美洲華人生物科學學會

Society of Chinese Bioscientists in America

The 18th Society of Chinese Bioscientists in America (SCBA) International Symposium

Meeting Program

July 27-31, 2022

Boston, MA, USA

Joyce Cummings Conference Center, 177 College Avenue, Medford, MA 02155

Map of the Joyce Cummings Conference Center (177 College Ave, Medfold, MA 02155)

Joyce Cummings Conference Center (see floor map at the end of this program book)

We want to be very clear that the meeting is on the TUFTS campus which will remain open to the public. The meeting spaces will not accommodate 3 feet social distancing, and masking will be as per TUFTS policy. These policies are subject to change in response to campus, local, and state COVID requirements.

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Program at a glance

	Day 1- July 28	Day 2- July 29	Day 3- July 30	Day 4- July 31
7:30 am	**Shuttle**			
8:00 am		**Shuttle**	**Shuttle**	**Shuttle**
8:00-8:45 am	Breakfast (Joyce Cummings Conference Center Lobby)			
9:00-9:45 am	Keynote 1	Keynote 2	Keynote 3	Presidential Award 1 & 2 Young Investigator's Award 1
9:45-10:15 am	Plenary 1	Plenary 3	Plenary 5	
10:15-10:45 am	Plenary 2	Plenary 4	Plenary 6	
10:45-11:10 am	**Break**	**Break**	**Break**	**Break**
11:10-12:40 pm	Dialogue with NIH	Session 11-15	Session 26-30	Young Investigator's Award 2 Election Results Travel/Poster Awards Meeting Remarks
12:40-2:00 pm	Lunch (Cafeteria) or Vendor Lunch Shows (1st floor conference room)	Lunch (Cafeteria)	Lunch (Cafeteria)	Lunch (Cafeteria)
2:00-3:30 pm	Session 1-5	Session 16-20	Session 31-35	**Departure**
3:30-4:00 pm	**Break**	**Break**	**Break**	
4:00-5:30 pm	Session 6-10	Session 21-25	Session 36-40	
5:30-7:00 pm	Poster 1	Meeting with editors	Poster 2	
7:00 pm	**Shuttle**		**Shuttle**	
7:00-9:00 pm	Dinner on your own	**Banquet**	Dinner on your own	
9:00 & 9:30 pm		**Shuttle**		

July 27 3:00 – 7:00 pm Arrival and registration (AC Hotel, Medford, MA)

5:00 – 7:00 pm Social time with snacks & drinks (AC Hotel, Medford, MA)

July 28	7:30 am	Shuttles leaving AC/Fairfield Medford and AC Cambridge to Joyce Cummings Center
	7:30 – 8:45 am	Breakfast (Joyce Cummings Center lobby)
	7:30 – 12:00 noon	Registration (Joyce Cummings Center lobby)
	8:45 – 9:00 am	**Welcome and Opening Remarks** (1st floor conference room)
		Hui Zheng, Immediate Past President, SCBA
		Shan-Lu Liu, President, SCBA
		Zhi-Ming Zheng and Lee Zou, Co-Chair of the Program Committee
	9:00 – 10:45 am	**Keynote and Named Lectures**
		Chairpersons: Paul Liu and Hua Yu
	9:00 – 9:45 am	Keynote Lecture**: William G. Kaelin Jr.**, M.D., 2019 Nobel Laureate, Dana-Farber Cancer Institute, Harvard Medical School
		The von Hippel-Lindau Tumor Suppressor Gene: Insights into Oxygen Sensing and Cancer
		Introduction by Wenyi Wei
	9:45 – 10:15 am	KT Jeang Lecture: **Edward Chu**, M.D., Director, Albert Einstein College of Medicine Cancer Center
		PHY906 as a Modulator of Cancer Chemotherapy: Where East Meets West
		Introduction by Paul Liu
	10:15 – 10:45 am	Tsai-Fan Yu Lecture: **Pei-Yong Shi**, Ph.D., Director, Sealy Institute for Drug Discovery, University of Texas Medical Branch
		SARS-CoV-2 Biology and Countermeasure Development
		Introduction by Hua Yu
	10:45 – 11:10 am	Coffee Break
	11:10 – 12:40 pm	**Dialogue between NIH Officials & SCBA Community: Concerns and Solutions** (1st floor conference room)
		Moderator: **Prof. Margaret Lewis,** Seton Hall University
		Introduction by Shan-Lu Liu

Panelists: U.S. Congressional Representative **Judy Chu** (Virtual)

Michael Lauer, M.D., Deputy Director for Extramural Research, NIH

Gang Chen, Ph.D., Carl Richard Soderberg Professor, MIT

Henry S. Tang, Co-founder of Committee of 100

12:40 – 2:00 pm Lunch (Cafeteria) and vendor lunch shows by ABclonal Technology and Vazyme Biotech (1st floor conference room)

2:00-3:30 pm Concurrent Workshops with Co-Chairs and Locations

1. SARS-CoV-2 and COVID-19: Jie Sun & Haitao Hu (1st floor conference room)

2:00-2:15	W1-1: **Jie Sun** (University of Virginia) ***Respiratory immunity and immunopathology following COVID-19***
2:15-2:30	W1-2: **Tian Wang** (University of Texas Medical Branch) ***Vaccines and Treatments Against SARS-CoV-2 Infection***
2:30-2:45	W1-3: **Jianwen Que** (Columbia University) ***Lung injury and regeneration following SARS-CoV-2 infection***
2:45-3:00	W1-4: **Haitao Hu** (University of Texas Medical Branch) ***Combination mRNA vaccination induces robust protection against SARS-CoV-2 variants of concern***
3:00-3:15	W1-5: **Hongpeng Jia** (Johns Hopkins University) ***The role of maternal SCV2 infection in shaping the antiviral immunity in offspring***
3:15-3:30	W1-6: **Shan-Lu Liu** (The Ohio State University) ***Cell-to-Cell Transmission of SARS-CoV-2, HIV-1 and EBOV***

2. Biotech & Single Cell Research: Chenghang Zong & Hongjie Li (2nd floor auditorium)

2:00-2:15	W2-1: **Hongjie Li** (Baylor College of Medicine) ***Fly Cell Atlas and Beyond***
2:15-2:30	W2-2: **Xiao Wang** (Massachusetts Institute of Technology) ***Translating spatial cell atlas to tissue function***
2:30-2:45	W2-3: **Yanxiang Deng** (Yale University) ***Spatial-CUT&Tag: Spatially resolved chromatin modification profiling at the cellular level***
2:45-3:00	W2-4: **Mingjie Dai** (Harvard Medical School) ***Highly scalable and flexible RNA diagnostics with One-Seq***
3:00-3:15	W2-5: **Chenghang Zong** (Baylor College of Medicine) ***Droplet-based High-Throughput Transcriptome Profiling of Individual Synapses Using Single-Cell Total-RNA-Seq***
3:15-3:30	W2-6: **Rongbin Zheng** (Boston Children's Hospital) ***Computational modeling of single cell metabolism in brown adipose tissue***

3. Neuroscience: Long-Jun Wu & Dandan Sun (2nd floor room 1)

2:00-2:15	W3-1: **Dandan Sun** (University of Pittsburgh) ***Modulating microglial energy metabolism in post-stroke brain repair***
2:15-2:30	W3-2: **Hui Zheng** (Baylor College of Medicine) ***A TFEB-mediated Lysosomal-to-Nucleus Signaling Pathway in Immune System Activation in Tauopathy***
2:30-2:45	W3-3: **Na Zhao** (Mayo Clinic) ***Microglial TREM2 expression modulates neuronal functions and amyloid development***
2:45-3:00	W3-4: **Ru-Rong Ji** (Duke University) ***Roles of pattern recognition receptors in primary sensory neurons for sensing danger signals and control of pain and itch***
3:00-3:15	W3-5: **Long-Jun Wu** (Mayo Clinic) ***Neuroimmune interaction: how microglia sense neuronal hyper- and hypoactivity***

4. Cancer Biology and Therapies: Wenliang Li & Jindan Yu (2nd floor room 2)

2:00-2:15	W4-1: **Jiaoti Huang** (Duke University) ***Cellular heterogeneity in therapy resistance and disease progression of prostate cancer***
2:15-2:30	W4-2: **Xiaoqi Liu** (University of Kentucky) ***Plk1 in prostate cancer progression and therapy resistance***
2:30-2:45	W4-3: **Jindan Yu** (Northwestern University) ***Epigenetic regulation of cancer metabolism leading to disease progression***
2:45-3:00	W4-4: **Wenliang Li** (University of Texas Health Science Center at Houston) ***Identifying and targeting novel kinase regulators for cancer metastasis***
3:00-3:15	W4-5: **Feng Yang** (Baylor College of Medicine) ***MAPK4 as a novel oncogenic driver for prostate cancer growth and therapy resistance***
3:15-3:30	W4-6: **Min Zhang** (University of Pittsburgh) ***A computational framework to characterize the cancer drug induced effect on aging using transcriptomic data***

5. Metabolism & Lipid: Li Qiang & Meilian Liu (2nd floor room 3)

2:00-2:15	W5-1: **Chih-Hao Lee** (Harvard University) ***Th2 cytokines in beige adipogenesis and metabolic health***
2:15-2:30	W5-2: **Meilian Liu** (University of New Mexico) ***Hormonal control of ILC2 development and activation in health and disease***
2:30-2:45	W5-3: **Beiyan Zhou** (University of Connecticut) ***Capture macrophage actions in diseased with novel single-cell based programs***
2:45-3:00	W5-4: **Qiong A. Wang** (City of Hope Medical Center) ***Adipogenesis and aging-associated adiposity***
3:00-3:15	W5-5: **Hai-Bin Ruan** (University of Minnesota) ***Myogenic (re)programing during brown adipose tissue development and aging***

3:30-4:00 pm Coffee Break

4:00 – 5:30 pm: Concurrent Workshops with Co-Chairs and Locations

6. SARS-CoV2 & COVID-19: Guangxiang Luo & Qiuhong Wang (1st floor conference room)

4:00-4:15	W6-1: **Qiuhong Wang** (The Ohio State University) ***The cold-adapted, temperature-sensitive SARS-CoV-2 strain TS11 is attenuated in Syrian hamsters and a candidate attenuated vaccine***
4:15-4:30	W6-2: **Pinghui Feng** (University of Southern California) ***Targeting a pyrimidine synthesis enzyme to combat SARS-CoV-2 infection.***
4:30-4:45	W6-3: **Yuntao Wu** (George Mason University) ***Development of a novel hybrid alphavirus-SARS-CoV-2 pseudovirion for rapid quantification of neutralization antibodies and antiviral drugs***
4:45-5:00	W6-4: **Tongqing Zhou** (National Institute of Allergy and Infectious Diseases) ***Structural basis of SARS-CoV-2 immune evasion and antibody neutralization***
5:00-5:15	W6-5: **Guangxiang (George) Luo** (University of Alabama at Birmingham) ***Cell culture models for investigation of SARS-CoV-2 infection and virus-host interaction***
5:15-5:30	W6-6: **Fei Gao** (Stanford University) ***Robust CD8+ and CD4+ T cell response to Pfizer/Biontech BNT162b2 vaccine and SARS-CoV- 2 infection in longitudual cohorts***

7. Cancer Biology & Therapies: Jing Yang & Dihua Yu (2nd floor auditorium)

4:00-4:15	W7-1: **Jing Yang** (University of California, San Diego) ***Regulation of epithelial-mesenchymal plasticity by matrix rigidity in carcinoma metastasis***
4:15-4:30	W7-2: **Min Yu** (University of Southern California) ***Circulating tumor cells inform mechanisms of breast cancer metastasis***
4:30-4:45	W7-3: **Huiping Liu** (Northwestern University) ***Circulating tumor stem cell clusters in breast cancer metastasis***
4:45-5:00	W7-4: **Siyuan Zhang** (University of Notre Dame) ***Mapping the role of neuronal niche in tumor brain metastasis progression***
5:00-5:15	W7-5: **Dihua Yu** (The University of Texas MD Anderson Cancer Center) ***EZH2 Promotes Breast Cancer Metastasis via methyltransferase-independent functions***
5:15-5:30	W7-6: **Bo Gao** (The University of Hong Kong) ***Post-translational Regulation of Wnt/PCP Signaling***

8. Neuroscience: Hong-Shuo Sun & Zhong-Ping Feng (2nd floor room 1)

4:00-4:15	W8-1: **Zhong-Ping Feng** (University of Toronto) ***Calcium-dependent mechanisms underlying learning & memory***
4:15-4:30	W8-2: **Hong-Shuo Sun** (University of Toronto) ***Ion channels in drug development for neuroprotection***
4:30-4:45	W8-3: **Jianhua Zhang** (University of Alabama at Birmingham) ***Protein O-GlcNAcylation and regulation of mitochondrial function in the brain***
4:45-5:00	W8-4: **Zhenning Yang** (Rutgers-The State University of New Jersey) ***Biomarkers of Circadian Disruption of Central and Peripheral Circadian Clocks in Night Shift Nurses in Working Environment***
5:15-5:30	W8-5: **Zilai Wang** (Chicago BioSolutions, Inc) ***Identification of GBM Subtype Specific small molecule inhibitors as potential therapeutics***

9. Genome Integrity & DNA Biology: Xiaobo Zhou & Honghuang Lin (2nd floor room 2)

4:00-4:15	W9-1: **Hailiang Huang** (Harvard Medical School) ***Pinning down genes and variants causal to the inflammatory bowel diseases***
4:15-4:30	W9-2: **Yin Shen** (University of California San Francisco) ***Dissect and characterize Alzheimer's disease risk variants***
4:30-4:45	W9-3: **Housheng Hansen He** (University of Toronto) ***Linking genetic and epigenetic risk in prostate cancer***
4:45-5:00	W9-4: **Xiaobo Zhou** (Harvard Medical School) ***Identification and characterization of the GWAS genetic variants and susceptible causal genes implicated in pulmonary diseases***
5:00-5:15	W9-5: **Jun-yuan Ji** (Tulane University) ***Role of Wnt signaling in regulating lipid homeostasis***
5:15-5:30	W9-6: **Binbin Ma** (Johns Hopkins University) ***Histone inheritance patterns in mammalian adult stem cells***

10. Metabolism: Wen-Xing Ding & Ling Yang (2nd floor room 3)

4:00-4:15	W10-1: **Mark Li** (University of Iowa) ***GILT-mediated lysosomal function maintains cardiac immuno-metabolic homeostasis in pressure-overload heart failure***
4:15-4:30	W10-2: **Bilon Khambu** (Tulane University) ***Autophagy regulates hepatic acetylome***
4:30-4:45	W10-3: **Wei-Xing Zong** (Rutgers-The State University of New Jersey) ***Bilateral roles of glutamine anabolism in cancer development and therapy***
4:45-5:00	W10-4: **Zhenyu Yue** (Icahn school of Medicine at Mount Sinai) ***The Landscape of Autophagy Degradation in the Brain***

	5:00-5:15	W10-5: **Jing Pu** (University of New Mexico) ***BORC-ARL8-HOPS Ensemble is Required for Lysosomal Cholesterol Egress through NPC2***
	5:15-5:30	W10-6: **Xuehong Zhang** (Harvard T. H. Chan School of Public Health) ***Sugar-Sweetened Beverage Intake and Liver Cancer Risk***

5:30 – 7:00 pm **Poster Session 1** (**Odd # poster,** Joyce Cummings Center Lobby)

7:00 pm Shuttles departing Joyce Cummings Center to AC/Fairfield and AC Cambridge

Dinner (on your own)

July 29 8:00 am Shuttles leaving AC/Fairfield Medford and AC Cambridge to Joyce Cummings Center

8:00 – 9:00 am Breakfast (Joyce Cummings Center Lobby)

8:00 – 12:00 noon Registration (Joyce Cummings Center Lobby)

9:00 – 10:45 am **Keynote and Plenary Lectures** (1st floor conference room)

Chairpersons: Wei Yang and Lee Zou

9:00 – 9:45 am Keynote Lecture: **Harvey J. Alter, M.D.,** 2020 Nobel Laureate, NIH

Hepatitis C: The end of the beginning and possibly the beginning of the end

Introduction by Zhi-Ming Zheng

9:45 – 10:15 am Plenary Lecture: **Louise T. Chow**, Ph.D., University of Alabama at Birmingham

Three-dimensional tissue models in vitro and in vivo to identify small molecular inhibitors of human papillomavirus diseases

Introduction by Wei Yang

10:15 – 10:45 am Plenary Lecture**: Kun-Liang Guan**, Ph.D., University of California, San Diego

Hippo signaling and cancer

Introduction by Lee Zou

10: 45 – 11: 10 am Coffee Break

11: 10 – 12: 40 pm **Concurrent Workshops with Co-Chairs and Locations**

11. HBV and Hepatitis: Haitao Guo & Jianming Hu (1st floor conference room)

11:10-11:25	W11-1: **Haitao Guo (**University of Pittsburgh) ***HBV cccDNA minichromosome: formation and epigenetics***
11:25-11:40	W11-2: **Jianming Hu** (Penn State University College of Medicine) ***Empty HBV Virions: Formation and Application***
11:40-11:55	W11-3: **Lishan Su** (University of Maryland School of Medicine) ***Modeling HBV Infection and Immunotherapy in Mice***

11:55-12:10	W11-4: **Zongdi Feng** (Nationwide Children's Hospital) ***The hepatitis E virus infectious cycle – an update***
12:10-12:25	W11-5: **Wei Jiang** (Medical University of South Carolina) ***Differential Expression of CREM/ICER Isoforms Is Associated with the Spontaneous Control of HIV Infection***
12:25-12:40	W11-6: Zhe Hu (China Agricultural University) ***The rapidly increased replication induced by mutations in multiple gene segments facilitates the adaptation of H9N2 avian influenza virus in vaccinated chickens***

12. Cancer Biology & Therapies: Jinsong Liu & Henry Heng (2nd floor auditorium)

11:10-11:25	W12-1: **Guang Peng** (The University of Texas MD Anderson Cancer Center) ***Transcriptomic mechanisms underlying the immune modulating function and therapeutic efficacy of PARP inhibitors***
11:25-11:40	W12-2: **Tao P. Wu** (Baylor College of Medicine) ***A New Method for Comprehensive Genomic Methylome Profiling in Cancer***
11:40-11:55	W12-3: **Henry H. Heng** (Wayne State University) ***Genome Chaos, Information Creation, and Cancer Emergence: A New Framework of Cancer Evolution***
11:55-12:10	W12-4: **Jinsong Liu** (The University of Texas MD Anderson Cancer Center) ***Giant cells: A new paradigm to understand human life and tumors at an organismal level***
12:10-12:25	W12-5: **Amy S. Yee** (Tufts University) ***CHA1: A New Combinatorial Therapy That Reciprocally Regulates Wnt and JAK/STAT/Interferon Signaling to Re-program Breast Tumors and the Tumor-Resident Landscape***

13. Neuroscience: Wenzhe Ho & Wenhui Hu (2nd floor room 1)

11:10-11:25	W13-1: **Wenhui Hu** (Temple University) ***Novel scalable and simplified system to generate microglia-containing cerebral organoids from human induced pluripotent stem cells***
11:25-11:40	W13-2: **Wen-Zhe Ho** (Temple University) ***HIV infection of human IPSC-derived microglia-containing cerebral organoids***
11:40-11:55	W13-3: **Guo-li Ming** (University of Pennsylvania) ***Engineering Organoid Models for Understanding Human Neurodevelopment and neurological disorders***
11:55-12:10	W13-4: **Yu-Chieh Wang** (Medical College of Wisconsin) ***Using Organoid Models to Investigate Neurodevelopmental Abnormalities Caused by a Congenital Disorder of Deglycosylation***
12:10-12:25	W13-5: **Xuejun Jiang** (Memorial Sloan Kettering Cancer Center) ***Lysosomal Stress, Mechanisms and Potential Effect on Neurodegeneration***
12:25-12:40	W13-6: **Xiao-Long Wang** (Temple University) ***Methamphetamine Enhances HIV Infection of iPSC-derived Microglia and Macrophages***

14. Metabolism: Qingchun Tong & Yong Xu (2nd floor room 2)

11:10-11:25	W14-1: **Allison W. Xu** (University of California, San Francisco) ***CNS control of metabolic functions over the lifespan***
11:25-11:40	W14-2: **Yanlin He** (Louisiana State University) ***Asprosin promotes feeding through SK channel-dependent activation of AgRP neurons***
11:40-11:55	W14-3: **Jonathan Z. Long** (Stanford University) ***An exercise-inducible metabolite that suppresses feeding and obesity***
11:55-12:10	W14-4: **Pingwen Xu** (The University of Illinois at Chicago) ***27 hydroxy-cholesterol acts on estrogen receptor alpha expressed by POMC neurons to modulate feeding behavior***
12:10-12:25	W14-5: **Hongxia Ren** (Indiana University School of Medicine) ***Investigating the Metabolic Function of an Orphan G Protein-coupled Receptor in the Intestinal Epithelium***

