommendation). These works provided new mechanisms for GPCR activated ion channel, which may be relevant to many physiological or pathological processes. Our fourth research aspect is the physiological and pathological functions of GPCRs. We have elucidated how biased signaling of CCK1R,β adrenergic receptors and adhesion GPCRs regulated different pancreatic islet function and homeostasis. We also identified that short term activation of β2AR signaling improved learning and memory by increased lactate metabolism and astrocyte-neuron lactate shuttle (ANLS), whereas long term stress or activation of β2AR signaling has harmful effect on learning and memory through a desensitization mechanism.

W72-3：Novel roles of PXR in liver diseases

Huichang Bi*

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Pregnane X receptor (PXR, NR1I2) is a ligand-activated transcription factor that belongs to the nuclear receptor superfamily. PXR is highly expressed in the liver and plays an integral role in the control of metabolism homeostasis as well as the liver pathophysiology. As a potential therapeutic target, fully understanding the biological and physiological properties and functions of PXR would be of great value. In this presentation, we will discuss the novel roles of PXR in liver diseases such as liver fibrosis, hepatic encephalopathy, cholestasis-related depression, and liver regeneration. Mechanistically, we found that PXR activation attenuates liver fibrosis by disrupting β-catenin/TCF4 interaction and downregulating leukocyte cell-derived chemotaxin 2 (LECT2) expression, PXR mitigates hyperammonemia and hepatic encephalopathy by regulating the key enzymes in urea cycle, and PXR activation promotes liver regeneration by activating yes-associated protein (YAP) signals. Furthermore, the effect of PXR on the nuclear translocation and post-translational modifications (PTMs) of YAP, the interactions between PXR and the above signals including YAP, β-catenin/TCF4 and LECT2 will be verified. These findings have implications for understanding the physiological functions of PXR and suggest the potential for the treatment of liver diseases.

W72-4：New strategy of drug therapy for breast cancer

Yan Cheng

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Triple-negative breast cancer （TNBC） is the most difficult subtype to treat due to limited targeted therapies. It is urgent to develop novel anti-cancer targeted strategies, especially for TNBC. We demonstrated the promoting effect of eEF-2K on energy metabolism in breast cancer cells, and revealing a new signaling pathway, eEF-2K-PP2A-cMyc-PKM2, in the regulating of glycolysis. In addition, we found that eEF-2K may serve as a biomarker for predicting therapeutic response and prognosis in patients receiving anti-PD-1 therapy, and eEF-2K inhibitor can enhance the efficacy of anti-PD-1 therapy. We further developed the first eEF2K molecular glue compound-C1, which promotes the proteasomal degradation of eEF2K by increasing the interaction between eEF2K and the ubiquitin E3 ligase βTRCP. C1 significantly inhibits the proliferation and metastasis of TNBC cells both in vitro and in vivo and in TNBC patient-derived organoids.

W72-5：The function, molecular mechanism, and therapeutic targeting of protein

Wen Liu*

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The STING-mediated type I interferon (IFN) signaling pathway has been shown to play critical roles in antitumor immunity, and cancers evolve a variety of mechanisms to limit the activation of this pathway to escape immune surveillance. Here, we demonstrate that an endoplasmic reticulum (ER) membrane-localized Jumonji C (JmjC) domain-containing protein, JMJD8, directly interacts with STING and inhibits STING-induced type I IFN responses to promote immune evasion and breast tumor growth. Mechanistically, JMJD8 competes with TBK1 for binding with the carboxyl (C)-terminus of STING, which blocks STING-TBK1 complex formation and restricts type I IFNs and IFN-stimulated genes (ISGs) expression. Consistently, JMJD8 knockdown leads to the increased expression of type I IFNs and ISGs as well as immune cell infiltration, reinforcing antitumor immunity to suppress tumor growth. Furthermore, JMJD8 knockdown significantly improves the efficacy of chemotherapy and immune checkpoint therapy in treating tumors, as which also take advantage of the STING-Type I IFN signaling. The clinical relevance is highlighted that JMJD8 is highly expressed in breast tumor samples in clinic, and the expression of JMJD8 is inversely correlated with that of type I IFN and ISGs as well as immune cell infiltration. A peptidic inhibitor targeting the interaction between JMJD8 and STING is potent in suppressing breast tumor growth both in vitro and in vivo. Combination therapy with this peptidic inhibitor and chemotherapy drug Etoposide or immunotherapy drug anti-PD-L1 antibody exhibits synergistic effects in suppressing breast tumor growth. Taken together, our study identifies JMJD8 as a critical regulator of STING-mediated type I IFN responses, and targeting the JMJD8 can trigger antitumor immunity, which represents a promising way of treating breast cancer in clinic.