15. Genome Integrity & DNA Biology: Bing Xia & Zhiyuan Shen (2nd floor room 3)

11:10-11:25	W15-1: **Hua Wang** (Harbor-UCLA Medical Center) ***METTL3 regulates the homology-dependent DNA repair pathway choice***
11:25-11:40	W15-2: **Yuan He** (Northwestern University) ***Structural basis of DNA double-strand break repair by NHEJ***
11:40-11:55	W15-3: **Yimeng Zhu** (Columbia University) ***Inhibition is not the same as deletion – lessons from mouse models with catalytically inactive mutations in DNA damage response factors – ATM, ATR, DNA-PKcs and PARPs***
11:55-12:10	W15-4: **Yanbin Zhang** (University of Miami) ***Defining the role of FANCA in breast cancer development and cell cycle progression***
12:10-12:25	W15-5: **Jian Jian Li** (University of California, Davis) ***Radioresistant glioblastoma cells enhance fat burning with CD47 mediated anti-phagocytosis***

12:40 – 2:00 pm Lunch (Cafeteria)

2:00 – 3:30 pm Concurrent Workshops with Co-Chairs and Locations

16. Tumor viruses & Cancers: Renfeng Li & Pinghui Feng (1st floor conference room)

2:00-2:15	W16-1: **Jun Zhao** (Cleveland Clinic) ***KSHV hijacks CAD to promote metabolic reprogramming and cell proliferation***
2:15-2:30	W16-2: **Shunbin Ning** (East Tennessee State University) ***New mechanistic insights into the host-virus interaction in EBV pathogenesis***
2:30-2:45	W16-3: **Fanxiu Zhu** (Florida State University) ***A non-catalytic and intrinsically disordered herpesviral protein rewires the host cellular signaling by impacting protein phosphorylation, ubiquitination, and sumoylation through SLiMs***

2:45-3:00	W16-4: **Xinghong Dai** (Case Western Reserve University) ***Cholesterol-driven spontaneous morphagenesis of HBV subviral particles***
3:00-3:15	W16-5: **Shou-Jiang (SJ) Gao** (University of Pittsburgh) ***Tumor suppression of arginine sensor CASTOR1 in viral and nonviral cancers***
3:15-3:30	W16-6: **Minh Tuyet Ma** (Rutgers-The State University of New Jersey) ***Chimeric Antigen Receptor (CAR)-Natural Killer (CAR-NK) Cells for Treatment of COVID Patients with Cancer***

17. Cancer Biology & Therapies: Bin Zheng & Wei-Xing Zong (2nd floor auditorium)

2:00-2:15	W17-1: **Boyi Gan** (University of Texas MD Anderson Cancer Center) ***Targeting Ferroptosis in Cancer Therapy***
2:15-2:30	W17-2: **Ning Wu** (Van Andel Institute) ***Excess glucose reduces optimal mitochondrial function by reducing membrane fluidity***
2:30-2:45	W17-3: **Hui Feng** (Boston University School of Medicine) ***The context-dependent role of the TCA cycle in MYC-driven tumor development***
2:45-3:00	W17-4: **Bin Zhang** (Northwestern University) ***Sustaining antitumor response of CD8+ T cells by distinct metabolic orchestration***
3:00-3:15	W17-5: **Bin Zheng** (Harvard Medical School) ***Repurposing phenformin for the treatment of cancer***

18. Lipid & Adipocytes: Kai Sun & Yu An (2nd floor room 1)

2:00-2:15	W18-1: **Shaotong Zhu** (Institute for Protein Innovation) ***High-Throughput Synthetic Antibody Discovery in Metabolic Diseases***
2:15-2:30	W18-2: **Yuwei Jiang** (The University of Illinois at Chicago) ***Lineage tracing of origin and fate of beige adipocytes in adult mice***
2:30-2:45	W18-3: **Yi Zhu** (Baylor College of Medicine) ***Connexin43 in the adipose tissue***
2:45-3:00	W18-4: **Yu A. An** (UT Health Science Center at Houston) ***Mitokines-mediated adipose tissue crosstalk***
3:00-3:15	W18-5: **Kai Sun** (University of Texas Health Science Center at Houston) ***Adipose Tissue Specific Ces1d Mediates Whole-Body Metabolic Homeostasis***
3:15-3:30	W18-6: **Fanyin Meng** (Indiana University School of Medicine) ***Deficiency of lin28 induces senescence of activated hepatic stellate cells and limits liver fibrosis during alcohol-induced liver injury***

19. Genome Integrity & DNA Biology: Xiaohua Wu & Li Lan (2nd floor room 2)

2:00-2:15	W19-1: **Hengyao Niu** (Indiana University, Bloomington) ***Deciphering the mechanism of processive ssDNA digestion by the Dna2-RPA ensemble***

2:15-2:30	W19-2: **Hong Wang** (North Carolina State University) ***DNA capture and loop extrusion dynamics by cohesin-NIPBL at the single-molecule level***
2:30-2:45	W19-3: **Huadong Pei** (Georgetown University) ***The role of histidine phosphorylation in DNA alkylation damage repair***
2:45-3:00	W19-4: **Li Lan** (Massachusetts General Hospital) ***Understanding R-loop and mRNA dependent repair pathway in cancer therapy***
3:00-3:15	W19-5: **Junjie Chen** (The University of Texas M. D. Anderson Cancer Center) T***ARGETING DNA DAMAGE RESPONSIVE PATHWAYS FOR CANCER THERAPY***
3:15-3:30	W19-6: **Jian Ouyang** (Harvard Medical School) ***RNA-dependent Homologous Recombination Repair DNA Double Strand Breaks***

20. Neuroscience: Hui Zheng & Xiaobo Mao (2nd floor room 3)

2:00-2:15	W20-1: **Yue-Ming Li** (Memorial Sloan Kettering Cancer Center) ***gamma-Secretase Modulation: from Hypoxia to Neuroinflammation***
2:15-2:30	W20-2: **Xiaobo Mao** (Johns Hopkins University) ***Pathogenic α-synuclein cell-to-cell transmission mechanism and related therapeutic development***
2:30-2:45	W20-3: **Yihong Ye** (National Institutes of Health) ***Abnormal triaging of misfolded proteins by adult neuronal ceroid lipofuscinosis-associated DNAJC5/CSPα mutants causes lipofuscin accumulation***
2:45-3:00	W20-4: **Junmin Peng** (St. Jude Children's Research Hospital) ***Proteomic landscape of Alzheimer's Disease: novel insights into pathogenesis***
3:00-3:15	W20-5: **Jiefu Li** (Janelia Research Campus, Howard Hughes Medical Institute) ***In Situ Cell-Surface Proteomics: Method Development and Applications in Neurobiology***
3:15-3:30	W20-6: **Pengbo Zhou** (Weill Medical College) ***G3BP1 modulates SPOP to promote prostate tumorigenesis***

3:30 – 4:00 pm Coffee Break

4:00 – 5:30 pm Concurrent Workshops with Co-Chairs and Locations

21. Immunotherapy: Jianzhu Chen & Lishan Su (1st floor small auditorium)

4:00-4:15	W21-1: **Jianzhu Chen** (Massachusetts Institute of Technology) ***Memory-Like NK Cells Armed with Neoepitope-Specific CAR for Treating Acute Myeloid Leukemia***
4:15-4:30	W21-2: **Qing Yi** (Houston Methodist Research Institute) ***Reprogramming lipid metabolism to restore tumor-specific CD8+ T cell function in tumor microenvironment***
4:30-4:45	W21-3: **Dong Feng Chen** (Harvard Medical School) ***Harnessing immunotherapy against neurodegeneration to restore vision***
4:45-5:00	W21-4: **Lili Qu** (University of Connecticut) ***Low disturbed flow induces dynamic immune compartment change in atherogenesis revealed by scRNA-seq***

5:00-5:15	W21-5: **Wenbin Lin** (The University of Chicago) ***Development of Innovative Nanotechnologies to Enhance Cancer Immunotherapy***

22. RNA Biology: Peixuan Guo & Daniel Binzel (2nd floor auditorium)

4:00-4:15	W22-1: **Peixuan Guo** (The Ohio State University) ***The dynamic, motile and deformative properties of RNA nanoparticles lead to efficient tumor vasculature targeting, fast renal excretion and rapid body clearance.***
4:15-4:30	W22-2: **Daniel Binzel** (The Ohio State University) ***Rational design for delivery and controlled release of siRNA from RNA nanoparticles via tumor targeting extracellular vesicles***
4:30-4:45	W22-3: **Wah Chiu** (Stanford University) ***Cryogenic Electron Microscopy of RNA Structures at Near Atomic Resolution***
4:45-5:00	W22-4: **Shi-Jie Chen** (University of Missouri, Columbia) ***Predicting RNA-small molecule interactions***
5:00-5:15	W22-5: **Shanqing Gu** (ExonanoRNA, LLC) ***Synthesis of RNA nanoparticles with cholesterol for ligand display on exosomes to target cancer cells for specific therapeutic drug delivery***
5:15-5:30	W22-6: **Peisong Gao** (Johns Hopkins School of Medicine) ***Mannose decorated exosomes with RNA nanoparticles harboring miR-511-3p protects against allergic asthma***

23. Cancer Biology & Immunity: Wanjun Chen & Ying Zhang (2nd floor room 1)

4:00-4:15	W23-1: **Wanjun Chen** (NIDCR, National Institutes of Health) ***TGFβ regulation of T-cell immunity***
4:15-4:30	W23-2: **Xiao-Jing Wang** (University of California Davis) ***Preclinical advances in combined TGFβ/PD-L1 inhibition in head and neck cancer and the implementation to clinical studies***
4:30-4:45	W23-3: **Zihai Li** (The Ohio State University) ***Targeting GARP to Thwart the TGF Pathway for Cancer Immunotherapy***
4:45-5:00	W23-4: **Xunrong Luo** (Duke University) ***Exploiting Apoptotic Cell Induced TGF for Transplantation Tolerance***
5:00-5:15	W23-5: **Hua Eleanor Yu** (City of Hope Comprehensive Cancer Center) ***STAT3: from fundamental discoveries to clinical translation***

24. Genome integrity & DNA biology: Zhongsheng You & Justin Leung (2nd floor room 2)

4:00-4:15	W24-1: **Yang Gao** (Rice University) ***Structural basis of DNA polymerase catalyzed DNA synthesis, error incorporation, and double-strand break repair***

4:15-4:30	W24-2: **Justin W Leung** (University of Arkansas for Medical Sciences) ***Molecular dissection of the evolutionarily diverse H2AX C-terminus in DNA repair***
4:30-4:45	W24-3: **Shan Yan** (University of North Carolina at Charlotte) ***Molecular mechanism of DNA single-strand break repair and signaling***
4:45-5:00	W24-4: **Junran Zhang** (The Ohio State University) ***Antioxidant system Trx1/Trx1R is a novel determinant of CHK1 inhibitor sensitivity in treating NSCLC via regulation of RRM1 oxidation/reduction***
5:00-5:15	W24-5: **Weihang Chai** (Loyola University Chicago) ***A novel tumor suppressor of colorectal cancer***
5:15-5:30	W24-6: **Xuwen Li** (Baylor College of Medicine) ***NT-seq: a Chemical-based Sequencing Method for Genomic Methylome Profiling***

25. Liver Metabolism: Huiping Zhou & Heather Francis (2nd floor room 3)

4:00-4:15	W25-1: **Tiangang Li** (University of Oklahoma Health Sciences Center) ***Pharmacological modulation of bile acid pool and its impact on non-alcoholic steatohepatitis and cholestasis in mice***
4:15-4:30	W25-2: **Grace L Guo** (Rutgers University) ***Effects of Overexpression of Fibroblast Growth Factor 15/19 on Hepatic Drug Metabolizing Enzymes***
4:30-4:45	W25-3: **Lindsey Kennedy** (Indiana University School of Medicine) ***Inhibition of endothelin receptor A (ET-A) reduces biliary damage, angiogenesis and liver fibrosis in cholestasis***
4:45-5:00	W25-4: **Wen-Xing Ding** (University of Kansas Medical Center) ***Regulation of Liver Cell Remodeling and Ductular Reaction by mTOR Activation in Alcoholic Hepatitis***
5:00-5:15	W25-5: **Xiaochao Ma** (University of Pittsburgh) ***EPP-related cholestasis: mechanism and novel therapy***
5:15-5:30	W25-6: **Xiaoqiang Qi** (University of Missouri, Columbia) ***Modulating gut microbiota to improve intrahepatic immunity against hepatocellular cancer***

5:30 – 6:30 pm	**Meet the Editors**
	Chairperson: Chris Lau
	Jia Cheng (Cell), Steve Mao and Zhaodong Li (Cancer Cell), Brian Plosky (Mol Cell), Mariela Zirlinger (Neuron), and Chris Lau (Cell & Bioscience) (2nd floor auditorium)
7:00 – 9:00 pm	**Banquet** (Joyce Cummings Center 1st floor)
9:00 pm	Shuttles departing Joyce Cummings Center to AC/Fairfield and AC Cambridge
9:30 pm	Shuttle departing Joyce Cummings Center to AC/Fairfield

July 30 8:00 am Shuttles leaving AC/Fairfield Medford and AC Cambridge to Joyce Cummings Center

8:00 – 9:00 am Breakfast (Joyce Cummings Center Lobby)

9:00 – 10:45 am **Keynote and Plenary Lectures (1st floor conference room)**

Chairpersons: Xinnian Dong, Duke University, and Hong Chen, Harvard University

9:00 – 9:45 am Keynote Lecture: **Virginia M.-Y. Lee,** Ph.D., 2020 Breakthrough Prize in Life Sciences recipient, University of Pennsylvania School of Medicine

Transmission of misfolded proteins in neurodegenerative disorders: a common mechanism of disease pathogenesis

Introduction by Hui Zheng

9:45 – 10:15 am Plenary Lecture: **Hao Wu**, Ph.D., Harvard Medical School

Inflammasomes at the crossroads of basic science and translation

Introduction by Xinnian Dong

10:15 – 10:45 am Plenary Lecture**: Zhi-Gang He,** Ph.D. Boston Children's Hospital, Harvard Medical School

Neural Regeneration after CNS Injury

Introduction by Hong Chen

10: 45 – 11: 10 am Coffee Break

11: 10 – 12: 40 pm **Concurrent Workshops with Co-Chairs and Locations**

26. RNA Biology: Chuan He & Xinshu Grace Xiao (1st floor conference room)

11:10-11:25	W26-1: **Xinshu Xiao** (University of California, Los Angeles) ***Widespread RNA hypoediting in schizophrenia and its molecular impact on mitochondrial function***
11:25-11:40	W26-2: **Zhi-Ming Zheng** (National Cancer Institute) ***The long noncoding RNA lnc-FANCI-2 restricts RAS signaling but maintains constitutive IFN response via TLR3 and MCAM in HPV-infected cervical cancer cells***
11:40-11:55	W26-3: **Shuo Gu** (National Cancer Institute) ***TENT2, TUT4, and TUT7 selectively regulate miRNA sequence and abundance***
11:55-12:10	W26-4: **Jianjun Chen** (Beckman Research Institute of City of Hope) ***METTL16 plays oncogenic roles in liver cancer and leukemia through distinct mechanisms***
12:10-12:25	W26-5: **Chuan He** (The University of Chicago) ***RNA methylation in gene expression regulation***
12:25-12:40	W26-6: **Xinlei Gao** (Boston Children's Hospital) ***Abundant m6A modification on RNA signifies strong expression maneuverability of tumor suppressors***

27. Cancer Biology and Therapies: Xinwei Wang & Chris Lau (2nd floor auditorium)

11:10-11:25	W27-1: **Gen-Sheng Feng** (University of California San Diego) ***Dissecting Shp2 function in hepatocytes unveils a new cell-cell communication mechanism under stress***
11:25-11:40	W27-2: **Yingzi Yang** (Harvard School of Dental Medicine) ***Generation of a novel mature liver organoid***
11:40-11:55	W27-3: **Lichun Ma** (National Cancer Institute) ***Spatial single-cell dissection of tumor and immune cells reveals stable lock-and-key features of a malignant ecosystem in liver cancer***
11:55-12:10	W27-4: **Yun-Fai Chris Lau** (University of California, San Francisco) ***Androgen receptor and AR variant 7 exacerbate the c-Myc-mediated hepatocarcinogenesis***
12:10-12:25	W27-5: **Mitchell Ho** (National Cancer Institute) ***Engineering CAR T cells Targeting GPC3 for liver cancer immunotherapy***
12:25-12:40	W27-6: **Yukai He** (Augusta University) ***Novel intermediate-avidity glypican-3 specific CARTs resist exhaustion and mediate durable antitumor effects against human hepatocellular carcinoma***

28. Neuroscience: Zhiping Pang & Ye Zhang (2nd floor room 1)

11:10-11:25	W28-1: **Xianhua Piao** (University of California, San Francisco) ***Microglial Mechanism of interneuron development***
11:25-11:40	W28-2: **Peng Jiang** (Rutgers University) ***Modeling microglia pathology in Down syndrome and Alzheimer's disease using human iPSC models***
11:40-11:55	W28-3: **Ye Zhang** (University of California, Los Angeles) ***Oligodendrocyte-lineage cell exocytosis and L-type prostaglandin D synthase promote oligodendrocyte development and myelination***
11:55-12:10	W28-4: **Chun-Li Zhang** (UT Southwestern Medical Center) ***In vivo multilineage reprogramming of adult astrocytes***
12:10-12:25	W28-5: **Sheng Zhang** (University of Texas Health Science Center at Houston) ***HAP40 in Huntingtin Regulation and Huntington's disease Pathogenesis***
12:25-12:40	W28-6: **Yi-Ping Hsueh** (Academia Sinica, Taipei) ***Phase separation and zinc-induced liquid-to gel phase transition modulate synaptic distribution and association of autism-linked CTTNBP2 and SHANK3***

29. Immunity & Immunotherapy: Shaun Zhang (2nd floor room 2)

11:10-11:25	W29-1: **Liang Deng** (Memorial Sloan Kettering Cancer Center) ***Reprogramming tumor microenvironment by a second-generation recombinant modified vaccinia virus Ankara***
11:25-11:40	W29-2: **Weiyi Peng** (University of Houston) ***Overcoming tumor immune resistance by targeting tumor intrinsic pathways***

11:40-11:55	W29-3: **Xiaotong Song** (Texas A&M University) ***Rational design of vaccinia virus for cancer therapy***
11:55-12:10	W29-4: **Yong Lu** (Houston Methodist Research Institute) ***Th9 cells represent a new paradigm of CD4+ T cells endowed with the ability to eradicate advanced tumors***
12:10-12:25	W29-5: **Nidhi Sahni** (The University of Texas MD Anderson Cancer Center) ***Delving into proteostasis network reveals an immunogenic vulnerability in mismatch repair deficient cancer***
12:25-12:40	W29-6: **Xin Wang** (Boston Children's Hospital) ***Cancer mutation landscape reveals marker genes and molecular regulators of immunotherapy efficacy***

30. Metabolism: Liqing Yu & Hongmin Ni (2nd floor room 3)

11:10-11:25	W30-1: **Hong-Min Ni** (University of Kansas Medical Center) ***The role of VMP1 in regulating hepatic lipoprotein secretion and NASH***
11:25-11:40	W30-2: X. **Charlie Dong** (Indiana University School of Medicine) ***Guarding against fatty liver disease by sirtuin 6***
11:40-11:55	W30-3: **Zhi Zhong** (Medical University of South Carolina) ***Histone deacetylase-11 (HDAC11) as a novel therapeutic target for non-alcoholic steatohapatitis (NASH)***
11:55-12:10	W30-4: **Wanqing Liu** (Wayne State University) ***Role of Fatty Acid Desaturase 1 (FADS1) in Non-alcoholic Fatty Liver Disease (NAFLD)***
12:10-12:25	W30-5: **Chaodong Wu** (Texas A&M University) ***Role of microbiota metabolite indole in NAFLD/NASH***
12:25-12:40	W30-6: **Lin Jia** (The University of Texas at Dallas) ***The Requirement and Sufficiency of Hepatocyte Toll-like Receptor 4 (TLR4) in Alcohol-associated Insulin Resistance***

12:40 – 2:00 pm Lunch (Cafeteria)