Workshop 73: New Horizons of Molecular Mechanisms in Cardiovascular Disease Research (Room 2F)

Chairs: Eugene Chen, Hong Chen

W73-1: Immune metabolic remodeling and cardiovascular disease

Xiaofeng Yang
Cardiovasc Sciences, Immunology and Pharmacology, Temple University Lewis Katz School of Medicine

I will discuss several novel concepts including: 1) aorta is an innate immune organ that provides microenvironment/niche for inflammation-recruited naïve immune cells trans-differentiate into effector immune subsets; 2) endothelial cells and vascular smooth muscle cells are innate immune cells; 3) early hyperlipidemia promote metabolomic reprogram to establish trained immunity (innate immune memory for inflammation enhancement); and 4) CD4+Foxp3+ regulatory T cells (Tregs) have immunosuppressive functions and programs that are sustained under pathological conditions.

W73-2: Novel mouse models to study smooth muscle cell biology

Jiliang Zhou
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Dysfunction in either embryonic or postnatal vascular smooth muscle cells (VSMCs) significantly contributes to the progression of various cardiovascular diseases. Therefore, it is crucial to elucidate the molecular mechanisms governing VSMC development and homeostasis. *MYH11* is the most faithful lineage gene for SMCs and has been utilized to develop tamoxifen-inducible Cre driver lines to achieve SMC-specific gene manipulation by crossing with mice carrying the loxP-flanked gene, preferably in adult mice. For studies in VSMCs during embryogenesis, the commonly used constitutive Cre driver is controlled by the *Tagln* gene promoter. However, this Cre driver displays Cre activity in multiple non-SMC populations including cardiomyocytes, introducing confounding effects. Additionally, most existing SMC-specific Cre drivers are generated using a transgenic approach that raises concerns about the random sites of integration and variable number of gene copies. To address these limitations, here we report a novel Cre mouse model generated by knock-in (KI) of a nuclear-localized Cre recombinase into the *Myh11* gene locus using homology recombination. We confirmed that the Cre activity precisely recapitulates endogenous *Myh11* expression by crossing the *Rosa26* mTmG dual fluorescence reporter mice. Moreover, *Myh11*-driven Cre can achieve an efficient deletion of the floxed allele of transcription factor Tead1 only in SMCs, while the Tead1 SMC-specific KO mice did not exhibit an overt phenotype, circumventing the embryonic lethal phenotype mediated by *Tagln*-driven Cre as we previously reported. These findings establish this novel driver line as a robust tool for tracing *Myh11*-positive SMC lineage and for manipulating gene function specifically in SMCs during embryonic development.

W73-3: G protein-coupled receptors in vasculature

Wei Kong

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G-protein coupled receptors (GPCRs) contribute significantly to ~30% of the global market share for therapeutic drugs due to their critical involvement in regulating diverse physiological processes. Despite their importance, the specific roles of various GPCRs in vasculature remain underexplored, and questions persist about the novel ligands and regulatory mechanisms. In response, our recent findings have uncovered new ligands and regulatory pathways for GPCRs in vascular tissues. We discovered that the adipokine FAM19A5 is a high-affinity ligand for the sphingosine-1-phosphate receptor 2 (S1PR2) to maintain vascular homeostasis. Additionally, Nidogen2, a basement membrane protein, was identified as a novel endogenous biased ligand for LGR4, mitigating vascular calcification. Our research also revealed that homocysteine allosterically activates the angiotensin II receptor (AT1) independent of classical ligands, promoting vascular inflammation. Furthermore, the matricellular protein COMP acts as an endogenous biased antagonist for AT1, specifically inhibiting the β-arrestin 2 signaling pathway and preventing the development of abdominal aortic aneurysms. These discoveries provide novel insights into GPCR ligand-receptor interactions and regulatory mechanisms in vasculature, laying the theoretical foundation for developing GPCR-targeted therapies for cardiovascular diseases.

W73-4: Coronary atherosclerosis: selective vascular vulnerability from mice to humans

Miao Wang

State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China.

Aims: Hypercholesterolemia and hypertension are major risk factors for atherosclerosis. This study determined their combined effects on coronary atherogenesis.