2: 00 – 3: 30 pm Concurrent Workshops with Co-Chairs and Locations

31. RNA Biology: Rui Zhao & Yongsheng Shi (1st floor conference room)

2:00-2:15	W31-1: **Yongsheng Shi** (University of California, Irvine) ***Anti-cancer compound JTE-607-mediated inhibition of pre-mRNA 3' processing is sequence-dependent***
2:15-2:30	W31-2: **Rui Zhao** (University of Colorado School of Medicine) ***Human PRPF39 is a new alternative splicing factor affecting weak 5' splice sites***
2:30-2:45	W31-3: **Wei Li** (University of California, Irvine) ***Alternative polyadenylation-wide association study (3'aTWAS) identifies novel APA-linked susceptibility genes in human disease.***
2:45-3:00	W31-4: **Chaolin Zhang** (Columbia University) ***MAPT splicing, tauopathies and lineage-specific regulation in the primate brain***

3:00-3:15	W31-5: **Xin Li** (University of Rochester Medical Center***) A new function of ribosome: guiding piRNA formation***
3:15-3:30	W31-6: **Jing Zeng** (Virginia Commonwealth University) ***Circular RNA Microarray Analysis in Mdr2-/- mice***

32. Immunity & Immunotherapy: Bangyan Stiles & Cynthia Ju (2nd floor auditorium)

2:00-2:15	W32-1: **Liyun Yuan** (University of Southern California) ***Outcomes of liver transplantation for combined hepatocellular cholangiocarcinoma: a single-center experience and literature review***
2:15-2:30	W32-2: **Cynthia Ju** (University of Texas Health Science Center at Houston) ***Role of Eosinophils in Acute Liver Injury***
2:30-2:45	W32-3: **Ekihiro Seki** (Cedars-Sinai Medical Center) ***Immune response mediated by extracellular matrix in liver fibrosis***
2:45-3:00	W32-4: **Bangyan L. Stiles** (University of Southern California) ***Steatosis Promote Liver Cancer Development by Inducing Chemokine Production From Kupffer Cells***
3:00-3:15	W32-5: **Tao Liu** (Harvard Medical School) ***Gasdermin B is an RNA sensor that promotes interferon response and airway inflammation***
3:15-3:30	W32-6: **Xianxin Hua** (University of Pennsylvania) ***Eradication of Gastrointestinal Cancers and Neuroendocrine Tumors by CDH17CAR T Cells Without Toxicity to Healthy Tissues***

33. Cancer Therapies: Li Jia (2nd floor room 1)

2:00-2:15	W33-1: **Kaifu Chen** (Boston Children's Hospital) ***MutaGene links deficiency of mutator genes to immunotherapy efficacy***
2:15-2:30	W33-2: **Thérèse Yingying Wu** (University of Houston) ***Mathematical Models of Cancer Therapies***
2:30-2:45	W33-3: **Li Jia** (Harvard Medical School) ***CRISRP screens reveal genetic determinants of PARP inhibitor sensitivity and resistance in prostate cancer***
2:45-3:00	W33-4: **Shi-Yong Sun** (Emory University) ***Managing acquired resistance to third generation EGFR inhibitors in lung cancer***
3:00-3:15	W33-5: **Gang Han** (University of Massachusetts Chan Medical School) ***Nano-optogenetic Cellular immunotherapy***

34. Cancer Biology & Therapies: Wei Zhang & Yi Sheng (2nd floor room 2)

2:00-2:15	W34-1: ***Yi Sheng*** (York University) ***An Integrated Structure-based Approach for the Development of MDM2 inhibitors***

2:15-2:30	W34-2: ***Wei Zhang*** (University of Guelph) ***Targeted protein degradation by ubiquitin variant induced proximity***
2:30-2:45	W34-3: ***Jun Yin*** (Georgia State University) ***Engineering ubiquitin transfer in the cell – the orthogonal way***
2:45-3:00	W34-4: ***Penghua Wang*** (University of Connecticut) ***UBXN3B Controls B Lymphopoiesis via BLNK***
3:00-3:15	W34-5: ***Nicole Wang*** (Baylor College of Medicine) ***CD44 is a checkpoint blockade for dendritic cell maturation in tumor immune suppression***

35. Antivirals: Jun Wang & Zhengqiang Wang (2nd floor room 3)

2:00-2:15	W35-1: **Jinhong Chang** (Baruch S. Blumberg Institute) ***A Yellow Fever Virus NS4B-Targeting Antiviral Compound Functions Through Disruption of the ER Membrane-Derived Replication Organelles and Stimulation of dsRNA-Mediated Immune Response***
2:15-2:30	W35-2: **Wenshe Liu** (Texas A&M University) ***Some Interesting Chemistry Involving the SARS-CoV-2 Main Protease***
2:30-2:45	W35-3: **Jun Wang** (Rutgers University) ***Rational design of SARS-CoV-2 main protease inhibitors with novel warheads and improved selectivity***
2:45-3:00	W35-4: **Zhengqiang Wang** (University of Minnesota) ***Targeting human cytomegalovirus (HCMV) pUL89 endonuclease for drug discovery***
3:00-3:15	W35-5: **Xiaoyang Yu** (University of Pittsburgh) ***Screening of an epigenetic compound library identifies BRD4 as a potential antiviral target for hepatitis B virus covalently closed circular DNA transcription***
3:15-3:30	W35-6: **Jiakai Hou** (University of Houston) ***Identification of host factors limiting SARS-CoV-2 cytopathic effects by genome-wide CRISPR drop-off screens***

3:30 – 4:00 pm Coffee Break

4:00 – 5: 30 pm **Concurrent Workshops with Co-Chairs and Locations**

36. Pathogens & Infections: Genhong Cheng & Xinnian Dong (1st floor conference room)

4:00-4:15	W36-1: **Genhong Cheng** (University of California Los Angeles) ***Broad antiviral agents based on innate immune response to viral infection***
4:15-4:30	W36-2: **Rui Lu** (Louisiana State University) ***SUMV-2, but not SUMV-1, mediates RNAi-independent antiviral immunity against Orsay virus during natural infection in C. elegans***
4:30-4:45	W36-3: **Xinnian Dong** (Duke University) ***Precision in plant immune regulation***
4:45-5:00	W36-4: **Wenjun Ma** (University of Missouri, Columbia) ***Influenza pathogenesis and cross-species infection***

5:00-5:15	W36-5: **Heng-Chi Lee** (University of Chicago) ***VASA helicase promote the phase separation of germ granules to ensure proper recognition of self and non-self nucleic acids***
5:15-5:30	W36-6: **Xiaozhe Xiong** (Boston Children's Hospital) ***Emerging enterococcus pore-forming toxins with MHC/HLA-I as receptors***

37. Precision Medicine: Hong Chen & Jinjun Shi (2nd floor auditorium)

4:00-4:15	W37-1: **Jinjun Shi** (Brigham and Women's Hospital) ***mRNA Nanomedicine for Precision Cancer Immunotherapy***
4:15-4:30	W37-2: **Yun Fang** (The University of Chicago) ***Precision nanomedicine treating vascular diseases***
4:30-4:45	W37-3: **Wei Sun** (University of Pittsburgh) ***Post-GWAS Functional Analysis to Define Genetic and Acquired Pathogenesis of Pulmonary Arterial Hypertension as A Complex Disease***
4:45-5:00	W37-4: **Lili Zhang** (Boston Children's Hospital) ***Endothelial Cell-Based Precision Therapy for Cardiovascular Disease***
5:00-5:15	W37-5: **Han Xu** (The University of Texas MD Anderson Cancer Center) ***Improving allele-specific genome editing with mismatched gRNA***
5:15-5:30	W37-6: **Alyssa J. Matz** (University of Connecticut) ***AtheroSpectrum reveals novel macrophage foam cell gene signatures associated with atherosclerotic cardiovascular disease risk***

38. Heart Disease in Model Systems: Zhe Han & Xiaolei Xu (2nd floor room 1)

4:00-4:15	W38-1: **Zhe Han** (University of Maryland School of Medicine) ***Single-cell profiling of the developing Drosophila heart***
4:15-4:30	W38-2: **Da-Zhi Wang** (Boston Children's Hospital) ***Molecular Regulation of Cardiac Function, Regeneration and Disease***
4:30-4:45	W38-3: **Jiandong Liu** (University of North Carolina, Chapel Hill) ***Coordinated transcriptome and cell state dynamics of non-myocytes in heart regeneration***
4:45-5:00	W38-4: **Mei Xin** (Cincinnati Children's Hospital Medical Center) ***Inhibition of adrenergic b1-AR/Gas signaling promotes cardiomyocyte proliferation through activation of RhoA-YAP axis***
5:00-5:15	W38-5: **Xiaolei Xu** (Mayo Clinic) ***Precision Medicine for Cardiomyopathies via Zebrafish Genetics***

39. Cancer Biology & Therapies: Qin Yan & Qing Zhang (2nd floor room 2)

4:00-4:15	W39-1: **Qing Zhang** (UT Southwestern Medical Center) ***Identification of New Therapeutic Targets in Kidney Cancer***
4:15-4:30	W39-2: **Haifeng Yang** (Thomas Jefferson University) ***Tumor suppression by BAP1: STING and Interferon pathway***
4:30-4:45	W39-3: **Yali Dou** (University of Southern California) ***Structural Insights on the MLL Complex for its Canonical and Non-canonical Functions in Cancer***
4:45-5:00	W39-4: **Liling Wan** (University of Pennsylvania) ***Targeting the chromatin reader ENL as a strategy against acute myeloid leukemia***
5:00-5:15	W39-5: **Qin Yan** (Yale School of Medicine) ***Epigenetic Regulation of Cancer Metastasis and Immune Evasion***
5:15-5:30	W39-6: **Weiwei Dang** (Baylor College of Medicine) ***Altered Super Enhancer – Promoter Interactions Mediated by YY1***

40. Drug Development: Shuxing Zhang & Han Liang (2nd floor room 3)

4:00-4:15	W40-1: **X. Shirley Liu** (GV20 Therapeutics) ***Genomics and AI-enabled Cancer Immunotherapeutics***
4:15-4:30	W40-2: **Han Liang** (The University of Texas MD Anderson Cancer Center) ***AI-driven Big Data Analysis for Drug Development***
4:30-4:45	W40-3: **Shuxing Zhang** (MD Anderson Cancer Center) ***Ultra-fast Deep Screening of >1,000 Cancer Cell Lines***
4:45-5:00	W40-4: **Lei Xie** (The City University of New York) ***Exploration of dark chemical, biological and patient spaces for precision drug discovery***
5:00-5:15	W40-5: **Songhai Tian** (Boston Children's Hospital) ***Targeted intracellular delivery of Cas13 and Cas9 nucleases using bacterial toxin-based platforms***

5:30 – 7:00 pm **Poster Session 2** (**Even # poster**, Joyce Cummings Center lobby)

7:00 pm Shuttles departing Joyce Cummings Center to AC/Fairfield and AC Cambridge

Dinner (on your own)

July 31 8:00 am Shuttles leaving AC/Fairfield Medford and AC Cambridge to Joyce Cummings Center

8:00 – -9:00 am Breakfast (Joyce Cummings Center lobby)

9:00 – 12:30 pm **SCBA Award Lectures and Closing Remarks** (1st floor conference room)

Chairpersons: Lishan Su and Renfeng Li

9:00 – 9:30 am	**Yang Shi,** Ph.D., SCBA Presidential Awardee, Oxford University, UK
	A tale of two cities/projects
	Introduction by Xi He
9:30 – 10:00 am	**Yi Zhang**, Ph.D., SCBA Presidential Awardee, Boston Children's Hospital, Harvard Medical School
	My journey on epigenetics: from basic mechanisms to clinical applications
	Introduction by Lishan Su
10:00 – 10:30 am	**Xiaochen Bai,** Ph.D., Kenneth Fong Young Investigator Awardee, UT Southwestern Medical School
	Structural basis for the activation of insulin receptor
	Introduction by Renfeng Li
10:30 – 11:00 am	Coffee break
11:00 – 11:30 am	**Stanley Qi**, Ph.D., Kenneth Fong Young Investigator Awardee, Stanford University
	Human Genome Engineering Beyond Editing for Therapy
	Introduction by Chris Lau
11:30 – 12:30 am	Award Presentations and Closing Remarks
	SCBA elections: Hui Zheng and Lishan Su
	Travel and Poster award announcement: Shan-Lu Liu and Kunxin Luo
	Closing remarks: Zhi-Ming Zheng, Lee Zou, Shan-Lu Liu
12:30 pm	Lunch (Cafeteria) and Departure

Oral Presentaion Abstracts

Workshop #1:

W1-1:

Respiratory immunity and immunopathology following COVID-19

Jie Sun[1, 2]

[1]Carter Immunology Center, University of Virginia, Charlottesville, VA, USA 22908

[2]Division of Infectious Disease and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, USA 22908

Systemic immunity following SARS-CoV-2 infection or vaccination has been well characterized, but the characteristics of mucosal immunity in the respiratory tract of COVID-19 vaccinated or convalescents remain largely elusive. Here we have characterized the humoral and cellular immune responses in the lung following COVID-19 vaccination or severe natural infection. We first found that chronic lung impairment was accompanied by persistent respiratory immune alterations in COVID-19 convalescents. We showed that functional SARS-CoV-2-specific memory T and B cells were enriched at the site of infection compared to those of blood. Detailed evaluation of the lung immune compartment revealed dysregulated respiratory $CD8^+$ T cell responses were associated with the impaired lung function following acute COVID-19. Single cell transcriptomic analysis identified the potential pathogenic subsets of respiratory $CD8^+$ T cells contributing to persistent tissue conditions following COVID-19. Additionally, our results showed that vaccinated individuals had significantly lower levels of neutralizing Ab and absence of mucosal B and T cell responses compared to those of COVID-19 convalescents, despite they possessed robust circulating cellular and humoral immunity in the blood. Our data have revealed pathophysiological and immune traits that may support the development of lung sequelae following SARS-CoV-2 pneumonia. Furthermore, our study provides the immunological evidence that the current COVID-19 vaccines are highly effective against severe disease development, but offer limited protection against breakthrough infection, especially by SARS-CoV-2 variants.

W1-2:

Vaccines and Treatments Against SARS-CoV-2 Infection

Tian Wang[1, 2]

[1] Department of Microbiology & Immunology, [2]Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX, 77555, USA.

The coronavirus disease 2019 (COVID-19) pandemic has made a serious impact on global public health over the past two years. Neither licensed vaccines nor treatments are available for humans. Multiple COVID-19 vaccine platforms have been tested with collaborative efforts in our study, including a modified porous silicon microparticle (PSM)-adjuvant to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD) vaccine and a multigenic SARS-CoV-2 vaccine based on an MVA vector expressing both viral nucleocapsid (N) and spike (S) proteins (MVA-S + N). These candidate vaccines generated potent and/or durable SARS-CoV-2- specific humoral and type 1 helper T (Th) cell- mediated immune responses in animals and protected host from SARS-CoV-2 and variants challenge. In addition, we found that dietary supplementation with active hexose correlated compound to enhance host protective immunity and treatment of inhibitors targeting inflammatory responses significantly reduced host susceptibility to SARS-CoV-2 infection in mice. These candidate vaccines and treatments are potentially important for prevention and control of COVID-19 morbidity and mortality following SARS-CoV-2 infection.

W1-3:

Lung injury and regeneration following SARS-CoV-2 infection

Huachao Huang, Yinshan Fang, Jianwen Que*

Columbia Center for Human Development, Department of Medicine, Columbia University Irving Medical Center. BB-801A, 650 West 168th Street, New York, 10032

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SARS-CoV-2 infection causes severe lung injuries at the early stage of the pandemics. Detachment and extensive loss of epithelial cells are the major presentations in the airways and alveoli. In response, stem/progenitor cells proliferate and attempt to repair the injured epithelium through cooperation with other cells including fibroblasts, immune cells and tuft cells which are dramatically expanded upon infection. When examined at the single cell level, it becomes clear that these different cell types coordinate to heal the lung.

W1-4:

Combination mRNA vaccination induces robust protection against SARS-CoV-2 variants of concern

Renee L. Hajnik[1], Jessica A. Plante[1], Yuejin Liang[1], Mohamad-Gabriel Alameh[2], Jinyi Tang[3], Chaojie Zhong[1], Awadalkareem Adam[1], Dionna Scharton[1], Grace H. Rafael[1], Yang Liu[4], Nicholas C. Hazell[1], Jiaren Sun[1], Lynn Soong[1], Pei-Yong Shi[4], Tian Wang[1], Jie Sun[3], Drew Weissman[2], Scott C. Weaver[1], Kenneth S. Plante[1], **Haitao Hu**[1]

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Emergence of SARS-CoV-2 variants of concern (VOCs), including the highly transmissible Omicron and Delta strains, has posed constant challenges to current COVID-19 vaccines that principally target the viral spike protein (S). Development of next-generation vaccines against SARS-CoV-2 variants is needed. Here, we report a nucleoside-modified mRNA vaccine that expresses the more conserved viral nucleoprotein (mRNA-N) and show that mRNA-N alone induced a modest but significant control of SARS-CoV-2. Critically, combining mRNA-N with the clinically approved S-expressing mRNA vaccine for immunization (mRNA-S+N) induced robust protection against both Delta and Omicron variants. In the hamster model, we demonstrated that, compared to mRNA-S alone, combination mRNA-S+N vaccination not only induced markedly more robust control of the Delta variant as well as the Omicron variant in the lungs, but also provided enhanced protection in the upper respiratory tract. *In vivo* cell depletion revealed a critical role of CD8 T cells in mediating the protective effect of mRNA-S+N vaccine against Omicron. Antigen-specific immune analyses indicated that N-specific immunity as well as augmented S-specific immunity were associated with enhanced protection by combination mRNA vaccination. Our findings suggest a potential next-generation vaccine approach for broad protection against SARS-CoV-2 variants.

W1-5:

The role of maternal SCV2 infection in shaping the antiviral immunity in offspring.

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The COVID-19 pandemic has affected hundreds of millions of people, including pregnant women. Although global efforts to curb the pandemic achieved somewhat encouraging results, the frequently emerging variant strains of the causing virus, SARS-CoV-2 (SCV2), hinder the resolution of this pandemic. Moreover, among the convalescent populations of COVID-19, some long-term adversary effects from the previous infection are observed. Pregnancy, in particular, is known to be associated with more severe diseases. However, whether infection of pregnant women with COVID-19 in different phases of gestation will affect host immune response, particularly the antiviral immunity, in their offspring is an open and important question, impacting clinical care for years to come.

Our study in a human cohort indicated that pregnant women with SCV2 infection exhibit increased angiotensin enzyme 2 (ACE2) in maternal serum but reduced ACE2 in infant blood compared to those who did not contract SCV2 during pregnancy. Moreover, proinflammatory cytokine IL-6 is elevated in both maternal and infant blood, hinting a possibility that transplacental delivered IL-6 is responsible for the decreased ACE2 in the infants. Further, inoculating mice with a mouse-adapted SCV2 as a model, we found that offspring from SCV2 infected dam at gestation day 14.5 (iDam) demonstrated an enhanced antiviral immune response, manifest by the reduced viral load in the lung, increased proinflammatory cytokine levels in serum, suggesting a maternal infection-induced immune imprint in the offspring. Strikingly, inhibition of ACE2 by maternal injection of an ACE2 inhibitor at e14.5 resembles the strengthened antiviral immune response in the offspring, as does the offspring from iDam, implying that the immune imprint is, at least in part, the prenatally reduced ACE2 mediated. Subsequently, we found that maternal SCV2 infection and amniotic reduction of ACE2 altered the chromatin accessibility of bone marrow cells in the offspring, together with reduced SDF-1 level and induced PU.1 gene expression. Therefore, the interim conclusion is that SCV2 infection during pregnancy induces proinflammatory cytokines, such as IL-6, which are transmitted into the fetal circulation and reduce ACE2 expression, leading to decreased SDF-1 levels in the BM of the fetus. Thus, the quiescent state of hematopoietic stem cell (HSC) is affected, the chromatin accessibility is increased, and PU.1 gene expression is induced, setting a prone to enhanced immune progenitor cell commitment and immune cell proliferation and release in response to viral insults. *Such an immune imprint predisposes the offspring to enhanced antiviral immunity at the cost of potential exacerbated inflammation.*

W1-6:

Cell-to-Cell Transmission of SARS-CoV-2, HIV-1 and EBOV

Cong Zeng, John P. Evans, Panke Qu, Yi-Min Zheng, and **Shan-Lu Liu**
The Ohio State University, Columbus, OH 43210, USA

Enveloped viruses spread in cultured cells and tissues via cell-free infection and cell-to-cell transmission, with the latter being much more efficient. Additionally, cell-to-cell transmission has the ability to evade antibody neutralization, accounting for efficient virus spread and pathogenesis. By using HIV lentiviral pseudotype and replication-competent VSV systems, we investigated roles of viral receptor, cell-cell fusion, and entry pathway in cell-to-cell transmission of humancoronaviruses with comparison with that of HIV and Ebolavirus (EBOV). We found that SARS- CoV-2 spike is more efficient in facilitating cell-to-cell transmission than SARS-CoV spike, whichreflects, in part, their differential cell-cell fusion activity. While cell-cell fusion enhances the cell-to-cell transmission of SARS-CoV-2, treatment of cocultured cells with endosomal entry inhibitors strongly impairs cell-to-cell transmission, suggesting endosomal membrane fusion as an underlying mechanism. Compared with cell-free infection, cell-to-cell transmission of SARS- CoV-2 is much more refractory to inhibition by neutralizing antibody or convalescent sera of COVID-19 patients. Interestingly, different from HIV and EBOV, the virus receptor ACE2 is notabsolutely required for the cell-to-cell transmission of SARS-CoV-2. Intriguingly, despite differences in cell-free infectivity, SARS-COV-2 authentic variants of concern Alpha and Beta have similar cell-to-cell transmission capability; this pattern is distinct from that of Delta and Omicron variants. Overall, our study reveals some unique features of cell-to-cell transmission of SARS-CoV-2 mediated by its spike, with important implications for a better understanding of SARS-CoV-2 spread and pathogenesis.