Methods and Results: A mouse model to accelerate coronary atherogenesis was induced by hypercholesterolemia via blocking two genome-wide association loci-apolipoprotein E and scavenger receptor B1 ($ApoE^{SA/SA}$) in combination with angiotensin II (AngII)-induced hypertension. The resulting atherosclerotic lesions exhibited endothelial erosion, myeloid cell infiltration, and plaque rupture, leading to spontaneous myocardial infarction, heart failure, and a male propensity for sudden death. AngII-treated $ApoE^{SA/SA}$ mice developed severe atherosclerosis in coronary arteries but not in femoral arteries, while norepinephrine-treated $ApoE^{SA/SA}$ mice did not develop coronary atherosclerosis, despite similar degree of hypertension. Proteomic analyses revealed notable differences between coronary and femoral arteries, which encompassed vasocontractility, extracellular matrix metabolism, angiotensinogen metabolism and inflammation. Endothelium-dependent dilatation of coronary arteries was highly susceptible to hypercholesterolemia and AngII-induced hypertension in contrast to femoral arteries. Losartan treatment restored coronary endothelium-dependent dilatation and eliminated coronary atherosclerosis. Additionally, coronary artery dilatation was more dependent on prostaglandins than femoral artery dilatation. Hypercholesterolemia and hypertension suppressed coronary prostaglandin biosynthesis and dilatation of coronary arteries. Conversely, an elevated coronary production of prostaglandins after methotrexate administration was

associated with improved endothelial dysfunction and better cardiovascular survival. In patients with hypercholesterolemia and hypertension, coronary arteries exhibited impaired endothelium-dependent dilatation, compared to internal mammary arteries. However, their endothelium-independent dilatation remained intact.
Conclusion: The combination of hypercholesterolemia and AngII-induced hypertension significantly increased coronary atherogenesis, which led to spontaneous myocardial infarction. This makes coronary arteries particularly vulnerable to AngII exposure and prostaglandin inhibition.

W73-5: Endogenous metabolites and vascular function
Lemin Zheng[1]
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In recent years, our group has focused on the role of endogenous metabolites in cardiovascular and cerebrovascular function. Blood-brain barrier (BBB) function deteriorates during aging, contributing to cognitive impairment and neurodegeneration. Using single-nucleus transcriptomics, we identified decreased connexin 43 (CX43) expression in cadherin-5+ (Cdh5+) cerebral vascular cells in naturally aging mice and confirmed it in human brain samples. Global or Cdh5+ cell-specific CX43 deletion in mice exacerbated BBB dysfunction during aging. The CX43-dependent effect was not due to its canonical gap junction function but was associated with reduced NAD+ levels and mitochondrial dysfunction through NAD+-dependent sirtuin 3 (SIRT3).CX43 interacts with and negatively regulates poly(ADP-ribose) polymerase 1 (PARP1). Pharmacologic inhibition of PARP1 by olaparib or nicotinamide mononucleotide (NMN) supplementation rescued NAD+ levels and alleviated aging-associated BBB leakage. These findings establish the endothelial CX43-PARP1-NAD+ pathway's role in vascular aging and identify a potential therapeutic strategy to combat aging-associated BBB leakage with neuroprotective implications.
Additionally, our research extends to non-invasive imaging of vascular diseases, particularly unstable atherosclerotic plaques. The current diagnostic challenge lies in directly identifying multi-site plaques and assessing their vulnerability, which is crucial for predicting the risk of atherosclerotic cardiovascular diseases (ASCVD). To address this, we developed an osteopontin (OPN)-specific nanoprobe (OPN Ab-Au/FeNiPO4@ICG) capable of both multiple spectra optoacoustic tomography (MSOT) and computed tomography (CT) imaging. This innovative nanoprobe enables systemic screening of vulnerable plaques by specifically targeting OPN-overexpressed foam cells and molecularly recognizing vulnerable plaques. By employing this dual-model nanoprobe strategy, we significantly advance the accurate diagnosis of ASCVD.

W73-6:Cellular communication network factors and vascular homeostasis
Zhiyong Lin

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Disruption of vascular homeostasis is central to the onset and progression of cardiovascular diseases, with properly regulated cell signaling being crucial for normal physiology. The Cellular Communication Network (CCN) protein family has emerged as a key player in vascular signaling, impacting critical biological processes such as cell adhesion, migration, proliferation, and cellular phenotype alterations. Studies in mouse models illuminate the significance of CCN proteins in vascular diseases, specifically abdominal aortic aneurysm (AAA) and atherosclerosis. Notably, recent research unveiled an unexpected regulatory role of CCN2 in the modulation of smooth muscle cell (SMC) behavior and reprogramming in AAA, partly through its interaction with TGFβ signaling. Moreover, murine models with a SMC-specific CCN2 deletion demonstrate exacerbated atherosclerosis, emphasizing CCN2's influence on lipid homeostasis and endoplasmic reticulum stress in SMCs. Similarly, decreased CCN3 expression in rodent models and human biopsies suggests a protective function in AAA, as CCN3 deficiency aggravates AAA phenotypes in mice while its overexpression attenuates AAA formation. Mechanistically, CCN3's modulation of the ERK1/2 pathway contributes to AAA development. Our findings collectively highlight the intricate involvement of CCN proteins in vascular biology, providing crucial insights into the underlying mechanisms of vascular diseases.

Workshop 74: Structures of Immune Complexes (Room 2G)

Chairs: Tianmin Fu

W74-1: Innate Immunity on Nucleic Acids: Signaling and Regulation

Pu Gao[1]

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Mammalian cells have evolved powerful innate immune mechanisms to detect pathogen- or host-derived nucleic acids in the cytosol, the most prominent of which are the cGAS-STING pathway for DNA and the RLR-MAVS pathway for RNA. In addition to their primary role in immune defense against pathogens and tumors, there is increasing evidence that these pathways are closely associated with autoimmune, metabolic and neurodegenerative disorders. In this talk, I will review our recent progress (both published and unpublished) in understanding the molecular mechanisms of nucleic acid-induced intra- and inter-cellular innate immune signaling, as well as interesting regulatory strategies mediated by both pathogens and host cells.

W74-2: One warrior with multiple weapons: Nezha anti-phage system

Yamei Yu[1]

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In response to the persistent exposure to phage infection, bacteria have evolved diverse antiviral defense mechanisms. I will present a bacterial two-component defense system consisting of a Sir2 NADase and a HerA helicase. Cryo-electron

microscopy reveals that Sir2 and HerA assemble into a 1MDa supramolecular octadecamer. Unexpectedly, this complex exhibits various enzymatic activities, including ATPase, NADase, helicase, and nuclease, which work together in a sophisticated manner to fulfill the antiphage function. Therefore, we name this defense system ‘‘Nezha’’ after a divine warrior in Chinese mythology who employs multiple weapons to defeat enemies. Our findings demonstrate that Nezha could sense phage infections,self-activate to arrest cell growth, eliminate phage genomes, and subsequently deactivate to allow for cell recovery. Collectively, Nezha represents a paradigmof sophisticated and multifaceted strategies bacteria use to defend against viral infections.

W74-3:Structural Insights into the Activation Mechanism of the Gabija Anti-Phage System

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Gabija is a highly widespread prokaryotic defense system that consists of two components, GajA and GajB. GajA functions as a DNA endonuclease that is inactive in the presence of ATP. GajB is required for Gabija's anti-phage defence but the function is unclear. To explore how the Gabija system is activated during phage infection, we report cryo-EM structures of Gabija in five states, including apo GajA, GajA in complex with DNA, GajA bound by ATP, apo GajA–GajB, and GajA–GajB in complex with ATP and Mg2+. GajA is a rhombus-shaped tetramer with its ATPase domain clustered at the centre and the topoisomerase–primase (Toprim) domain located peripherally. ATP binding at the ATPase domain stabilizes the insertion region within the ATPase domain, keeping the Toprim domain in a closed state. Upon ATP depletion by phages, the Toprim domain opens to bind and cleave the DNA substrate. GajB, which docks on GajA, is activated by the cleaved DNA, ultimately leading to prokaryotic cell death. Our study presents a mechanistic landscape of Gabija activation.