Workshop #2:

W2-1:

Fly Cell Atlas and Beyond

Hongjie Li

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The past decade has witnessed the emergence and rapid development of a host of single-cell sequencing technologies, including single-cell RNA sequencing (scRNA-seq). The *Drosophila* fruit fly is a premier model organism to study fundamental and evolutionarily conserved mechanisms ranging from development, aging and diseases. Combining scRNA-seq with powerful genetic tools holds a great potential for making new discoveries. For this talk, I will first talk about applications of scRNA-seq for studying brain development and then share the Fly Cell Atlas project.

W2-2:

Translating spatial cell atlas to tissue function

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Spatially charting molecular cell types at single-cell resolution across the entire three-dimensional (3D) volume of the brain is critical to illustrating the molecular basis of the tissue anatomy and functions. Recent development of spatial transcriptomic methods has enabled scalable profiling of transcriptome-defined spatial cell atlas. Yet, there is still a big gap between spatial cell atlas and tissue function. In this presentation, I will introduce a few experimental and computational advances in our lab that further enable multi-modality deep profiling of cell types and states *in situ*, bridging single-cell molecular profiles with single-cell functional status in intact biological tissues and accelerating gene-to-function discoveries in development and diseases.

W2-3:

Spatial-CUT&Tag: Spatially resolved chromatin modification profiling at the cellular level

Yanxiang Deng[1] and Rong Fan[1,2]

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Spatial omics emerged as a new frontier of biological and biomedical research. Despite recent breakthroughs in single-cell sequencing that have enabled the profiling of the epigenome in single cells, it remains challenging to integrate the spatial information of individual cells in the tissue of origin. Here, we present spatial-CUT&Tag for spatially resolved genome-wide profiling of histone modifications by combining in situ CUT&Tag chemistry, microfluidic deterministic barcoding, and next-generation sequencing. Spatially resolved chromatin states in mouse embryos revealed tissue-type-specific epigenetic regulations in concordance with ENCODE references and provide spatial information at tissue scale. Spatial-CUT&Tag revealed epigenetic control of the cortical layer development and spatial patterning of cell types determined by histone modification in mouse brain. Single-cell epigenomes can be derived in situ by identifying 20-micrometer pixels containing only one nucleus using immunofluorescence imaging. Spatial chromatin modification profiling in tissue may offer new opportunities to study epigenetic regulation, cell function, and fate decision in normal physiology and pathogenesis.

W2-4:

Highly scalable and flexible RNA diagnostics with One-Seq

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Highly scalable molecular diagnostics is essential for effective pandemic control and prevention. Next-generation sequencing (NGS) promises highly multiplexed testing as well as viral sequencing, however current methods require complex pre-processing steps (e.g. RNA extraction and individual RT-PCR reactions). Here we report a new method, One-Seq, that allows immediate sample pooling after collection, thus enabling highly scalable testing. Specifically, we introduced a one-step reaction system to eliminate RNA extraction without compromising sensitivity, and a molecular protector strategy to eliminate individual RT-PCR reactions but still allow accurate readout, despite a large dynamic range in the sample pool. In COVID clinical specimens, our method demonstrated multiplexed testing with quantitative readout and a wide dynamic range (6 logs), sensitive detection of positive samples (Ct<35) down to 360 gce/ml, and simultaneous reports targeted viral sequences for variant identification. Our method is highly sensitive (LoD = 5 gce), highly scalable (estimated 200,000-350,000 tests per day on a high-throughput sequencer), affordable (<$1 amortized cost) and fast (8-15 hr turn-around time). We envision that our method can be ultimately implemented for whole-population, multi-virus surveillance, allowing for effective pandemic control, as well as early warning of emerging infectious viruses, preventing the next global outbreak.

W2-5:

Droplet-based High-Throughput Transcriptome Profiling of Individual Synapses Using Single-Cell Total-RNA-Seq

Chenghang Zong

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Synapses are crucial structures that mediate signal transmission between neurons in complex neural circuits. There is considerable morphological and electrophysiological heterogeneity among individual synapses, but we still lack a high-throughput method to profile this heterogeneity among individual synaptosomes. Here we developed the first droplet-based single-cell total-RNA-seq method that profiles the transcriptomic heterogeneity of individual neurites, the primary components of which are synaptosomes. We refer to the transcriptome of single synaptosomes as synaptome. Here we profiled the synaptome for both human and mouse brain samples. We were able to characterize the landscape of local splicing occurred in synapses and associate specific subclusters of synapses with distinct subclusters of neuronal nuclei. We were also able to identify novel synaptopathy genes in AD mice that cannot be detected by single-nucleus transcriptome profiling. Overall, our results show that this platform, which we call Multiple-Annealing-and-Tailing-based Quantitative scRNA-seq in Droplets (MATQ-Drop), provides a high-throughput

synaptosome transcriptome profiling tool that will facilitate future discoveries in neuroscience.

W2-6:

Computational modeling of single cell metabolism in brown adipose tissue

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Obesity and metabolic syndrome are rapidly increasing worldwide, leading to high morbidity and mortality in Type2 diabetes (T2D) and cardiovascular diseases. Brown adipose tissue (BAT) is specialized to dissipate chemical energy in the form of heat in thermogenesis. Therapeutical activation of BAT is recognized as a promising method to combat obesity and metabolic diseases.Many recent studies have been focused on identifying the metabolic pathways related to the activation of BAT using bulk metabolomics. However, like other tissue types, BAT consists of many cell types, including adipocytes, immune cells, and stromal cells. Therefore, a comprehensive analysis of the cellular metabolic heterogeneity of BAT is urgently needed. Although a few single-cell metabolomics were reported recently, the techniques are much unmatured. Instead, single-cell RNA sequencing (scRNA-seq) techniques have been successfully utilized to study cellular heterogeneity in an unprecedented high resolution in many tissues, including BAT. Here, we aimed to develop novel computational methods to analyze the metabolic heterogeneity of different cell types in BAT from scRNA-seq data. We integrated metabolic resources of metabolic reactions and enzymes from the Human Metabolome Database (HMDB), Reconstruction of metabolic network (Recon), Kyoto Encyclopedia of Genes and Genomes (KEGG), as well as literature. We successfully built a computational model to impute metabolic heterogeneities from scRNA-seq data of mouse BAT. The model successfully identified cold- sensitive metabolic pathways by analyzing scRNA-seq data of mouse BAT from thermoneutral (30 ℃ for 7 days), room temperature (22 ℃), and cold exposure (4 ℃) for 2 and 7 days. The analysis revealed new mechanism of brown adipocyte activation. Our bioinformatic modeling and analyses demonstrate the power of studying single-cell metabolism using scRNA-seq datasets. The new computational methods and biological findings regarding the metabolic effect of BAT activation will be beneficial for the metabolism research community and metabolic disorder patients.

Workshop #3:

W3-1:

Modulating microglial energy metabolism in post-stroke brain repair.

Dandan Sun

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The balance between glycolysis and OXPHOS is important for the transition between the damaging immune responses and their restorative functions in many immune cells. Therefore, immune dysfunction/dysregulation is closely associated with loss of balance in immune energy metabolism homeostasis in many diseases, such as cancer, Alzheimer's disease, stroke, etc. This talk will discuss our research on modulating a key pH regulator Na/H exchanger (NHE1) function in stimulating microglial OXPHOS metabolism and phagocytosis functions in stroke brain repair. In addition, I will also discuss our recent study that blockade of NHE1 protein improved glioma tumor immunity and OXPHOS function of myeloid cells in experimental glioma models.

W3-2:

A TFEB-mediated Lysosomal-to-Nucleus Signaling Pathway in Immune System Activation in Tauopathy

Hui Zheng

Huffington Center on Aging, Baylor College of Medicine

Accumulating evidence implicates that hyperphosphorylated and misfolded Tau, the main components of neurofibrillary tangles in Alzheimer's disease and other tauopathy diseases, is subject to degradation by the autophagy-lysosomal pathway. TFEB was discovered as a master regulator of lysosomal biogenesis whose activity is mediated by phosphorylation and cytoplasmic to nucleus translocation. Our studies revealed that neuronal TFEB is highly efficacious in clearing Tau/NFT pathology whereas astroglial TFEB prevents Tau spreading. We found that pathological Tau acts as an upstream inducer of TFEB, which in turn mediates the transcription of multiple submits of the vacuolar ATPase (v-ATPase) critical for lysosomal acidification. To decipher the functional role of the TFEB-v-ATPase regulation in vivo, we created a knock-in mouse line in which the TFEB-v-ATPase signaling axis is specifically disrupted. Molecular, morphological and functional phenotyping revealed a critical role of the TFEB-v-ATPase signaling pathway in lysosomal homeostasis under physiological conditions and in proper activation of microglia and astrocytes in response to Tau pathology, thus uncovering a novel lysosome-immune relationship.

W3-3:

Microglial TREM2 expression modulates neuronal functions and amyloid development

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The triggering receptor expressed on myeloid cells 2 (TREM2) is a cell surface receptor that is expressed exclusively in the myeloid lineage cells, in particular microglia in the brain. *TREM2* variant p.R47H has been linked to the risk for Alzheimer's disease (AD) with an effect size comparable to that of the *APOE4* gene allele. However, how human TREM2 or TREM2-R47H modulates microglial functions under physiological and pathological conditions is unclear. Here we generated conditional mouse models expressing either the human wild-type TREM2 (TREM2-WT) or its R47H variant (TREM2-R47H). Specifically, human TREM2 expression was conditionally induced in microglia by a *Cx3cr1-CreER* driver upon which microglial and neuronal functions, microglia-neuron interactions, pathology and related- toxicity, as well as molecular profiles at the single cell level were comprehensively assessed. In non-amyloid background, we found a strong stimulation of microglia activation at both functional and molecular levels after TREM2-WT expression; however, TREM2-R47H expression did not induce microglia activation signature, consistent with a loss-of-function as previously reported. Furthermore, TREM2-WT expression enhanced associative memory and synaptic plasticity; whereas TREM2-R47H expression led to neuronal hyperactivity and impaired pre-synaptic transmission, demonstrating differential functions of TREM2-WT and TREM2-R47H in modulating neuronal functions. Upon breeding to the 5xFAD amyloid mouse model, we found that expression of TREM2-WT at early amyloid seeding stage, but not during amyloid rapid growth or saturation stage, significantly reduced the amyloid deposition by accelerating the Aβ clearance. However, the expression of TREM2-R47H during amyloid rapid growth period exacerbated amyloid burden. Further sing-cell RNA-seq analysis revealed the protective role of TREM2-WT in restricting the spread of disease-associated microglial signature; while TREM2-R47H exacerbated the transcriptional signature of antigen presentation pathway. Altogether, our study demonstrates distinct roles of TREM2 in modulating neuronal function and AD pathology, revealing underlying molecular mechanisms critical for guiding development of TREM2-targeted therapy to treat AD.

W3-4:

Roles of pattern recognition receptors in primary sensory neurons for sensing danger signals and control of pain and itch

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Sensory neurons are activated by physical and chemical stimuli, eliciting sensations such as temperature, touch, pain, and itch. From an evolutionary perspective, sensing danger is essential for organismal survival. Upon infection and injury, immune cells respond to pathogen/damage-associated molecular patterns (PAMPs/DAMPs) through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), and produce inflammatory mediators that activate sensory neurons through neuro-immune interactions. Our lab and other labs have demonstrated that sensory neurons in dorsal root ganglia and trigeminal ganglia express TLRs such as TLR3, TLR4, TLR5, and TLR7 and the

downstream signaling molecules such as MyD88. Interestingly, TLRs can directly sense danger signals (e.g., single and double-stranded RNAs, miR-let-7b, and miR-711) after injury or during infection, leading to pain, itch, or analgesia, in a context-dependent manner. In addition to slow-acting canonical TLR signaling via MyD88 and NF-kB, TLRs function uniquely in sensory neurons through non-canonical coupling to ion channels, such as TRP channels (TRPA1, TRPV1). This non-conical signaling enables rapid modulation of neuronal activity, leading to behavioral responses of pain or itch. The rapid neuronal activation induced by PAMPs and DAMPs can also orchestrate local immune reaction. In addition to TLRs, sensory neurons also express DNA sensor STING and Type I interferon receptor (IFNRA) that are critical regulators of physiological nociception. I will discuss how sensory neurons utilize TLRs and other PRR pathways to detect danger signals in their environment.

W3-5:
Neuroimmune interaction: how microglia sense neuronal hyper- and hypoactivity
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Microglia are innate immune cells of the central nervous system (CNS) and are sensitive to extracellular cues. Brain injuries, inflammation, and pathology evoke dynamic structural responses in microglia, altering their morphology and motility. In addition, microglia respond to and engage in neuronal activity—a facet of their biology that exists alongside their pathological responses. Using in vivo imaging approaches, we found that microglia increase their process dynamics and surveillance in response to either hyperactive or hypoactive network activity in seizure and anethesia repectively. Interestingly, microglia sense neuronal hyperactivity via P2Y12 receptors while they sense hypoactivity via adrenergic beta2 receptors. Using microglia calcium imaging and chemogenetic manipulation in awake mice, we further demonstrate that microglia calcium is attuned to neuronal activity. Specifically, microglia increase calcium signaling in response to bi-directional shifts in neuronal activity. Together, our studies elucidate how microglia sense neuronal activity and later influence neuronal function following their engagement. The dynamic function of microglia in monitoring and dictating neuronal activity is critical for brain hemostasis and repair in health and disease.

Workshop #4:

W4-1:

Cellular heterogeneity in therapy resistance and disease progression of prostate cancer

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Prostate cancer (PCa) cells are heterogeneous, consisting of bulk luminal cells expressing androgen receptor (AR) and prostate specific antigen (PSA) as well as a minor component of neuroendocrine (NE) cells. Our work over the years has shown that clinically used drugs targeting AR signaling effectively inhibit luminal cells but NE cells are spared because they do not express AR and are androgen-independent. Such cellular heterogeneity contributes to therapy resistance and disease progression to the castration resistant stage (CRPC). We have developed a novel approache targeting NE cells which, in combination with AR targeting, achieves superior therapeutic efficacy. We recently performed single cell RNA sequencing of tumor cells from primary PCa, CRPC and small cell neuroendocrine carcinoma (SCNC), an aggressive histologic variant PCa that often emerges after therapy failure. Our work demonstrates that ~0.5% of tumor cells in primary, untreated PCa bears genomic and molecular features of CRPC cells, revealing an additional layer of cellular heterogeneity and suggesting that the pre-existing CRPC-like cells may be selected by hormonal therapy leading to the eventual therapy failure and disease progression.

W4-2:

Plk1 in prostate cancer progression and therapy resistance.

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Polo-like kinase 1 (Plk1), a critical regulator of cell cycle progression, has been postulated to have a pro-tumorigenesis function. Integrating our recently generated prostate-specific Plk1 knock-in genetically engineered mouse model (GEM) and the transcriptome data of human prostate cancer (PCa) patients, we aimed to establish an oncogenic role for Plk1 in PCa progression. To elucidate the underlying mechanism, we investigated the relationship between PLK1 and the tumor microenvironment (TME). After the mRNA expression of Plk1 was identified in human PCa from TCGA database, staining scores for Plk1 in clinical tissue microarray (TMA) were evaluated by a pathologist. We also designed a prostate-specific GEM mouse study to characterize the oncogenic role of Plk1 overexpression in PCa. For mechanism study, a series of *in vitro* assays using human PCa cells and inducible macrophages, as well as murine prostatic-epithelial cells and murine bone marrow-derived macrophages, were designed to uncover regulation of Plk1 in macrophage polarization and the interaction between malignant cells and immune cells within TME. Finally, the efficacy of the STAT6 inhibitor on advanced PCa was determined. Mechanistically, we found that Plk1 overexpression induces phosphorylation of JAK3 and activation of the JAK/STAT6 pathway in cancer cells, which has a pro-tumorigenic impact on TME by enabling macrophage polarization towards the M2 phenotype and ultimately accelerates PCa development. Pharmaceutical inhibition of STAT6 signaling significantly reverses the Plk1-mediated M2 polarization and, subsequent, the progression of PCa. Our findings provide clinical and pre-clinical evidence to characterize the promoting role of Plk1 in PCa and novel insights into regulation of Plk1 in prostatic TME. In addtion, we also provide strong support for clinical potential of targeting STAT6 for advanced PCa therapy.

W4-3:

Epigenetic regulation of cancer metabolism leading to disease progression

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HOXB13, a homeodomain transcription factor, critically regulates androgen receptor (AR) function and promotes androgen-dependent prostate cancer (PCa) growth. However, the functions of HOXB13 in an AR-independent context remain elusive. Here we report an essential role of HOXB13 in directly suppressing lipogenic transcriptional programs in both AR-positive and -negative PCa cells. The MEIS domain (aa70-150) of HOXB13 interacts with the histone deacetylase HDAC3, which is disrupted by HOXB13 G84E mutation that has been associated with early-onset PCa. Thus, HOXB13 wildtype (WT), but not G84E mutant, recruits HDAC3 to lipogenic enhancers to catalyze histone de-acetylation and suppress lipogenic programs. HOXB13 knockdown unleashes the expression of key lipogenic regulators such as fatty acid synthase (FASN), requiring HDAC3. Analysis of human tissues revealed that HOXB13 is lost in about 30% of metastatic castration-resistant PCa, at least in part, through DNA hypermethylation. Functionally, loss of HOXB13 leads to massive lipid accumulation in PCa cells, thereby promoting cell motility *in vitro* and fueling xenograft tumor metastasis *in vivo*, which is mitigated by pharmaceutical inhibitors of FASN. In summary, our study discovers an essential AR-independent function of HOXB13 in repressing *de novo* lipogenesis and inhibiting tumor metastasis and defines a subclass of PCa that may benefit from lipogenic pathway inhibitors.

W4-4:

Identifying and targeting novel kinase regulators for cancer metastasis

Wenliang Li
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Metastasis is responsible for >90% of cancer death. However, metastasis is still poorly understood and the current approaches to prevent or treat human metastatic cancers are largely unsuccessful. Through RNAi and cDNA functional screening, genomics analysis, and functional validations, we have identified several critical but previously unknown/understudied kinase regulators for metastasis. As an example, shRNA screening revealed that GPCR-kinase 3 (GRK3) is essential preferentially for highly metastatic cancer cells as compared to lowly metastatic cancer cells. GRK3 is significantly overexpressed in metastatic prostate tumors from patients, especially in the aggressive variant of prostate cancers, so called treatment-related neuroendocrine prostate cancer (NEPC). We further found that GRK3 is a key missing link between two prominent phenotypes of NEPC, i.e. angiogenesis and neuroendocrine feature. Mechanistically, our preliminary study suggests that GRK3 enhances epigenetic repressor activity of histone deacetylase 2 (HDAC2). Through compound library screening, we have identified several compounds that block the kinase activity of GRK3 much more potently than that of GRK2, the closest-related kinase to GRK3. Of note, our GRK3

inhibitors could significantly inhibit NEPC cell growth in culture and in mouse xenografts. Another group of potential metastasis regulators we are studying are new EMT-regulating kinases from our kinome cDNA screening for epithelial-mesenchymal transition (EMT). For example, mixed lineage kinase ZAK positively regulates EMT phenotypes and migration of breast cancer cells in culture, as well as metastasis in mice. ZAK overexpression correlates with poor prognosis in breast cancer patients. We are carrying out in silicon docking analysis and chemical genomics to identify potent and specific inhibitors for ZAK as research tools and drug candidates for metastatic cancers.