W74-4: DNA methylation activates retron Ec86 oligomers for antiphage defense

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First discovered in the 1980s, retrons are bacterial genetic elements consisting of a reverse transcriptase and a non-coding RNA (ncRNA). Retrons, together with its cognate effector, mediate antiphage defence in bacteria, but the full picture of how retron systems sense invading phages and mediate defense remains to be elucidated. Here, we use cryo-electron microscopy to determine the structures of the Retron Ec86 complex. The Ec86 reverse transcriptase exhibits a characteristic right-hand-like fold, and reverse-transcribes part of the ncRNA into satellite, msDNA (multicopy single-stranded DNA, a DNA-RNA hybrid) that we show wraps around the reverse transcriptase electropositive surface. The Ec86 effector adopts a two-lobe fold and directly binds reverse transcriptase and msDNA. We identified a phage-encoded DNA

cytosine methyltransferase (Dcm) as the trigger of the Ec86 defence system and show that Ec86 senses msDNA methylation to be activated. We further determined the structure of a tripartite retron Ec86 supramolecular assembly, which is primed for activation by Dcm, and demonstrated that the activated system confers defense through depletion of nucleoside derivatives. These findings emphasize the role of retrons being a second line of defense and highlight an emerging theme of anti-phage defense through supramolecular complex assemblies.

W74-5: Targeting PD-1H in the Microenviroment of Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is characterized by the malignant growth of myeloid cells, leading to bone marrow failure. AML has a 5-year overall survival rate of less than 30%. Despite advances in our understanding of the pathology and genetics of the disease, chemotherapy has remained the mainstay of AML treatment for decades. While some patients benefit from hematopoietic stem cell transplantation (HSCT) and targeted therapies, relapse and refractory disease remain common challenges. Recent breakthroughs in immunotherapy, particularly immune checkpoint blockade (ICB) therapy in solid tumors, have sparked interest in their applicability to AML. However, the unique immunosuppressive milieu of AML's tumor microenvironment (TME) poses distinct challenges, as evidenced by the limited success of ICB treatment in AML. This gap in effective immunotherapeutic options for AML underscores the need for a deeper understanding of its unique TME and an exploration of novel immune checkpoint pathways. PD-1H (Programmed Death-1 Homolog, also known as VISTA, or VSIR) is an immune inhibitory molecule predominantly expressed on the surface of hematopoietic cells. Our recent study has identified PD-1H as a distinct immune checkpoint molecule predominantly expressed in human AML. We observed high expression of PD-1H in bone marrow blasts of AML patients, while normal myeloid cell subsets and T cells also express PD-1H. Using syngeneic and humanized AML mouse models, we demonstrated that PD-1H overexpression facilitated AML cell growth, primarily by circumventing T cell-mediated immune responses. Crucially, blocking AML cell-surface PD-1H through antibody blockade or genetic knockout significantly impeded AML progression by enhancing T cell activity. Furthermore, deleting PD-1H from host normal myeloid cells hindered AML advancement. Combining PD-1H blockade with anti-PD therapy exhibited a synergistic antileukemia effect. These findings underscore PD-1H as a promising therapeutic target for combating human AML.

W74-6: Structural transitions enable IL-18 maturation and signaling

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Interleukin-1 (IL-1) family cytokines are key regulators of inflammation and host defense. Unlike most cytokines, several IL-1 family members, including IL-1β and IL-18 are synthesized as non-inflammatory pro-proteins. Only upon cleavage, typically by inflammasome-associated caspases, is the inflammatory activity of IL-1β and IL-18 unveiled. A structural explanation of how pro-IL-1 family cytokines are selected as substrates for caspase cleavage, and why cytokine cleavage is necessary for inflammatory activity, is unclear. Here, we report the cryogenic electron microscop structure of caspase-1 in complex with pro-IL-18, and the nuclear magnetic resonance structure of apo pro-IL-18. Our studies reveal the molecular mechanisms for the complete pathway of recognition and cleavage of pro-IL-18 by human caspase-1 and suggest diverse ways with which inflammatory caspases process their substrates.

Workshop 75: Cancer Immunotherapy beyond ICB: Cytokine-, CAR-NK-, and TCR-Immunotherapy Progress (Room 3A)

Chairs: Yangxin Fu, Dongfang Liu

W75-1: New strategies of antibody based fusion proteins for tumor immunity
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Cytokines are third signals for T cell activation and function. We propose that tumor-infiltrating T cells (TIL) become exhausted due to limited T cell-driven cytokines inside TME. Systemic delivery cytokines activate both innate and adaptive immune cells causing severe toxicity. Targeting molecules preferentially expressed on TIL with cytokines with cis-delivery could rejuvenate exhausted T cells and reduce its toxicity through periphery NK and T cells.

Prolonged tumor antigen exposed T cells express much high level of PD-1 than periphery NK and T cells. To better target PD-1^{high} TIL, we engineered anti- PD-1 based cytokines. To selectively deliver IL-2 to PD-1+ CD8+ tumor-infiltrating lymphocytes (TILs), we engineered a low-affinity IL-2 paired with anti–PD-1 (PD-1–laIL-2), which reduced affinity to peripheral Treg cells but enhanced avidity to PD-1+ CD8+ TILs. PD-1–laIL-2 exerted better tumor control and lower toxicity than single or mixed treatments.

Exogenous IL-15 could further expand TILs and thus synergize with αPD-L1 therapy. However, systemic delivery of IL-15 extensively expands peripheral NK cells, causing severe toxicity. To redirect IL-15 to intratumoral PD-1+ CD8+ T effector cells instead of NK cells for better tumor control and lower toxicity, we engineered an anti–PD-1 fusion with IL-15, whose activity was geographically concealed by immunoglobulin Fc region with an engineered linker (αPD-1-IL-15-R) to bypass systemic NK cells. Systematic administration of αPD-1-IL-15-R elicited extraordinary

antitumor efficacy with undetectable toxicity. Collectively, these results highlight that PD-1 based cytokines can target and reactivate tumor-specific TILs for tumor regression as a unique strategy with stronger efficacy and lower toxicity.

W75-2: Molecular Mechanisms of CD147-CAR-NK for Hepatocellular Carcinoma Treatment

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Recent clinical trials testing cancer immunotherapies have shown promising results for the treatment of various cancers. One such therapy involves engineering immune cells to express chimeric antigen receptors (CAR), which combine tumor antigen specificity with immune cell activation in a single receptor. The adoptive transfer of these CAR-modified immune cells (especially T cells, CAR T) into patients has shown remarkable success in treating multiple refractory blood cancers. However, in order to achieve the promise of CAR-modified immune cells in treating solid tumor cancers, further advances will be required. Here, we devise a strategy for targeting hepatocellular carcinoma (HCC, one of the deadliest malignancies). We report that T and NK cells transduced with a CAR that recognizes the surface marker, CD147, also known as Basigin (BSG) or extracellular matrix metalloproteinase inducer (EMMPRIN), can effectively kill various malignant HCC cell lines in vitro, and HCC tumors in xenograft and patient-derived xenograft mouse models. In this study, we also added the ability to express autocrine IL-15 to our CD147-CAR-NK cells (now 'CD147-IL15-CAR-NK' cells) to augment their ability to persist and kill within the immunosuppressive HCC tumor microenvironment (TME). Our data show that autocrine expression of IL-15 can improve CAR-NK cell function by promoting integrin extended-open conformation and therefore superior immunological synase (IS) quality and stronger adaptive immunity. In conclusion, these findings support the therapeutic potential of CD147-CAR-NK cells for HCC patients. The results of these studies will also streamline the path to clinical trials of CD147-IL15-CAR-NK cells for adoptive cell therapy for the treatment of HCC. This study could be translated to the treatment of other CD147 positive solid tumor cancers as well in the future.

W75-3: Metabolic regulation of tumor associated macrophages via the mTOR signaling

Bing Su, Ph.D.
KC Wang Endowed Chair Professor, Shanghai Jiao-Tong University School of Medicine, Shanghai Institute of Immunology

Mechanistic target of rapamycin (mTOR) is a well-studied Thr/Ser kinase functioning mainly through two distinct multi-protein complexes, namely the mTOR complex (mTORC) 1 and mTORC2. Sty1/Spc1-interacting protein 1 (Sin1) is an essential component for mTORC2 integrity and activity involved in regulating immune cell development, trafficking, metabolism, and function. We found that deletion of Sin1 in tumor associated macrophages (TAMs) augmented antitumor immunity via a type I interferon (IFN)-mediated $CD8^+$ T cell response. Mechanistically, Sin1-mTORC2 signaling was needed for active lipid metabolism in TAMs which accumulated cholesterol to block STING-mediated type-I IFN production. Our findings may

provide help developing a strategy to improve cancer immunotherapy by targeting Sin1-mTORC2 in TAMs.