W4-5:

MAPK4 as a novel oncogenic driver for prostate cancer growth and therapy resistance

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Prostate cancer (PCa) is the second leading cause of cancer death in American men. Androgen receptor (AR) signaling is essential for PCa cell growth/survival and remains a key therapeutic target for lethal castration-resistant PCa (CRPC). GATA2 is a pioneer transcription factor crucial for inducing AR expression/activation. MAPK4 is an atypical MAPK without known function in human cancers. Our pioneering works demonstrated that MAPK4 promotes tumor progression via noncanonical activation of AKT. MAPK4 also activates AR by enhancing GATA2 transcriptional expression and stabilizing GATA2 protein through repression of GATA2 ubiquitination/degradation. Concerted activation of both GATA2/AR and AKT by MAPK4 promoted PCa cell proliferation, anchorage-independent growth, xenograft growth, and castration resistance. Conversely, knockdown of MAPK4 decreased activation of both AR and AKT and inhibited PCa cell and xenograft growth, including castration-resistant growth. Both GATA2/AR and AKT activation were necessary for MAPK4 tumor-promoting activity. Interestingly, combined overexpression of GATA2 plus a constitutively activated AKT was sufficient to drive PCa growth and castration resistance, shedding light on an alternative, MAPK4-independent tumor-promoting pathway in human PCa. We concluded that MAPK4 promotes PCa growth and castration resistance by cooperating parallel pathways of activating GATA2/AR and AKT and that MAPK4 is a novel therapeutic target in PCa, especially CRPC.

W4-6:

A computational framework to characterize the cancer drug induced effect on aging usingtranscriptomic data

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The cancer patient prognosis has been significantly improved due to early diagnosis and more effective treatments. However, cancer treatments such as chemotherapies that help the cancer survivors fight their diseases may change or accelerate their aging trajectories in the long term. Cancer treatments induced aging- related conditions are emerging as significant health burdens for cancer survivors. Emerging evidence hasshown that transcriptome and other types of “omics” data can be used to study molecular changes and trajectory of the aging process. Our analyses demonstrated that the aging-associated gene expression in theGenotype-Tissue Expression (GTEx) dataset can recapitulate the well-established aging hallmarks. Integrating the drug-induced transcriptomic data in LINCS L1000 (L1000) dataset and normal aging transcriptomic data in GTEx dataset, we next characterized the drug-induced transcriptomic changes of 28FDA approved cancer drugs in brain, kidney, muscle, and adipose tissues. Further drug-aging interaction analysis identified 34 potential drug regulated aging events. Those events include aging accelerating effects of vandetanib (Caprelsa®) and dasatinib (Sprycel®) in brain and muscle, respectively. Our result also demonstrated aging protective effect of vorinostat (Zolinza®), everolimus (Afinitor®), and bosutinib (Bosulif®) in brain.

Workshop #5:

W5-1:

Th2 cytokines in beige adipogenesis and metabolic health

Chih-Hao Lee, PhD,
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Recent evidence has demonstrated a clear role for immune signaling in metabolic regulation. The concept of meta-inflammation originates from several original observations describing that in obesity, adipose tissue produces proinflammatory cytokines and that infiltrating macrophages are a major source of these inflammatory mediators. Subsequent studies further identified a spectrum of pro- and anti-inflammatory immune cells in major metabolic tissues that are, in principle, associated with (and may contribute to) the obese and lean state, respectively. It is becoming evident that resident immune cells sense the local environment and participate in crosstalk with the tissues in which they reside to recalibrate the "metabolic setpoint". Obesity-induced chronic inflammation represents a maladaptation of immune cells to sustained nutrient surplus. In contrast, physiological processes, such as endurance exercise and cold exposure, trigger adaptive responses that could lead to enhanced metabolic fitness. Therefore, understanding mechanisms through which immune signaling restores physiological states will help identify new therapeutic opportunities to treat metabolic diseases. The role of Th2 cytokines, notably IL-13, in beige adipogenesis and maintenance of metabolic homeostasis will be discussed.

W5-2:

Hormonal control of ILC2 development and activation in health and disease

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ILC2s are present in metabolically active organs such as adipose tissue and play a critical role in the maintenance of metabolic homeostasis at tissue and systemic levels. However, the mechanisms underlying the development and activation of ILC2s remain poorly defined. Here we show that IL-33, a potent ILC2 activator, stimulates phosphorylation of AMPK at Thr^{172} via TAK1 in primary ILC2s, which provides a feedback mechanism to inhibit IL-33-induced NFκB activation and IL-13 production. Treating ILC2s with adipokine adiponectin or adiponectin receptor agonist, AdipoRon, activated AMPK and decreased IL-33-stimulated activation of ILC2s. In contrast, adiponectin deficiency promotes ILC2 development and activation, leading to upregulated thermogenic gene expression in adipose tissue of cold exposed mice. ILC2 deficiency or blocking ILC2 function by neutralization of the IL-33 receptor with anti-ST2 diminished the suppressive effect of adiponectin on cold-induced adipose thermogenesis and energy expenditure. Furthermore, depleting adiponectin receptor 1 (AdipoR1) and AdipoR2 in ILC2 increased resident ILC2 population in adipose tissue and substantially drove favorable metabolic phenotype. Taken together, our study uncovers a novel mechanism underlying the hormonal control of ILC2 and reveals that adiponectin is a key regulator of ILC2 function via AMPK-mediated negative regulation of IL-33 signaling.

W5-3:

Capture macrophage actions in diseased with novel single-cell based programs

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Macrophages are widely distributed immune cells that play central roles in a variety of physiological and pathological processes, including obesity and cardiovascular disease. They are highly plastic cells that execute diverse functions according to signaling and environmental cues. While macrophages have traditionally been understood to polarize to either pro-inflammatory M1-like or anti-inflammatory M2-like states, evidence has shown that they exist in a spectrum of states between those two phenotypic extremes.
M1-like macrophages exacerbate inflammation and promote insulin resistance in obesity-related diseases, while M2-like macrophages reduce inflammation, promoting insulin sensitivity. However, polarization markers are expressed inconsistently in adipose tissue macrophages, and they additionally exhibit phenotypes differing from the M1/M2 paradigm. In atherosclerotic cardiovascular disease, activated plaque macrophages can also exist in a range of pro-inflammatory or anti-inflammatory states. Some of these macrophages scavenge lipids, developing into heterogeneous foam cell populations.
To better characterize the multifaceted actions of macrophages in human disease, we have designed a set of novel computational tools: MacSpectrum and AtheroSpectrum. These tools provide information on the inflammatory polarization status, differentiation, and foaming of macrophages in both human and mouse samples, allowing for better characterization of macrophage subpopulations based on their function. Using these tools, we identified disease-relevant cell states in obesity and cardiovascular disease, including the new concept that macrophage-derived foam cell formation can follow two distinct programs: homeostatic non-inflammatory or pathogenic inflammatory foaming programs. Applying these annotation systems also facilitated the development of risk prediction models for cardiovascular diseases and diabetes.

W5-4:

Adipogenesis and aging-associated adiposity

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The average fat mass in adults increases dramatically with age, and older people often suffer from visceral obesity and related adverse metabolic disorders. Unfortunately, how aging leads to fat accumulation is poorly understood. It is known that fat cell (adipocyte) turnover is very low in young mice, similar to that in young humans. Here, we find that mice mimic age-related fat expansion in humans. In vivo lineage tracing shows that massive adipogenesis (the generation of new adipocytes), especially in the visceral fat, is triggered during aging. Thus, in contrast to most types of adult stem cells that exhibit a reduced ability to proliferate and differentiate, the adipogenic potential of adipocyte progenitor cells (APCs) is unlocked by aging. In vivo transplantation and 3D imaging of transplants show that APCs in aged mice cell-autonomously gain high adipogenic capacity. Single-cell RNA sequencing analyses reveal that aging globally remodels APCs. Herein, we identify a novel committed preadipocyte population that is age-specific (CP-A), existing

both in mice and humans, with a global activation of proliferation and adipogenesis pathways. CP-A cells display high proliferation and adipogenesis activity, both in vivo and in vitro. Macrophages may regulate the remodeling of APCs and the generation of CP-A cells during aging. Together, these findings define a new fundamental mechanism involved in fat tissue aging and offer prospects for preventing and treating age-related metabolic disorders.

W5-5:
Myogenic (re)programing during brown adipose tissue development and aging
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Brown adipose tissue (BAT) is a thermogenic organ that promotes metabolic fitness. Brown adipocytes arise from myogenic progenitors and the repression of muscle gene program is necessary for the developmental establishment of BAT. In adult humans, metabolically active BAT mainly exists in the supraclavicular area; however, its prevalence and activity decrease in obesity and aging. Developmental origins of supraclavicular BAT and mechanisms governing the age-dependent BAT involution remain unknown. Using lineage tracing mouse models, we found that supraclavicular brown adipocytes are not derived from the *Pax3*$^{+}$/*Myf5*$^{+}$ precursor cells in the epaxial dermomyotome. PPARγ deficiency in *Myf5*+ cells did not cause atrophy of supraclavicular BAT. Instead, we identified that the supraclavicular brown adipocytes are progeny cells of a developmental lineage that also gives rise to neck myocytes. PPARγ deficiency in this lineage caused BAT paucity in the supraclavicular region and compromised thermogenesis upon cold challenge. Meanwhile, we discovered that age-associated decline in BAT thermogenesis is associated with progressive occurrence of brown adipocytes that activate myogenic genes. Depleting these myogenically active, functionally incomplete brown adipocytes improves thermogenic function of aged BAT. In summary, our results demonstrate that there are region-specific myogenic precursors that contribute to BAT at different anatomical locations during development. Repressing myogenic programing may revitalize BAT function for the treatment of aging-related metabolic disorders.

Workshop #6:

W6-1:

The cold-adapted, temperature-sensitive SARS-CoV-2 strain TS11 is attenuated in Syrian hamsters and a candidate attenuated vaccine

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of COVID-19 pandemic. Current vaccines are effective in preventing severe COVID-19 cases and deaths. However, they do not prevent infection/virus transmission and as the virus continues to evolve, S mutants have emerged and will continue to emerge that can escape current vaccine-induced immunity. Live attenuated vaccines (LAVs) replicate in the respiratory/oral mucosa, mimic natural infection, and can induce mucosal and systemic immune responses to the full repertoire of SARS-CoV-2 components and may induce better strain coverage and duration of protection than current vaccines. We generated a temperature-sensitive (TS) SARS-CoV-2 mutant TS11 via cold-adaptation of the SARS-CoV-2 WA1 strain and tested the pathogenicity of TS11 in Syrian hamsters. TS11 replicated at > 4 Log_{10}-higher titers at 32°C ($1.8x10^7$ plaque forming units (PFU) /mL) than at 39°C. Genomic sequence analyses showed that TS11 has multiple mutations, including those in nsp3, a 12-amino acid-deletion spanning the furin cleavage site of the S protein and a 371-nucleotide-deletion spanning the ORF7b-ORF8 genes. Eighteen hamsters were randomly assigned to two groups (n=9 per group) and were inoculated intranasally with a high dose (4×10^4 PFU) of WA1 or TS11. The TS11-inoculated hamsters gradually gained body weight from 1 to 13 days post-inoculation (dpi) and did not show clinical signs. However, the WA1-inoculated hamsters lost body weight starting from 2 dpi and reached peak weight loss at 6 dpi. The hamsters in both groups started shedding infectious virus in nasal washes from 1 dpi, peaked at 1/3 dpi, and stopped shedding from 6 dpi. No or extremely low levels of infectious virus were detected in both groups at 13 dpi. These results suggests that TS11 mutant was attenuated in hamsters. Challenge study is ongoing to test whether TS11 can be a LAV candidate for COVID-19.

W6-2:

Targeting a pyrimidine synthesis enzyme to combat SARS-CoV-2 infection.

Pinghui Feng

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) evolves rapidly under the pressure of host immunity as evidenced by waves of emerging variants despite of effective vaccinations, highlighting the need of complementing antivirals. We report that targeting a pyrimidine synthesis enzyme restores inflammatory response and depletes nucleotide pool to impede SARS-CoV-2 infection. SARS-CoV-2 deploys Nsp9 to activate carbamoyl-phosphate synthetase, aspartate transcarbamoylase and dihydroorotase (CAD) that catalyzes the rate-limiting steps of *de novo* pyrimidine synthesis. Activated CAD not only fuels *de novo* nucleotide synthesis, but also deamidates RelA. While RelA deamidation shuts down NF-κB activation and consequent inflammatory response, it upregulates key glycolytic enzymes to promote aerobic glycolysis that provides metabolites for *de novo* nucleotide synthesis. A *de novo* synthesized small molecule inhibitor of CAD restores antiviral inflammatory response and depletes the pyrimidine pool, thus efficiently impeding SARS-CoV-2 replication in mouse models. Targeting an essential cellular metabolic enzyme thus offers an antiviral strategy that would be more refractory to SARS-CoV-2 genetic changes.

W6-3:

Development of a novel hybrid alphavirus-SARS-CoV-2 pseudovirion for rapid quantification of neutralization antibodies and antiviral drugs

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SARS-CoV-2 spike protein (S) pseudotyped viruses are commonly used for quantifying antiviral drugs and neutralizing antibodies. Here we describe the development of a new hybrid alphavirus-SARS-CoV-2 pseudovirion (Ha-CoV-2), which is a non-replicating SARS-CoV-2 virus-like particle composed of viral structural proteins (S, M, N, and E) and a RNA genome derived from a fast expressing alphaviral vector. We validated Ha-CoV-2 for rapid quantification of neutralization antibodies, antiviral drugs, and viral variants (e.g. delta). In addition, as a proof-of-concept, we used Ha-CoV-2 to quantified the neutralizing antibodies from an infected and vaccinated individual, and found that the one dose vaccination with Moderna mRNA-1273 has greatly increased the anti-serum titer for approximately 6 fold. The post-vaccination serum can differently neutralize all 9 variants tested. These results demonstrated that Ha-CoV-2 can be used as a robust platform for rapid quantification of neutralizing antibodies against SARS-CoV-2 and its emerging variants.

W6-4:

Structural basis of SARS-CoV-2 immune evasion and antibody neutralization

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The acquisition and accumulation of mutations in the SARS-CoV-2 variants of concern (VOC) have enhanced the viral transmissibility and conferred resistance to neutralization by vaccinee and convalescent sera. To investigate the mechanisms of viral immune evasion and provide insights into the effective antibody neutralization, we isolated monoclonal antibodies targeting the SARS-CoV-2 spike from convalescent samples, evaluated their ability to bind and neutralize VOCs as well as pseudoviruses containing individual VOC mutations in the RBD region, and determined cryo-EM structures of SARS-CoV-2 spike in complex with antibody or antibody combinations. Our functional and structural studies showed how mutations in VOCs conferred immune evasion and identified antibodies that retained significant inhibitory activities against VOCs including the Omicron variants. Moreover, we identified antibodies cocktails with synergistic neutralization activity and superior activity in suppressing viral escape. Overall, our studies revealed structural mechanisms for immune evasion and provided structural details of antibodies binding modes and targeting sites that permitted broad neutralization against different SARS-CoV-2 VOCs, including the newly emerged BA.4 and BA.5, suggesting potential targets for the development of universal vaccines and therapeutic antibodies.

W6-5:
Cell culture models for investigation of SARS-CoV-2 infection and virus-host interaction

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SARS-CoV-2 can infect and replicate in many different cell types, including Vero E6 and Calu-3. However, SARS2 virus exhibits a remarkable difference in cytopathogenicity among different cell types. Although Vero E6 has been broadly used for SARS-CoV-2 infection and plaque formation, it cannot be used for testing interferon sensitivity and does not support robust plaque formation for some SARS-CoV-2 variants. To overcome these shortcomings, we have constructed a stable ACE2-expressing Hela cell line that supports robust SARS-CoV-2 infection and plaque formation. Using this cell culture model, we have demonstrated that SARS-CoV-2 can spread from cell to cell in an ACE2-dependent manner. It does not spread to cells that do not express the ACE2 receptor, which is different from recent findings reported by others. More significantly, we have found that virus-neutralizing antibodies induced by SARS-CoV-2 infection and mRNA vaccines could also block SARS-CoV-2 cell-to-cell transmission. We have also developed stable cell lines expressing viral replicase proteins that can support reporters-based replicon replication. These new cell culture models can be used for interrogating the roles of viral and host factors in SARS-CoV-2 replication besides their usefulness as platforms for high throughput screens to identify novel antiviral drugs as well as for genome profiling studies.

W6-6:
Robust CD8+ and CD4+ T cell response to Pfizer/Biontech BNT162b2 vaccine and SARS-CoV-2 infection in longitudual cohorts

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As of May 15, 2022, about 343 million Pfizer-BioNTech COVID-19 vaccine doses had been administered in the United States. The mRNA-based vaccine formulations have demonstrated high-efficacy mediated by efficient recruitment of both the innate and adaptive immune responses. Several studies have shown that mRNA vaccines can induce durable neutralizing antibodies that can be detected in circulation several months after the second dose. Unfortunately, quantification of the durability of SARS-CoV-2 specific T cell responses after vaccination is limited. In this study, we used the recent developed “spheromer” technology, a next generation pMHC multimerization platform that prove to capture higher number of antigen-specific T cells compared to other pMHC multimers (e.g. tetramer), to study both CD8+ and CD4+ T cell responseafter vaccination. To study the diverse T cell response to vaccination and infection, we involved multipul cohorts including (1) healthy vaccinees, (2) COVID-19 patients and (3) vaccinees who recovered from COVID-19, from whom blood was collected at sequential timepoints from the first dose to 4 months post second dose, which fill a long-standing need in human immunology. We designed a panel of 19 epitopes spanning the entire spike protein to characterize epitope-specific CD8+ and CD4+ T cell responses. We probed a total of >275 blood samples. Overall, BNT162b2 vaccination rapidly induced polyfunctional CD8+ and CD4+ T cell responses, which contribute to its remarkable efficacy. Surprisingly, the vaccine elicted CD8+ T cell response showed later peaking time than other vaccines, which could be a unique feature of mRNA platform.Vaccination led to the rapid induction of effector T cells concomitant with an increase of memoryT cells. In contrast, we observed significantly lower levels of T cells after infection, especially in the CD8+ T cell compartment with a skewing of the response hierarchy among the tested epitopes. Comparing with vaccination, low-levels of virus-specific memory CD8+ T cells detected in patient samples. Finally, given the high community-wide viral exposure burden, we also evaluated the CD8+ and CD4+ T cell response in donors who received vaccination after the recovery of COVID-19. Intriguingly, we observed a significant decrease in the frequency of spike-specific CD8+ T cells with attenuated functionality. Our data delineated novel dynamic of virus-specific T cell response elicited by vaccination and infection, and highlights the need to develop efficient vaccination strategies to specifically boost the anti-viral CD8+ T cell responses in the context of hybrid immunity.

Workshop #7:

W7-1:

Regulation of epithelial-mesenchymal plasticity by matrix rigidity in carcinoma metastasis

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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. We also identified the EphA2/LYN complex to be mechanically regulated by matrix stiffness to control TWIST1/G3BP2 interaction, EMT and tumor dissemination. 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. Activation of novel mechanoregulators in response to tumor rigidity suggests them to be attractive targets for anti-metastasis therapeutics.

W7-2:

Circulating tumor cells inform mechanisms of breast cancer metastasis

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Hematogenous metastasis is a complicated and inefficient multistep process by which tumor cells spread via blood circulation to form secondary tumors in distant organs throughout the body. 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: lung, bone, brain. 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. Serial *in vivo* inoculation showed increased organotropisms, further proving the intrinsic tendency of these cells to initiate metastasis in specific organs. Via genetic, epigenetic and transcriptional analyses, we revealed genes associated with organotropic features and identified drivers for brain metastasis. Through functional validation, we found that the semaphorin family cell surface receptor SEMA4D and oncogene MYC work together to promote brain metastasis, by promoting blood-brain barrier (BBB) transmigration and colonization, two orthogonal steps of brain metastasis. To further investigate the metastatic capacity of CTCs, our recent research discovered a "hypoxia-exposure memory" that provides a long-lasting effect from the solid tumors on CTCs to promote metastasis. Ongoing research is focusing on elucidating the underlying mechanisms of this novel finding in CTCs, with the goal to uncover vulnerabilities for novel therapies.