W75-4: High-throughput Deciphering of Disease-Relevant TCR Repertoire
Meng Michelle Xu
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Linking phenotypic changes with the antigen specificity conferred by T cell receptors (TCRs) offers valuable insights into in vivo T cell responsiveness across various diseases and immunotherapies. Here, we developed PRECISE-seq, a method that integrates multi-omics T cell analysis with contact-dependent proximity labeling for rapid screening of disease-relevant T cell repertoires with functional interpretation at the single-cell level. We demonstrated the accuracy and sensitivity of PRECISE-seq in retrieving T cell clonotypes with diverse TCR potency against human cytomegalovirus (CMV) infection in peripheral blood. Our findings reveal that CMV-specific T cells with high-potency TCRs undergo an acquisition of an exhausted state. Moreover, our analysis of naturally occurring polyclonal T cell responses against tumors indicates that most tumor-reactive T cells in colon cancer are skewed toward a unique, natural killer (NK)-like exhausted state. Notably, PD-1 blockade leads to the reinvigoration of tumor-reactive T cells, driving a transition from the NK-like exhausted phenotype to an effector-like phenotype. Importantly, this effector revival in tumor-infiltrating T cells correlates with the clinical responsiveness to anti-PD-1 therapy. In summary, our study underscores PRECISE-seq as a promising tool for dissecting in vivo T cell responsiveness and for predictive biomarker discovery across diseases and immunotherapies.

Workshop 76: Precise Medicine and Therapy (Room 3B)

Chairs: Weidong Pan and Wei Yang

W76-1: Design, synthesis and biological evaluation of glycosphingolipid derivatives as potential antitumor agents
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Glycosphingolipids (GSLs) are components of all animal cell membranes and are involved in many cellular functions including proliferation, adhesion, motility, and differentiation. Ganglioside GM3 (NeuAcα3Galβ4Glcβ1Cer), the first and simplest member in the metabolic series of a GSLs family containing sialic acids (N-acetyl- and N-glycolyl-neuraminic acids and their O-acyl derivatives), is known as one of the most abundant tumor-associated carbohydrate antigens on several types of tumors. Glycosphingolipid structures, and their changes associated with biological functions, have been the central focus of our studies, since structural change is the starting point for understanding biological significance, and enzymatic/genetic mechanisms.
We discuss here the design, synthesis and biological evaluation of several GM3 analogues which were prepared by using modern glycochemistry methods.

W76-2: Bioresponsive Drug Delivery

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College of Pharmaceutical Sciences, Zhejiang University

Spurred by recent advances in materials chemistry, molecular pharmaceutics and nanobiotechnology, stimuli-responsive "smart" systems offer opportunities for precisely delivering drugs in dose-, spatial- and temporal-controlled manners. In this talk, I will discuss our ongoing efforts in developing physiological signal-triggered bioinspired drug delivery systems. I will first present the glucose-responsive synthetic systems for biomimetic delivery of insulin for diabetes treatment. Development of smart insulin patches will be emphasized. I will further discuss the local and targeted delivery of immunomodulatory therapeutics for enhanced cancer therapy. Our latest studies utilizing platelets, cell conjugates and sprayed gels for delivery of immune checkpoint inhibitors will be specifically introduced.

W76-3: Identification of the protective mutations of TRPM2 in Parkinson's disease

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As a sensor of oxidative stress, the transient receptor potential melastatin 2 (TRPM2) channel is a non-selective cation channel that primarily permeates calcium ions. Research has shown that the TRPM2 channel is involved in various diseases such as stroke, inflammation, diabetes, and liver ischemia-reperfusion injury. Recently, mutations in TRPM2 that accelerate channel inactivation have been identified in patients with Parkinson's disease (PD). However, the effect of these mutations on PD patients remains unclear. Our recent study found that TRPM2 channel selectively induced dopaminergic neuron death by causing mitochondrial hyperfusion, suggesting that TRPM2 plays a significant role in PD pathogenesis. We further conducted genome-wide exome sequencing in a large cohort of PD patients and identified multiple novel TRPM2 mutations, some of which lack channel function. Moreover, we explored the effects of these mutations on PD pathogenesis using induced pluripotent stem cells (iPSCs) combined with CRISPR-Cas9 technology. Our findings indicate that loss-of-function TRPM2 mutations in PD patients are protective rather than harmful. Overall, our studies provide new insights for potential cell therapy approaches for PD in the future.

W76-4: Artemisinin alleviates Bortezomib-induced peripheral neuropathy: findings from experiment models and a preliminary clinical study

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The bortezomib (BTZ)-induced peripheral neuropathy (BIPN) remains a frequently occurring adverse effect, the mechanisms of which remain largely unclear. We previously found lysosomal dysfunction of Schwann cells underlies the toxicity of BIPN. In this study, we established an *in vitro* screening model to assess, and identified artemisinin (ART) as a promising candidate to rescue the lysosomal activity of Schwann cells. ART significantly reversed BTZ-induced lysosomal dysfunction in RSC96 Schwann cells. ART was found to mitigate the demyelination and reduce conduction deficits of sciatic nerve of BTZ-treated mice, as well as prevent their mechanical hyperalgesia. Mechanistically, ART was shown to counteract the inhibitory effects of BTZ on the expression and nuclear translocation of TFEB in RSC96 Schwann cells. Importantly, ART did not impede the antineoplastic efficacy of BTZ against lymphoblast RPMI8226 cells. Of note, we conducted a preliminary clinical study to evaluate the efficacy of ART in multiple myeloma patients experiencing BIPN. Oral administration of ART for 3 months substantially alleviated of BIPN symptoms in 7 patients. Collectively, these findings support the hypothesis that ART can restore lysosomal function in Schwann cells and consequently ameliorate the manifestations of BIPN. As such, ART holds promise as a potential therapy for the treatment of BIPN.

W76-5: Chemical Modulators Targeting the Oncogenic m6A Demthylase FTO

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The N6-methyladenosine (m6A), the most prevalent mRNA modification, has emerged in recent years as a new layer of post-transcriptional regulatory mechanism controlling gene expression in eukaryotes. The m6A methylation influences nearly every step of RNA metabolism, and thus broadly affects gene expression at multiple levels. Increasing evidence has revealed connectivity between reversible RNA m6A methylation with tumorigenesis, metastasis and immune evasion via the dynamic alteration of m6A-marked mRNA transcripts. FTO, the first m6A demethylase, revitalized the interest in m6A and created new momentum for the biology of RNA epigenetics, which has been associated to promote leukemogenesis and other types of cancers. Our investigations into FTO inhibitors have yielded substantial advances through a structure-based rational design approach, resulting in the development of highly selective and potent chemical inhibitors. Our inhibitors have proved to eficiently altere FTO-mediated aberrant epitranscriptome and significantly inhibite acute myeloid leukemia (AML) progression in vivo. We also have developed FTO-targeting proteolysis targeting chimera (PROTAC) degraders and demonstrated that FTO degradation exerted antileukemic effects. Collectively, these chemical modulators that selectively targeting the oncogenic FTO demethylase will promote in-depth studies on the regulation of gene expression and potentially accelerate anticancer target discovery.

W76-6: Long Non-coding RNAs: Multi-dimensional Regulators of Cellular Activity

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Subcellular structures, composed of organelles and biomolecular condensates, necessitate specific molecules to uphold metabolic homeostasis. Research, including our own, has demonstrated the involvement of long noncoding RNAs (lncRNAs) in diverse biological proceedings. Nonetheless, understanding the precise function of lncRNAs within these subcellular compartments remains an incomplete endeavor. To address this, we charted the localization of organelle-associated lncRNAs through a technique combining organelle isolation by endogenous antibodies and purification by density gradient centrifugation. Furthermore, our studies have uncovered the molecular mechanisms by which lncRNAs associated with organelles modulate a range of cellular functions, such as energy metabolism, cholesterol metabolism, Hippo-YAP signaling, redox homeostasis, and organelle interactions. These findings highlight their pivotal roles in pathologies like obesity, non-alcoholic fatty liver disease, and cancer. Therefore, clarifying the spatiotemporal distribution of lncRNAs within cells and their compartment-specific functions will advance RNA biology and health care.

W76-7: Design, Synthesis of Fangchinoline Derivatives as Anti-tumor Agents and their Mechanisms of Action

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Chinese Medicinal plants are good sources for search of new drugs and potential ingredients for drug candidates. Our group focused on the search of naturally occurring bioactive compounds from Guizhou medicinal plants for the development of new anti-cancer drugs. This presentation focus on the case study of an anti-tumor natural lead compound, fangchinoline, isolated from the dried roots of *Stephania tetrandra* as well as the design, synthesis and evaluation of the bioactivity of its derivatives as anti-tumor agents will also be discussed. The bioassay results showed that most of the derivatives possess better activities against certain cancer cell lines. The mechanism of anti-tumor activity study was also studied for a fangchinoline derivative, **HL23**. The results revealed that, it might act as an autophagy inducer as well as HDACi agent and may targeted on EGFR. These results help shed light in the new anti-cancer drug development from the lead natural compound, fangchinoline.