W7-3:

Circulating tumor stem cell clusters in breast cancer metastasis

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Circulating tumor cells (CTCs) are a vital component of liquid biopsy for longitudinal monitoring of residual disease and therapy response. CTCs with stem cell properties are considered the initiating seeds of distant metastases. Our studies and others found that compared to single CTCs, multicellular clusters of circulating tumor stem cells (CTSC) possess 25-50 times higher metastatic propensity and predict worse prognosis (*Cancer Discovery* 2019; *Nat Commu* 2021). We have established multiple complementary methods to characterize CTSC clusters, including mass spectrometry and flow cytometry profiling, immunofluorescence staining and histology analyses of CTCs in-situ, and intravital imaging of migrating tumor cells and CTSCs *in vivo*. We discovered that individual CTSCs aggregate into clusters near the vasculature and in the blood to promote survival, enhance stemness, and enable transendothelial dissemination. Furthermore, we identified stemness markers and cell adhesion molecules CD44 and ICAM1 that mediate CTSC cluster formation and tumor cell-endothelial cell interactions to empower stemness and metastasis. Machine learning-based structural analyses facilitated the identification of CD44 and ICAM1 molecular regions responsible for homotypic CTSC cluster formation. Proof-of-concept neutralization antibodies against these driver molecules inhibit CTSC cluster formation and reduce metastatic burden of triple negative breast cancer.

W7-4:

Mapping the role of neuronal niche in tumor brain metastasis progression

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Metastatic brain tumor is the most devastating form brain tumor with a median survival of less than six months. Metastatic colonization to the brain - from a single disseminated tumor cell to an overt tumor at the secondary organ brain - represents a major bottleneck of the whole metastatic cascade. Metastatic progression at the brain is a multi-step, evolutionary process that is accomplished through a consistent interplay between disseminated tumor cells and brain microenvironment - "the niche". The brain metastatic niche is a myriad of diverse cell types that have been speculated to contribute to the brain tumor and brain metastasis development, including endothelial cells,

astrocytes, neural stem/progenitor cells (NSC hereafter) and increasingly appreciated brain immune cells. How do the NSC and its progenies respond to brain metastatic colonization and regulate brain immune landscape and the metastatic outcome has yet to be systematically investigated. In this study, we revealed that NSC migrates to brain colonized tumor cells (seeds), forming early metatstic niche. The present of NSC in the niche is functionally essential for brain metastasis progression. Using NSC lineage tracing mouse models, coupled with single-cell RNA-seq (CITE-seq), we examined the lineage dyanmics of NSC evolutionary trajectory during the brain metastasis progression. The NSC homing to metastatic seeds and NSC derived secretory factors constitute an immune suppressive metastatic niche that potentially leads to a uncontrolled brain metatstic outgrowth. Our study delineated the neuron-immune cross-talk at the brain microenvironment. Mechanistic insights will provide guiding principles for treating devastating brain metastasis by modulating brain microenvironmental neuronal-immune homeostasis.

W7-5:
EZH2 Promotes Breast Cancer Metastasis via methyltransferase-independent functions
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Metastases occur at overwhelmingly high incidneces in patients with late-stage breast cancers, and is the major cause of death. Unfortunately, there are no curative therapies available for treating metastases. Enhancer of zeste homolog 2 (EZH2), a well-known histone methyltransferase that is an enzymatic subunit of the polycomb repressive complex 2 (PRC2) and that epigenetically represses gene expression through trimethylation of histone H3 at lysine (K) 27 (H3K27me3). We found that EZH2 expression is frequently increased in brain and bone metastases of breast cancers. In brain metastatic cells, EZH2) is phosphorylated at tyrosine-696 (pY696-EZH2) by nuclear-localized Src tyrosine kinase. Phosphorylation of EZH2 at Y696 changes its binding preference from histone H3 to RNA polymerase II, which consequently switches EZH2's function from a methyltransferase to a transcription factor that increases *c-JUN* expression. *c-JUN* upregulates G-CSF, which recruits immunosuppressive neutrophils into the brain to drive metastasis outgrowth. Immune checkpoint blockade therapies combined with Src inhibitors impeded brain metastasis in multiple mouse models.
In bone metastatic cells, EZH2 transcriptionally increases integrin β1 expression, which activates focal adhesion kinase (FAK). FAK phosphorylates TGFβ receptor type I at tyrosine 182 to enhance its binding to TGFβ receptor type II, thereby activating TGFβ signaling that promotes bone metastases. Clinically-applicable FAK inhibitors, but not EZH2 methyltransferase inhibitors, effectively inhibited breast cancer bone metastasis in vivo. These findings indicate that EZH2 can function methyltransferase-independently as a transcription factor to facilitate metastases, which could be clinically targeted for metastasis treatment.

W7-6:
Post-translational Regulation of Wnt/PCP Signaling
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Planar cell polarity (PCP) is an evolutionarily conserved fundamental mechanism that conveys directional information to orchestrate polarized cellular behaviors in a broad range of morphogenic and physiological processes. PCP signaling is governed by a set of highly conserved core proteins that are distributed asymmetrically at the cell surface. Disruption of PCP signaling results in a variety of developmental abnormalities such as neural tube defects and skeletal dysplasia, whereas its aberrant activation promotes cancer malignancy. Despite the critical role of PCP signaling in development and disease, how it is controlled remains poorly understood. By focusing on two core PCP proteins, Vangl1 and Vangl2, our previous studies showed that Wnt-induced Vangl phosphorylation is dose-dependently required for PCP, and Wnt5a plays both instructive and permissive roles in regulating PCP. Our recent studies further identified CUL3-KBTBD7 regulates Vangl stability through the ubiquitin-proteasome system. Vangl directly bound to p97/VCP via a highly conserved VCP-interacting motif. This interaction governed the access of KBTBD7 to Vangl, resulting in Vangl poly-ubiquitination and endoplasmic reticulum-associated degradation (ERAD). We found that Wnt5a prevents Vangl ubiquitination and ERAD by inducing Vangl phosphorylation, which highlights the importance of Wnt signaling and ERAD in the quality and activity control of PCP proteins. Together, our findings revealed a new regulatory mechanism of Wnt/PCP signaling through Vangl ubiquitination and phosphorylation. References: Yang et al. Cell Research. 2017 Dec;27(12):1466-1484. Gao et al. Development. 2018 Apr 13;145(8). Feng et al. Science Advances. 2021 May 14;7(20):eabg2099.

Workshop #8:

W8-1:

Calcium-dependent mechanisms underlying learning & memory

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Calcium ions serve as critical signalling elements essential for neuronal development, maturation, and survival. An optimal intracellular calcium level is required for the cellular and subcellular processes, and is tightly regulated to ensure proper functions of neurons. The cytoplamic calcium conducting channels permitting transient influx of extracellular calcium ions are one of the major mechanisms that regulate the intracellular calcium levels. The transient elevated free calcium can be amplified further by calcium released from the internal stores. Calcium-dependent signaling processes require calcium-binding proteins. In this talk, I will discuss the functional insight into regulatory mechanisms of calcium signaling during neurodevelopment and maturation, under physiological and pathophysiological conditions, including learning and memory.

W8-2:

Ion channels in drug development for neuroprotection

Hong-Shuo Sun

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Stroke is one of the leading causes of mortality in the world based on WHO statistics. Stroke research and the related neuroprotection studies have been focused on excitotoxicity and the traditional glutamate mechanism in cerebral ischemia for decades. Thus, the drug development of stroke treatment is an important direction in stroke research. Recently, the non-traditional non-glutamate mechanisms have attracted some attention in cerebral ischemia and stroke research. Non-glutamate mechanisms, which include K(ATP) channels, Transient receptor potential (TRP) channels, Acid-sensing ion channel (ASIC), hemichannels, chloride channels, ion exchangers and other nonselective cation channels, etc., also lead to intracellular ionic imbalance and neuronal cell death in cerebral ischemia and stroke. Ion channels are the third largest class of drug targets for therapeutic drug development. Ion channel pharmacology is an important research direction for drug discovery and potential new therapeutic development for human diseases, including brain disorders and stroke. In this talk, we will discuss some examples of the non-glutamate mechanisms in indentifying drug development targets and leads using both in-vitro and in-vivo experiments for potential future new drug discovery for cerebral ischemia and stroke.

W8-3:

Protein O-GlcNAcylation and regulation of mitochondrial function in the brain

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Mitochondrial dysfunction plays an important role in Alzheimer's and Parkinson's diseases. We examined how the stress-sensing post-translational O-GlcNAcylation pathway may play a role in regulating mitochondrial function in mouse models and human samples. We first tested the impact of in vivo administration of an inhibitor of the O-GlcNAcase enzyme (OGA) that removes the modification from proteins. We tested whether key factors in O-GlcNAcylation are correlated with mitochondrial electron transport activities and proteins in mice with and without OGA inhibition. We found that the networks associated with O-GlcNAcylation enzymes and activities with mitochondrial parameters, autophagy-related proteins as well as neurodegenerative disease-related proteins exhibited sex and OGA inhibition-dependent differences. We next explored these networks in Alzheimer's and Parkinson's diseases post-mortem brain samples. Taken together, these studies provide a framework of interconnectivity for multiple O-GlcNAc-dependent pathways in mouse brain of relevance to aging and sex/age-dependent neurodegenerative pathogenesis and response to potential therapies.

W8-4:

Biomarkers of Circadian Disruption of Central and Peripheral Circadian Clocks in Night Shift Nurses in Working Environment

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Persistent night shift that disrupts circadian rhythm is strongly associated with female breast cancer. To develop biomarkers of circadian disruption, we assessed central clock markers, melatonin and cortisol in plasma, and peripheral clock genes expression and clock regulatory factors (NAD^+/NADH and SIRT1 deacetylase activity) in peripheral blood mononuclear cells (PBMCs) over 24 hours in day (12) and night (10) shift nurses. In day shift nurses, cortisol appeared to reach the lowest level at midnight and highest level in the morning; melatonin reached the highest level right before dawn and decreased later in the morning to the lowest in the afternoon, both showing significant rhythmicity with opposite phases at the same morning time. Major clock genes (*PER2* and *PER3*) mRNA expression also showed 24-hour rhythmswith peak values in the morning. In contrast, night shift nurses showed disrupted circadian rhythms with significant lower cortisol and clock genes expression levels in the morning compared to the day shift nurses.Melatonin levels and rhythms were similar, suggesting a misalignment of melatonin and cortisol. Molecular clock regulators, NAD^+/NADH and SIRT1 activity, neither showed clear 24-hour circadian rhythm in day shift nurses, nor any significant changes in the night vs day shift nurses. The changes of these markers wererepeated with similar findings at early and late night timepoints in additional night (20) and day (19) shift nurses. To our knowledge, this is the first study to assess the circadian disruption in cortisol and clock genesexpression and their misalignment with melatonin in night shift workers in the working environment. Morning cortisol and clock gene mRNA expression levels in peripheral blood should be further investigated as biomarkers of circadian disruption in shift workers.

W8-5:
Identification of GBM Subtype Specific small molecule inhibitors as potential therapeutics

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Glioblastoma (GBM) is a grade four glioma and is the most common and lethal cancer of the central nervous system (CNS) with extreme cellular and molecular heterogeneity. Despite comprehensive genomic profiling and molecular characterization and numerous clinical trials, no targeted agents have been FDA approved in the past decade. Previously we have demonstrated the generation of mouse models of two functionally distinct GBM subtypes initiated from distinct cells of origin in genetically engineered mice, and the development of a cell lineage based GBM stratification which has allowed manifestation of starkly differential susceptibility of Type 1 and Type 2 GBM to anti-cancer therapies. As we have shown, the Type 2/II GBM primary cells are sensitive to the ErbB2 inhibitor, Tucatinib, as well as the multi-tyrosine kinase inhibitor Dasatinib. Thus, subtype specific small molecule inhibitors have potential for improved patient outcomes in GBM therapy. In the current study, we have developed a robust, cell-based high-throughput screening (HTS) platform on both Type 1 and Type 2 cells in a parallel and comparative setting, which allows us to reduce the number of false positives in hit selection and identify subtype specific inhibitors for Type 1 or Type 2 GBM. We adopted experimental features that afford improved physiological relevance, such as the use of primary early-passage tumor cells and serum-free conditions, and a new modified culture medium supplemented with EGF and Nrg1, a more balanced condition than that with EGF and FGF, which has long been used in the field and is biased toward type 1 cells. We envisioned that findings from these studies will not only provide the basis for further development of GBM subtype specific small molecule inhibitors, but may also help understand subtype specific signaling mechanisms and development of new strategies against GBM, and will have a long-term impact on 46% of human GBM cases categorized under Type I and II for their targeted therapy and precision medicine.

Workshop #9:

W9-1:

Pinning down genes and variants causal to the inflammatory bowel diseases

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Inflammatory bowel diseases (IBD) are a group of chronic, debilitating disorders of the gastrointestinal tract. As of 2017, there were 6.8 million people diagnosed with IBD globally, with increasing incidence and prevalence worldwide, especially in recently industrialized countries likely due to the modernization and westernization of the populations. Most large-scale IBD genetic studies studied common variants in people of European ancestries, potentially missing important biological insights from rare variants and from other ancestral populations. To address this gap in scientific knowledge while advancing global health equity, it is imperative to include variants of all frequencies and all diverse populations across the world in IBD genetics research. Here, as the initial step towards this goal, we present latest IBD genetics findings from a large-scale sequencing study of European ancestry and an East Asian IBD genetics study. Genome-wide association studies (GWAS) have identified hundreds of loci associated with Crohns disease (CD), a subtype of IBD. However, as with all complex diseases, robust identification of the genes dysregulated by non-coding variants typically driving GWAS discoveries has been challenging. Here we analyzed sequence data from more than 30,000 CD patients and 80,000 population controls. We directly implicate ten genes in general onset CD for the first time via association to coding variation, four of which lie within established CD GWAS loci. In addition to reiterating the central role of innate and adaptive immune cells as well as autophagy in CD pathogenesis, these newly associated genes highlight the emerging role of mesenchymal cells in the development and maintenance of intestinal inflammation. European populations only host a proportion of human genetic variants. Studying IBD genetics in all populations is the only way to have a full picture of IBD genetic risk. Here we report the largest IBD study of East Asian ancestry (EAS) including 14,393 cases and 15,456 controls. We found 80 IBD-associated genetic loci in EAS alone and 320 when meta-analyzed with ~370,000 European subjects (~30,000 cases), among which 81 are novel, including *ADAP1* (P14R) and *GIT2* (N387S) that were implicated by EAS enriched coding variants. In known IBD-associated loci, EAS enriched coding variants directly implicated many genes for the first time including *CELA3B (*R79W*) and SHC1* (A205V). We found common variants confer similar genetic effect in most IBD loci across ancestries with a few notable exceptions such as *TNFSF15* and *CSF2RB.* We showed that multi-ancestry samples enable a more accurate genetic risk model, highlighting the importance of including global populations for equitable deployment of genetic risk prediction. Our study represents a much-needed breakthrough in IBD genetics by extending IBD genetics to rare variants and East Asian populations for the first time. Novel findings and resources from this study will drive critical discoveries to uncover fundamental aspects of IBD pathogenesis that are relevant to the East Asia populations and across the world.

W9-2:

Dissect and characterize Alzheimer's disease risk variants

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Genome-wide association studies (GWAS) identified thousands of non-coding variants associated with late-onset Alzheimer's disease (LOAD). Strikingly, candidate cis-regulatory elements (cCREs) harbor a disproportionally large number of variants associated with Alzheimer's disease (AD), leading to the theory that genetic variations in cCREs contribute substantially to AD. In fact, cCREs in microglia demonstrate the most significant enrichment for AD heritability compared to other brain cell types. However, whether and how these GWAS variants contribute to AD remain elusive. Here, we combine fine-mapping and experimental characterization to annotate variants to function. First, by integrating genetic information and 3D epigenome annotation in human pluripotent stem cell (hPSC)-derived microglia. Second, we further establish functional AD risk variants and their target genes by CRISPRi screening and targeted single-cell RNA sequencing in microglia. In addition, we provide base-pair evaluation of functional impact by allelic analysis and prime editing. Finally, we demonstrate multiple physiological effects exerted by AD risk SNPs in microglia. Our work represents the first systematic experimental characterization of AD fine-mapped variants with substantial evidence of their functional roles in transcriptional regulation and downstream physiological conditions in microglia. Our strategy provides a roadmap for advancing genetic association to experimentally validated cell type-specific phenotypes and mechanisms.

W9-3:

Linking genetic and epigenetic risk in prostate cancer

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Prostate cancer is one of the most heritable diseases. Genome wide association studies (GWAS) have identified 270 prostate cancer risk loci that are enriched for cis-regulatory elements (CREs). It is important to identify the underlying functional mechanisms and their target genes. A key approach in doing so is to assess the association between variants in CREs and gene expression, also known as, cis-eQTL analysis. Our CRISPRi screen of 270 prostate cancer risk SNP-containing CREs (rCREs) identified the most essential one as an enhancer of MYC, and the regulation is directed by a DNA methylation mediated CTCF binding in between the risk enhancer and MYC promoter. In addition, association between the risk SNP and MYC expression (cis-eQTL) is only evident in population with high methylation at the CTCF binding site. This resolves a long-standing conundrum that although MYC has always been speculated as a causal gene in the 8q24.21 locus, no association between risk SNPs genotypes and MYC expression (eQTL) has been observed. To systematically evaluate this kind of DNA methylation modulating SNP and gene interaction, we developed a new eQTL mapping method, termed as DNA methylation modulated eQTL (abbreviated as memo-eQTL). This led to the identification of 2,270 DNA memo-eQTLs and novel causal mechanisms of many risk SNPs in prostate cancer and beyond. This study not only enhances our understanding of prostate cancer genetic risk, ethnic disparities and enhancer biology, but also identifies novel causal genes for biomarker and therapeutic target discovery.

W9-4:

Identification and characterization of the GWAS genetic variants and susceptible causal genes implicated in pulmonary diseases

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Genome-wide association studies (GWAS) have identified ~82 loci associated with chronic obstructive lung diseases (COPD), the third leading cause of death worldwide. However, majority of

these loci lack annotation to the disease causal genes and pathways regulated by genetic determinants driving disease progression. Integrative approaches have been applied to identify functional variants in various COPD GWAS loci including massively parallel reporter assays and CRISPRi-mediated screening. Furthermore, genetic deficient mouse models targeting the top COPD GWAS genes such as HHIP (Hedgehog Interacting Protein) and FAM13A (family with sequence similarity 13 member A) have uncovered novel insights into the COPD pathogenesis including the Wnt pathway and cellular metabolism. We found that genetic susceptible genes including FAM13A and DSP modulate cellular metabolic reprograming during lung epithelial cell repair/regeneration, thus fine-tune lung epithelial repair capacity, which further contribute to COPD development. Our study has focused on molecular characterization of the most significant COPD GWAS loci, as an exemplar to demonstrate both the challenges and the opportunities of translating GWAS discoveries to novel disease mechanisms, which provides generalizable approaches for other complex diseases as well.

W9-5:

Role of Wnt signaling in regulating lipid homeostasis

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Wnt signaling plays crucial roles in regulating normal development in metazoans, and components of the Wnt signaling pathway are often mutated in a variety of human cancers. The role of Wnt signaling in regulating adipogenesis has been extensively studied, but its role in regulating cellular lipid homeostasis is poorly understood. Unlike mammalian systems, adipogenesis, lipogenesis, lipolysis and fatty acid β-oxidation are temporally separated during the life cycle of *Drosophila*, thus we used *Drosophila* as an experimental model to elucidate the mechanisms of how Wnt signaling regulates lipid homeostasis in larval adipocytes. Specifically, we have observed that hyperactive Wnt signaling in *Drosophila* larvae reduces lipid accumulation in larval adipocytes, which can be strongly rescued by feeding the larvae with several proteosome inhibitors, such as Bortezomib (BTZ). Hyperactive Wnt signaling in adipocytes increases the levels of most free fatty acids but reduces the levels of various triglycerides. In addition, our transcriptomic and proteomic analyses using larvae with hyperactive Wnt signaling with or without Bortezomib treatment led us to identify genes involved in regulation of lipid mobilization, such as *PLIN1* (*Perilipin1*), *PLIN2* (*Perilipin2*), and genes regulating fatty acid degradation. Both *PLIN1* and *PLIN2* are significantly reduced by elevated Wnt signaling, but these effects are strongly reversed by BTZ treatment. Moreover, our genetic analyses suggest that adipocyte defects caused by hyperactive Wnt signaling can be enhanced by depleting *PLIN1*, while ectopic expression of *PLIN1* and *PLIN2* can potently rescue these adipocyte defects. Currently, we are testing whether Wnt signaling directly regulates the transcription of these genes encoding factors that control lipid mobilization in adipocytes. Taken together, our results suggest that Wnt signaling plays important roles in regulating cellular lipid homeostasis by promoting lipolysis and fatty acid oxidation.

W9-6:

Histone inheritance patterns in mammalian adult stem cells

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A long-standing question in stem cell field is how distinct cell fates are defined with one stem cell asymmetric division. Histones, a major carrier of epigenetic information, play important roles in regulatingdifferential gene expression and cell fate decisions. Our previous studies have shown asymmetric histone inheritance during asymmetric divisions of *Drosophila* male germline stem cells and intestinal stem cells. However, it is unclear whether asymmetric histone inheritance is applicable to mammalian stem cells. To address this question, we are using spermatogonial stem cells (SSCs) in mouse testes as a model system tostudy histone inheritance patterns *in vivo*. First, we explored the division modes of SSC populations with published stem cell markers. Intriguingly, we found that ~9% of spermatogonial pairs (GFRa1+/Tex14+) exhibited asymmetric distribution of both Eomes and Id4, key transcription factors in stem cells. A similarpercentage (~10%) of spermatogonial pairs (GFRa1+/Tex14+) that derived from SSCs divisions displayed asymmetric histone H4 and H3 distribution. To confirm that the asymmetric histone inheritance patterns correlate with distinct cell fates, we incorporated the Id4-EGFP; H4-mscarlet mice and showed that lower histone H4 cells are the Id4-EGFP^{high} cells, which represents the stem daughter cell. Furthermore, using a heat-shock induced regeneration regime, we found that asymmetrically dividing mSSCs with asymmetric histone H4 patterns significantly increased to 18.36%. These results indicate the asymmetric histone inheritance likely contributes to establishing distinct cell fates during stem cell asymmetric divisions, whichcould be important for both tissue homeostasis and regeneration. In the future, we will further explore old versus new histone inheritance patterns and the biological significance of asymmetric histone inheritance in the mouse SSCs system. Together, these results will greatly enhance our understanding of how stem cells redistribute epigenetic information during asymmetric divisions and whether this is a conserved phenomenon across different tissue contexts and species.

Workshop #10:

W10-1:

GILT-mediated lysosomal function maintains cardiac immuno-metabolic homeostasis in pressure-overload heart failure

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Heart failure (HF) remains the leading cause of death globally. The progression of HF is characterized by cardiac mitochondrial dysfunction and aberrant inflammatory responses. Although the lysosome has been recognized as the central player for maintaining immuno-metabolic homeostasis in diverse organs in health and disease, the mechanistic insights into the regulation of lysosome-dependent immuno-metabolism in the heart are lacking. Lysosomal reductase Gamma Interferon-Inducible Thiol Reductase (GILT) is the only identified lysosomal reductase that controls more than 11 lysosomal enzymes, and a single nucleotide polymorphism in the coding sequence of GILT has been implicated in promoting cardiovascular risk. Here, we show that GILT expression and activity are reduced in hearts from human patients and mice with HF, respectively. Moreover, cardiomyocyte-specific deletion of GILT (GILT-cKO) results in left ventricular remodeling and dysfunction in the setting of pressure overload. At the cellular level, cardiac GILT deficiency leads to impaired mitochondrial respiration, elevated mitochondrial oxidative stress, and increased NLR Family Pyrin Domain Containing 3(NLRP3)-dependent inflammation in the heart. Mechanistically, inhibition of NLRP3 in primary cardiomyocytes ameliorated the mitochondrial dysfunction in the GILT deficient cells, implicating a causative role of the lysosome-inflammasome axis on regulating cardiac mitochondrial function. Together, these findings elucidate a functional link between cardiac lysosomes, inflammatory responses and mitochondrial respiration. Knowledge gained from this study might speed the development of therapeutic agents to treat patients with HF.

W10-2:

Autophagy regulates hepatic acetylome

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The hepatic acetylome consists of the complete set of proteins acetylated, and its level reflects cell's metabolic status. However, little is known about how the hepatic acetylome is maintained. Here, we show that macroautophagy hereafter referred to as the autophagy, an intracellular lysosomal degradative pathway, regulates the level of hepatic acetylome. The total hepatic acetylome (nuclear, cytosolic, mitochondrial, membrane) in autophagy-deficient or autophagy-defective liver showed remarkably low levels when compared to normal liver. The lower hepatic acetylome was not caused by cellular injury commonly observed in autophagy-deficient conditions. In contrast, autophagy activation by fasting or rapamycin treatment elevated hepatic acetylome levels. Furthermore, mechanistic studies demonstrated that liver autophagy functions maintain the level of acetyl-CoA, a central intermediary metabolite required for protein acetylation. Autophagy impairment significantly decreased hepatic acetyl-CoA by transcriptionally downregulating key acetyl-CoA synthesizing enzymes- *Acly*, *AceCS1*, *AceCS2*, *Mcd* and *Pdha1*. Notably, the restoration of hepatic acetyl-CoA rescued the lowered hepatic acetylome and partially protected against liver injury and inflammation in autophagy-deficient livers. In conclusion, autophagy regulates hepatic acetylome as a critical mechanism to protect against liver injury and associated liver pathologies.

Key words*: Hepatic Acetylome, autophagy, acetyl coenzyme-A, liver injury*

W10-3:

Bilateral roles of glutamine anabolism in cancer development and therapy

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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. We have recently shown that genetic ablation of GS in the pancreas reduces pancreatic ductal carcinoma (PDAC) development 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 waste, next to the urea cycle, in the liver and kidney. As dysregulated urea cycle is implicated in cancer development, the impact of GS' ammonia clearance function has not been explored in cancer. We used a mouse model where GS is specifically disrupted in the liver and found that.genetic ablation of hepatic GS accelerates the onset of liver tumors in several mouse models that involve β-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. Therefore, while GS plays a pro-tumorigenic role in some cancers such as PDAC, GS-mediated ammonia clearance serves as a tumor-suppressing mechanism in livers that harbor β-catenin activation mutations.

W10-4:

The Landscape of Autophagy Degradation in the Brain

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Autophagy is a lysosomal degradation pathway and plays a critical role in regulating cellular and tissue homeostasis. Emerging evidence has linked impairment of autophagy to neurodegenerative diseases and neurodevelopmental disorders. However, the precise process of autophagy in human

brains remains poorly defined. The mechanism for how autophagy is disrupted in human neurological disorders remains unclear. We recently employed mouse genetics, human iPSC neurons, quantitative proteomics, and molecular and cellular biology, to systematically investigate autophagy targets in neurons and glia. Our analysis reveals common and distinct autophagy cargoes in neurons *verse* glia. Focusing on neuronal autophagy cargoes, we have identified proteins that are enriched in multiple cellular pathways as primary autophagy targets, including ER, synaptic vesicle, and PKA kinase complexes. We have also identified novel autophagy receptors that plays a role in mediating selective autophagy in neurons. Our work thus reveals the landscape of autophagy degradation and regulation in mouse brains and human neurons. It underscores the complexity of the autophagy functions in brains and sheds light on the mechanisms for neuroprotective function of autophagy.

W10-5:

BORC-ARL8-HOPS Ensemble is Required for Lysosomal Cholesterol Egress through NPC2

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Lysosomes stand at the end of endocytosis and autophagy pathways, and thus serve as a distribution point for cholesterol ingested from the extracellular space as well as recycled from intracellular organelles, respectively. Lysosomal cholesterol is delivered to other organelles for membrane biogenesis and synthesis of steroid hormones, bile acids and vitamin D. In addition, lysosomal cholesterol regulates the SREBP2 pathway that controls cholesterol synthesis and uptake. Many proteins, including the cholesterol transport proteins NPC1 and NPC2 regulate cholesterol export from lysosomes, thus contributing to cellular cholesterol homeostasis. In our study, we identify BORC, ARL8 and HOPS as additional factors that are required for cholesterol egress from lysosomes and for storage of esterified cholesterol in lipid droplets. Depletion of BORC, ARL8 or HOPS does not alter the localization of the lysosomal transmembrane cholesterol transporter NPC1 to degradative compartments, but decreases the association of the luminal transporter NPC2 and increases NPC2 secretion. BORC-ARL8-HOPS depletion also increases lysosomal degradation of CI-MPR, which normally sorts NPC2 to the endosomal-lysosomal system and then is recycled to the trans-Golgi network (TGN). These defects likely result from impaired HOPS-dependent fusion of endosomal-lysosomal organelles and an uncharacterized function of HOPS in CI-MPR recycling. Our study demonstrates that the BORC-ARL8-HOPS ensemble is required for cholesterol egress from lysosomes by enabling CI-MPR-dependent trafficking of NPC2 to the endosomal-lysosomal system.

W10-6:

Sugar-Sweetened Beverage Intake and Liver Cancer Risk

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Background and Aims: Intake of sugar-sweetened beverage (SSB), a postulated risk factor for obesity, diabetes, and cardiovascular disease, may drive insulin resistance and inflammation which are strongly implicated in liver carcinogenesis. However, evidence on the association between SSB intake and liver cancer is scarce. We hypothesized that higher SSB intake would be associated with a greater risk of liver cancer. **Method:** We included 97,601 women aged 50-79 years from the Women's Health Initiative Observational Study and Dietary Modification Trial comparison arm. SSB intake was defined as the sum of soft drinks and fruit drinks (1 serving=one 12 fl. oz can or 355 ml), which was assessed by a validated food

frequency questionnaire administered at baseline between 1993 and 1998. Incident liver cancers were reported by self-administered questionnaires and further confirmed by medical record review. Cox proportional hazards regression models were used to estimate multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) with adjustment for age, race and ethnicity, education, alcohol intake, smoking status, body mass index, non-steroidal anti-inflammatory drug use, physical activity, total caloric intake, and history of diabetes. **Results:** After a median of 19.5 years follow-up, 206 women had confirmed liver cancer. Approximately 6.8% of women consumed ≥1 serving/day of SSB at baseline. Higher SSB intake was associated with a 99% greater risk of liver cancer ($HR_{\geq 1/day \text{ vs never to } <3/month}$=1.99, 95%CI=1.25-3.18, P linear trend=0.004) as compared to intake of <3 servings/month. Non-statistically significant positive associations with liver cancer were observed for fruit drinks ($HR_{\geq 1/day \text{ vs never to } <3/month}$=1.83, 95%CI=0.80-4.17) and soft drinks ($HR_{\geq 1/day \text{ vs never to } <3/month}$=1.79, 95%CI=1.05-3.07). Results were similar after further adjustment for coffee/tea intake, or history of liver diseases, or when liver cancer cases diagnosed within the first 2 years of follow-up or those with history of diabetes were excluded. Substitution analyses indicated replacing SSB with water and/or coffee or tea could significantly lower liver cancer risk. **Conclusion:** Our findings suggest SSB as a potential modifiable risk factor for liver cancer in postmenopausal women. Studies in men and diverse populations are needed to examine these associations more comprehensively. If our findings confirmed, reducing SSB consumption might serve as a public health strategy to reduce liver cancer burden.

Workshop #11:

W11-1:

HBV cccDNA minichromosome: formation and epigenetics

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Chronic hepatitis B virus (HBV) infection remains a significant public health burden worldwide. HBV covalently closed circular DNA (cccDNA) is essential to the virus life cycle by serving as the persistent form of viral genome and transcription template, its complete elimination or inactivation during chronic infection is considered critical to a cure but has not been achieved by current antivirals. cccDNA is formed through a DNA repair process of the viral genomic relaxed circular DNA (rcDNA), and once formed, cccDNA exists in a stable episomal minichromosome decorated with host histones and nonhistone proteins. Accumulating evidence suggests that epigenetic modifications of cccDNA contribute to viral replication and the outcome of chronic HBV infection. Furthermore, HBV X protein (HBx) is known as a cccDNA transcription activator and essential for maintenance of cccDNA at transcriptionally active epigenetic state. Here, I will discuss the current progress on HBV cccDNA formation and epigenetics research, focusing on our recent studies that utilized proteomic approach to identify host factors involved in cccDNA formation and epigenetics (*Marchetti et al, J. Virol. 2022; Kim et al, PLoS Pathog. 2022*). Elucidating the molecular mechanisms of cccDNA biosynthesis and epigenetic regulation could allow us to elaborate new strategies for addressing the unmet clinical need.

W11-2:

Empty HBV Virions: Formation and Application

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Hepatitis B virus (HBV) is a para-retrovirus or retroid virus that contains a double-stranded DNA genome and replicates this DNA via reverse transcription of a RNA pregenome. Viral reverse transcription takes place within a capsid upon packaging of the RNA and the viral reverse transcriptase. A major characteristic of HBV replication is the preferential selection of capsids containing the double-stranded DNA, but not those containing the RNA or the single-stranded DNA replication intermediate, for envelopment during virion secretion. The complete HBV virion particles thus contain an outer envelope, studded with the viral envelope proteins, that encloses the capsid, which, in turn, encapsidates the double-stranded DNA genome. Furthermore, HBV morphogenesis is characterized by the release of subviral particles that are several orders of magnitude more abundant than the complete virions. One class of subviral particles is the classical HBV surface antigen particles (HBsAg, or Australian antigen) that contain only the viral envelope proteins, whereas the more recently discovered genome-free (empty) virions contain both the envelope and capsid but no genome. In addition, low levels of RNA-containing virions are secreted, as well. I will briefly discuss the complete and incomplete HBV particles. I will then focus on the discovery of empty HBV virions and explore their utility as a diagnostic biomarker and potential vaccine candidate. I will also discuss briefly the potential functions of the empty virions.

W11-3:

Modeling HBV Infection and Immunotherapy in Mice

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The mechanisms of chronic HBV infection and immunopathogenesis are poorly understood, due to a lack of relevant robust animal models. We have developed the AAV-based liver delivery platform in wild type mice to study HBV persistence and immunotolerance, as well as immunotherapy. We have also developed humanized mouse models with human immune system and/or human liver cells by reconstituting highly immunodeficient mice with human hematopoietic stem cells and liver progenitor cells (NBT-hu HSC+/-Hep mice). We detect human liver-specific chronic liver inflammation and fibrosis in HBV infected humanized mice. Chronic HBV-induced liver inflammation showed high level of human macrophages with M2-like phenotypes, associated with tissue fibrosis in infected animals. Importantly, similar M2-like macrophage accumulation was confirmed in chronic hepatitis virus infected patients with liver diseases. Furthermore, we demonstrate that HBV preferentially induced M2-related genes in macrophages. Finally, HBV infection and its associated immunopathogenesis were prevented by modulating host immune M2 responses. Our study demonstrates that different models of HBV persistence in mice with immune and liver cells provide valuable *in vivo* platforms for studying HBV infection, immune responses/immunopathogenesis and associated liver diseases, as well as for developing novel therapeutics targeting viral and host factors. We have discovered that HIV-1 infection in NBT-hu HSC/HepSC mice also induced M2-like macrophages; and HIV-1 infection/cART enhances liver diseases in humanized mice via the pDC/IFN-I axis with induction of M2-like macrophages in the liver. We have also developed the Hu-HSC/HEP mouse model with adult human hepatocytes and allogeneic CD34+ HSC and showed they support efficient infection by both HIV and HBV. Our findings will advance the field of HBV+/- HIV-1 research on HBV infection and immune pathogenesis, and shed light on developing novel treatments for viral liver diseases in CHB patients +/- HIV-1 co-infection.

W11-4:

The hepatitis E virus infectious cycle – an update

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The hepatitis E virus (HEV) is an enterically transmitted RNA virus and a major cause of acute hepatitis worldwide. In recent years, cases of persistent HEV infection in immunocompromised inviduals have been rising which is alarming. HEV has a unusual life cycle in that it exists in two different virion forms: a naked form that is shed into the feces and an enveloped form that circulates in the bloodstream. The enveloped form, so called eHEV, is infectious, despite lacking virally encoded envelope proteins. While the biogenesis of eHEV is thought to be similar to that of exosomes, little is known about how these particles enter the cell and initiate an infection. Our recent study suggests that eHEV uses a novel entry mechanism involving the degradation of its membrane within late endosomes/lysosomes, as evidenced by a substantial loss of infectivity in cells depleted of NPC1 (a cholesterol extractor) or treated with an inhitor of lysosomal acid lipase. This novel entry mechanism may provide hints on the role of antibody in HEV infection. Neutralizing anti-capsid antibodies do not recognize the free eHEV particles, yet they effectively block eHEV-mediated spread in cell culture. Thus, antibodies may prevent eHEV uncoating in the late endosomes/lysosomes upon

the degradation of the viral membrane. A better understanding of the eHEV entry and neutralization mechanisms is expected to advance our knowledge about this infection and may inform better strategies to prevent and treat HEV-associated diseases.

W11-5:

Differential Expression of CREM/ICER Isoforms Is Associated with the Spontaneous Control of HIV Infection

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A rare subset of HIV-infected individuals, termed elite controllers (ECs), can maintain long-term control over HIV replication in the absence of antiretroviral therapy (ART). To elucidate the biological mechanism of resistance to HIV replication at the molecular and cellular levels, we performed RNA sequencing and identified alternative splicing variants from ECs, HIV-infected individuals undergoing ART, ART-naive HIV-infected individuals, and healthy controls. Weidentified differential gene expression patterns that are specific to ECs and may influence HIV resistance, including alternative RNA splicing and exon usage variants of the CREM/ICER gene(cyclic AMP [cAMP]-responsive element modulator/inducible cAMP early repressors) (ANOVA tests). The knockout and knockdown of EC-increased ICER exons (exons 15 and 16) resulted insignificantly increased HIV infection in a CD4 T cell line and primary CD4 T cells ($P < 0.05$, non-parametric MannWhitney tests). Overexpression of EC-increased ICER isoforms decreased HIVinfection in primary CD4 T cells ($P < 0.05$). Furthermore, ICER regulated HIV long terminal repeat (LTR) promoter activity in a Tat-dependent manner. Together, these results suggest that ICER isan HIV host factor that may contribute to the HIV resistance of ECs. These findings will help elucidate the mechanisms of HIV control by ECs and may yield a new approach for treatment of HIV.

W11-6:

The rapidly increased replication induced by mutations in multiple gene segments facilitates the adaptation of H9N2 avian influenza virus in vaccinated chickens

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H9N2 subtype avian influenza virus is the main pathogen that endangers the poultry industry in China and causes serious economic loss. H9 virus is not only widespread in poultry, but also can cross the interspecies barrier to infect a variety of mammals, including humans, threatening public health. Although China has been using inactivated vaccine for a long time to prevent and control H9N2 subtype AIVs in chickens, the virus is still widespread in poultry. We used next generation sequencing of chicken-derived viruses from niave, placebo-controlled trial of vaccine efficacy to characterize the diversity of H9 virus and to define the impact of vaccine-induced immunity on within host populations. In our study population, we found that viruses can improve its replication ability through genome-wide cooperative mutation. The variation of HA leads to the linkage variation of other gene fragments, which may be the cause of the increase in the diversity of the whole genome, especially the induction of the linkage variation of the polymerase gene, leading to the change of its host specificity. At the same time, we compared the effects of homologous vaccine and allogenic vaccine on the virus, and found that homologous vaccine had less evolutionary bottleneck than allogenic vaccine, which may lead to stable mutation. Vaccine immune pressure resulted in increased genetic diversity of H9 avian influenza, which provided a larger quasispecies for the virus to produce strains adapted to mammals. Contrary to what has been suggested in epidemiological systems, our data indicate that H9N2 influenza vaccination has significant impact on intrahost diversity in natural infection.

Workshop #12:

W12-1:

Transcriptomic mechanisms underlying the immune modulating function and therapeutic efficacy of PARP inhibitors

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Poly-(ADP-ribose) polymerase (PARP) inhibitors (PARPis) have shown remarkable therapeutic efficacy against BRCA1/2 mutant ovarian and breast cancers. The current understanding of underlying mechanisms responsible for the efficacy of PARPis is through synthetic lethality, whereby blockade of single-stranded DNA damage repair, leads to an accumulation of toxic DNA double strand breaks specifically in cancer cells with defects in homologous recombination (HR) repair (BRCAness). This concept of 'BRCAness' provides a mechanistic paradigm for the sensitivity of such tumors to PARPis. However, clinical studies in ovarian cancer have demonstrated that PARPi as a maintenance drug exhibits a similar efficacy in both BRCA-mutant and wildtype cisplatin-sensitive ovarian tumors. More recently, , multiple clinical trials testing the combination of PARPis with programmed cell death-1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors (PD-1/L1is) showed there was no clear correlation between durable responses to the combination and known predictive DNA damage/repair biomarkers for PARPis, e.g. BRCA1/2 mutations. These data suggest that the mechanisms underlying the efficacy of PARPis as immunomodulating agents may be different from or independent of the existing paradigm of therapeutic mechanism of PARPis based on synthetic lethality with DDR defects. Here, through single cell transcriptomic analysis, we show that PARPis induce immunogenic transcriptomic changes and modulate immune reposes, which contribute to their therapeutic effects independent of BRCA1/2 mutations. Furthermore, we observed that PARPis increase PARP1 binding/trapping to the STING promoter, which transcriptionally upregulates cancer cell intrinsic STING expression, a key innate immune response gene, potentially through a catalytic independent function of PARP1. In addition, our data showed that PARP1i can revert epigentic silencing of STING induced by the altered LKB1-mediating metabolic signlaing. Ultimately, these PARPi-induced transcriptomic changes promote antitumor immune responses independent of BRCAness. Our results may provide a mechanistic rationale for using PARPis as immunomodulatory agents to harness therapeutic efficacy of immune checkpoint blockade.

W12-2:

A New Method for Comprehensive Genomic Methylome Profiling in Cancer

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DNA methylation has been shown to have broad influences on DNA replication, cell cycle, and gene expression regulation. In addition to the canonical *C5*-methylcytosine (5mC), other two non-canonical forms of DNA methylation, *N4*-methylcytosine (N4mC) and *N6*-methyladenine (6mdA) have also been reported in eukaryotes. We and others identified that 6mdA exists and plays critical functional roles in mouse stem cells and human brain tumors. However, there are few technologies to obtain high-resolution genomic maps of all three types of DNA methylation (genomic methylome profiling). Most DNA methylation sequencing methods were developed for 5mC, such as bisulfite sequencing. Although the 3rd-generation single-molecule real-time (SMRT) or Nanopore-based sequencing can be applied to identify the modified bases, both methods request high sequencing coverage, and neither can efficiently detect all three forms in high fidelity. Multiple antibody-based or enzyme-based approaches have been developed for 6mdA profiling, yet the protocols are complicated or highly specialized.

To help to understand the complete picture of the DNA epigenomic spectrum in human tissues and diseases, we developed a DNA methylome sequencing method, the nitrite-treatment followed by PCR and NGS sequencing (***NT-Seq***), that can simultaneously map the 5mC, N4mC, and 6mdA, at single-base resolution in human genomes. NT-Seq successfully overcomes the technological obstacles of several prevalent methods for DNA methylome profiling. This simple, low-cost, high-fidelity strategy can analyze the frequency and distribution of all three methylated residues in human genomes and thoroughly improve the methylome profiling in cancer research.

W12-3:

Genome Chaos, Information Creation, and Cancer Emergence: A New Framework of Cancer Evolution

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Cancer evolution is usually considered a stepwise Darwinian process characterized by the accumulation of a few common gene mutations which either serve as breakers of tumor suppressors or oncogenes activating signaling pathways, leading to uncontrolled proliferation. However, increasing clinical evidence, particularly data from Cancer Genome Sequencing Project, has challenged the long-accepted somatic gene mutation theory of cancer evolution. First, identifying recurrent patterns of gene mutations for a given cancer type is difficult. Second, chromosomal changes, including various structural or numerical genome chaos (e.g. chromothripsis, chromoplexy, micronuclei clusters, and polyploid giant cancer cells) are overwhelming and act as common drivers for macroevolution. Together, these multiple genomic and non-genomic changes call for a new evolutionary paradigm to understand the pattern of cancer evolution. In this presentation, we invoke the Genome Architecture Theory, an alternative conceptual framework that departs from gene-based theories. Using the Genome Architecture Theory, we discuss the relationship between stress-induced genome reorganization, the creation and preservation of karyotype coding, and two-phased cancer evolution (genome alteration-mediated punctuated macroevolution followed by gene mutation and epigenetic alteration-mediated microevolution). Specifically, we will emphasize how new system information unifies diverse molecular mechanisms. Such discussions will not only create a new landscape of cancer research but also provide new platforms to reestablish genome system-based, information-focused genomic and evolutionary theories. For additional information, see Heng, H.H., 2015. Debating Cancer: The Paradox in Cancer Research. Heng, H.H., 2019. Genome Chaos: Rethinking Genetics, Evolution, and Molecular

Medicine. Academic Press, San Diego, CA doi:10.1016/C2016-0-05291-9.

W12-4:

Giant cells: A new paradigm to understand human life and tumors at an organismal level

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Human tumors have been traditionally considered a genetic-based disease characterized by dysregulation of cell cycle due to somatic mutations. However, this traditional conceptualization of cancer cannot explain the numerous mutations and structural alterations in normal and embryonic tissues, and it does not address the lack of tissue differentiation and high nuclear atypia based on histology, the criteria that pathologists use to diagnose a malignancy (cancer). In the postgenomic era, enormous amounts of cancer-related data are generated daily, but very little knowledge has been gained from these data regarding cancer or cancer patient care. The cancer research community is experiencing a conceptual crisis and is searching for a new paradigm to guide future research. Here I propose a new theory to explain human tumors: Life is perpetuated through a giant egg cell via fertilization (sex), and tumors are parasitic evil twins of life that also arise from either a sexual or an asexual origin. For tumors of sexual origin, the giant egg cell undergoes cleavage to form blastomeres to rapidly increase genomic contents and generate embryoblasts (inner cell mass). Following uterine implantation, the inner cell mass either develops into a baby or develops into a differentiated tumor resembling its tissue of origin if development of one or more germ layers is arrested before full differentiation. For tumors of asexual origin, senescent somatic cells undergo senescence escape via genomic duplication or multiplication, giving rise to polyploid giant cancer cells (PGCCs), a somatic equivalent of blastomeres, which leads to formation of undifferentiated tumors with high level of nuclear atypia. The switch from conceiving of cancer as a mitosis-based proliferation to conceiving of cancer as a giant cell-mediated embryogenic or embryogenic-like process provides a new paradigm to decode the complexity of human life and tumors at an organismal level.

W12-5:

CHA1: A New Combinatorial Therapy That Reciprocally Regulates Wnt and JAK/STAT/Interferon Signaling to Re-program Breast Tumors and the Tumor-Resident Landscape

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Triple negative breast cancers (TNBC) pose exceptional challenges with fatal brain metastasesas a clear and unmet need. Immune checkpoint inhibitors (ICIs) are promising therapeutic strategies, but most TNBC are resistant, or “cold” tumors, due to lack of tumor-resident immuneengagement. No FDA-approved therapies exist which promote a “cold-to-hot” transition or induce the important biomarker PD-L1, often used for ICI clinical decision-making. Maximal ICI susceptibility, or a full “cold-to-hot” transition, requires reciprocal Wnt signaling inhibition and Jak/STAT/interferon signaling activation. We report a new compound combination (CHA1) that fits the above criteria. CHA1 is comprised of EGCG (epigallocatechin-3-gallate; green-tea compound) and decitabine (DNA-methyltransferase (DNMT1) inhibitor; 5’deaza-cytidine; FDA-approved for hematologic malignancies). We used immune-compromised and syngeneic TNBC pre-clinical models to investigate tumor-intrinsic and tumor-resident T-cell effects, respectively. All results required CHA1 (but not EGCG or decitabine alone) and utilized attainable human dose equivalences with manageable safety profiles. CHA1 triggered efficient Wnt signaling inhibition by elevating Wnt pathway inhibitors (HBP1 and SFRP1) and traversed the blood-brain barrier to reduce both tumor and brain metastatic growth. Transcriptomic and expression analyses revealed that CHA1 treatment effectuated a robust tumor-intrinsic JAK/STAT/IFN response 1) to induce PDL1 and 2) to induce antigen presentation and processing genes, including MHC-1, MHC-2 and numerous genes attributed to professional antigen-presenting cells; 3) to induce CD8+-T-cell infiltration and activation. Additionally, CHA1 pre-treatment improved anti-PDL1 efficacy in a syngeneic setting. Lastly, we derived a composite gene signature emblematic of CHA1 treatment and of a favorable clinical prognosis in-silico. Together, our work supports a model in which CHA1 influences epigenetics, Wnt and Jak/STAT/IFN signaling mechanisms—all to reprogram an epithelial-mesenchymal TNBC tumor to express antigen-presenting properties and to recruit and activate tumor-resident CD8+-T cells. We discuss our findings in the context of cancer biology and immunity with implications for improving ICI susceptibility for TNBC.

Workshop #13:

W13-1:

Novel scalable and simplified system to generate microglia-containing cerebral organoids from human induced pluripotent stem cells

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Human cerebral organoid (CO) is a three-dimensional (3D) cell culture system that recapitulates the developing human brain. While CO has proved an invaluable tool for studying neurological disorders in a more clinically relevant matter, there have still been several shortcomings including CO variability and reproducibility as well as lack or underrepresentation of certain cell types typically found in the brain. As the technology to generate COs has continued to improve, more efficient and streamlined protocols have addressed some of these issues. Here we present a novel scalable and simplified system to generate microglia-containing CO (MCO). We characterize the cell types and dynamic development of MCOs and validate that these MCOs harbor microglia, astrocytes, neurons, and neural stem/progenitor cells, maturing in a manner that reflects human brain development, as determined by immunohistochemistry, flow cytometry and single cell RNA sequencing. We introduce a novel technique for the generation of embryoid bodies directly from inducible pluripotent stem cells (iPSCs) that involve simplified steps of transitioning directly from 3D cultures as well as orbital shaking culture in standard 6-well culture plate. This allows for the generation of MCOs with an easy-to-use system that is affordable and accessible by any general labs.

W13-2:

HIV infection of human IPSC-derived microglia-containing cerebral organoids

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Studying the mechanism(s) of HIV-associated neurocognitive disorders (HAND) requires an appropriate in vitro brain model. In this study, we developed a human induced pluripotent stem cell (hiPSC)-derived cerebral organoids which contain not only astrocytes, neurons, and neural stem/progenitor cells, but also microglial cells, the primary target of HIV infection in the central nerve system. We demonstrated that the microglia containing cerebral organoids (MCOs) expressed both the HIV major entry receptor (CD4) and coreceptors (CXCR4 and CCR5) and could be productively infected by HIV. The infected MCOs showed a steady and significant increase of HIV gag gene mRNA and p24 protein expression, which was followed by latent infection with only detectable HIV gag DNA and little expression of the viral gag mRNA and p24 protein in the infected MCOs. The HIV latent infection was confirmed by the RNAscope in situ hybridization using the antisense probes of the virus. The addition of the combination of the HIV replication stimulants (TNF-□, PMA and SAHA) to the latently infected MCO cultures could reactivate the HIV replication evidenced by elevated expression of HIV gag mRNA and P24 protein. The existence of replication-competent intact HIV provirus in the reactivated MCOs was also demonstrated by the viral outgrowth assay using HutR5 cells. The single-cell RNA sequencing analysis of HIV-infected MCOs revealed that microglia is the major target of the virus. These observations suggest that the human iPSC- derived MCOs be an alternative and suitable *in vitro* brain model for studying both acute and latent HIV infection of microglia in the presence of other major brain cells, which is crucial to examine the mechanism(s) underlying the immunopathogenesis of HAND.

*These authors contributed equally to this study; **Corresponding authors

W13-3:

Engineering Organoid Models for Understanding Human Neurodevelopment and neurological disorders

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Human Induced pluripotent stem cells (hiPSCs) has 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. Brain organoid cultures from human iPSCs have been recently developed to recapitulate the cellular composition and the cytoarchitecture of the developing brain. These hiPSC based organoid model systems offer unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development and in modeling neurodevelopmental and neurological disorders. I will discuss our recent work in engineering organoids with brain region specific identity and applying these models to understand human brain development and neurotropism of SARS-CoV-2.

W13-4:

Using Organoid Models to Investigate Neurodevelopmental Abnormalities Caused by a Congenital Disorder of Deglycosylation

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Human cerebral organoids (COs) represent an increasingly noticed platform for investigating defective neurodevelopment caused by genetic disorders. Mutations in *N*-glycanase 1 (NGLY1) can lead to NGLY1 deficiency, a congenital disorder of deglycosylation, in patients. NGLY1-deficiency patients often show clinical presentations in the brain, including microcephaly and other neurological disorders. Despite the discovery of NGLY1-deficiency patients, how NGLY1 dysfunction affects neurodevelopment in a human-relevant system remains largely unknown. Using COs developed from human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), we modeled and investigated how NGLY1 dysfunction disturbs early brain development. While NGLY1 loss had limited impact on the undifferentiated cells, COs developed from NGLY1-deficient hESCs showed defective formation of upper-layer neurons and attenuation of signaling critical for sustaining radial glia. NGLY1-deficient CO cells were more vulnerable to multiple stressors. Transcriptomic analysis revealed premature neuronal differentiation accompanied by abnormal downregulation of secreted and transcription factors, including TTR, IGFBP2, and ID4, in NGLY1-deficient COs. NGLY1 malfunction also dysregulated ID4 and enhanced neuronal differentiation in CO transplants developed in vivo. Expressing NGLY1 led to IGFBP2 and ID4 upregulation in CO cells developed from NGLY1-deficiency patients' hiPSCs. In addition, treatment with recombinant IGFBP2 enhanced ID4 expression, STAT3 signaling, and proliferation of NGLY1-deficient CO cells. Overall, our findings in NGLY1-defective human COs indicate that dysregulation of stress responses and neural precursor

differentiation underlies the brain abnormalities observed in NGLY1-deficiency patients.

W13-5:
Lysosomal Stress, Mechanisms and Potential Effect on Neorodegeneration
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Autophagy, a cellular catabolic pathway mediated by lysosomal degradation, plays important roles in multiple physiological and pathological processes. Deregulation of the autophagy-lysosome pathway has been implicated in pathogenesis of neurodegeneration. 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. Here we will describe our finding that lysosomal stress can trigger TFEB activation in a manner that is independent of mTORC1 suppression but instead requires an enhanced protein phosphatase 2A (PP2A)-B55α activity to counter the function of mTORC1. We will also discuss the mechanism by which lysosomal stress stimulates PP2A activity towards TFEB and the potential therapeutic implication of this mechanism in the treatment of Alzheimer's disease.

W13-6:
Methamphetamine Enhances HIV Infection of iPSC-derived Microglia and Macrophages
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Because of the overlapping impact of methamphetamine (METH), a potent addictive psychostimulant, and HIV on the CNS, it becomes increasingly important to understand the role of interplays between METH and HIV in the pathogenesis of HIV-associated neurocognitive disorders (HAND). However, studies of HAND have been hampered by difficulties in collecting primary microglial cells from autopsy or biopsy ofHIV patients. Recent success in generating human cells from induced pluripotent stem cell lines (iPSCs) now offers a great opportunity to investigate the impact of METH on HIV infection of microglial cells. In this study, we demonstrated that METH at concentration as low as 10 μM could significantly enhance HIVinfection of human iPSC-derived microglia (iMg) and primary human macrophages, which was evidencedby significantly increased expression of the HIV gag gene, p24 protein and reverse transcriptase activity inthe treated cells. To depict the question of how METH undermines the host cells innate immunity and induces the HIV replication in microglia and macrophages, we exam the effect of METH on Toll like receptor 3 (TLR3)-mediated interferon (IFN) signaling pathway. METH suppressed the intracellular expression of the IFNs (IFN-α/β) and the anti-HIV IFN-stimulated genes (ISGs), particularly, the HIV egress inhibitors (viperin and tetherin) in iMg and macrophages. In addition, METH treatment of iMg andmacrophages facilitated the expression of the inflammatory cytokines (IL-1 beta and IL-8). These data provide the experimental evidence to support the notion that METH use inhibits the specific intracellular immunity against HIV and induces the inflammatory cellular factors, which facilitate HIV infection/replication in microglia and macrophages.

Workshop #14:

W14-1:

CNS control of metabolic functions over the lifespan

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The central melanocortin system is critical for regulation of energy metabolism, and Agouti-Related Protein (AgRP) is the sole orexigenic component of the system. AgRP is conserved across mammalian species, and no null mutation of the *AGRP* gene has been found in humans, suggesting that AGRP is important for survival in humans. Neurons expressing AgRP are potent drivers of feeding. However, the functions of AgRP are still not-well defined and mice lacking AgRP have normal food intake. In this presentation, I will describe our recent research on the metabolic functions of AgRP, as well as Ankyrin Repeat and SOCS Box Containing 4 (ASB4), a protein that is negatively regulated by AgRP. I will highlight the interactions between genes and environmental factors in impacting energy metabolism during early development, adulthood, and advanced aging.

W14-2:

Asprosin promotes feeding through SK channel-dependent activation of AgRP neurons

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Asprosin is a fasting induced hormone that activates Agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARH) for optimal maintenance of appetite and body weight. Protein Tyrosine Phosphatase Receptor δ (Ptprd), a membrane-bound phosphatase receptor, was recently identified as the orexigenic asprosin-receptor in AgRP neurons. However, the intracellular mechanisms responsible for asprosin/Ptprd-mediated activation of AgRP neurons remain unknown. Here, we demonstrate that the small conductance of the calcium-activated potassium (SK) channel is necessary for mediating the stimulatory effects of asprosin and Ptprd on AgRP neurons. Specifically, we found that genetic asprosin deficiency increased the SK current and reduced AgRP neuron activity, both of which were restored upon *ex vivo* and *in vivo* asprosin replenishment. Conversely, chronic elevation of plasma asprosin inhibited the SK current and activated AgRP neurons, leading to enhanced food intake, weight gain and glucose intolerance. AgRP neuron-specific deletion of SK3 (a subtype of the SK channel highly expressed in AgRP neurons) blocked the stimulatory effects of asprosin on AgRP neurons and prevented asprosin-induced overeating. Further, pharmacological blockade or genetic knockdown of the asprosin orexigenic receptor, Ptprd, abolished asprosin's effects on modulation of the SK current and AgRP neuron activity. Taken together, our results demonstrate an essential role for potassium conductance via SK3 in asprosin/Ptprd-induced AgRP neuron activation and hyperphagia.

W14-3:

An exercise-inducible metabolite that suppresses feeding and obesity

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Exercise confers robust protection against obesity, type 2 diabetes, and other cardiometabolic diseases. However, the molecular and cellular mechanisms that mediate the metabolic benefits of physical activity remain unclear. Here we show that exercise stimulates production of Lac-Phe, a blood-borne signaling metabolite that suppresses feeding and obesity. Lac-Phe biosynthesis from lactate occurs in CNDP2+ cells including immune cells, epithelial cells, and mesenchymal stem cells localized to diverse organs. In diet-induced obese mice, pharmacological elevation of circulating Lac-Phe reduces food intake without affecting movement or energy expenditure. Chronic administration of Lac-Phe decreases adiposity and body weight and improves glucose homeostasis. Conversely, genetic ablation of Lac-Phe biosynthesis in mice increases food intake and obesity following exercise training. Lastly, dramatic

activity-inducible elevations of circulating Lac-Phe levels are also observed in humans and racehorses, establishing this metabolite to be a robust molecular effector associated with physical activity across multiple activity modalities and mammalian species. These data define a conserved exercise-inducible metabolite that controls food intake and influences systemic energy balance.

W14-4:

27 hydroxy-cholesterol acts on estrogen receptor alpha expressed by POMC neurons to modulate feeding behavior

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Oxysterols are metabolites of cholesterol produced in peripheral tissues to eliminate cholesterol. 27-hydroxycholesterol (27HC) is the most abundant oxysterol and can cross the blood-brain barrier. Interestingly, 27HC has recently been identified as an endogenous selective estrogen receptor modulator (SERM) for both estrogen receptor α and β (ERα/β). Considering the regulatory effects of brain estrogen/ERα signaling on energy metabolism, we hypothesize that the endogenous SERM 27HC binds with ERα in the arcuate nucleus of the hypothalamus (ARH) of the brain to modulate energy homeostasis. In supporting this point of view, we found that a single acute intracerebroventricular (ICV) injection of 27HC inhibited food intake in both male and female mice. The reduced food intake was attributed to decreased meal size and increased intermeal intervals. This anorexigenic effect was also associated with the increased c-fos expression in the pro-opiomelanocortin neurons in ARH ($POMC^{ARH}$). Using brain slice patch-clamp recording, we consistently showed that 27HC dose-dependently actives $POMC^{ARH}$ neurons in an ERα-dependent manner, suggesting a mediating role of ERα expressed by $POMC^{ARH}$ neurons. Notably, we further revealed that the inhibitory effects of 27HC on food intake were blocked by antagonists for ERα or POMC downstream melanocortin 3/4 receptors. In addition, chemogenetic inhibition of $POMC^{ARH}$ neurons also blunted the anorexigenic effects of 27HC in mice. Collectively, these results support a model that 27HC acutely inhibits food intake by acting on ERα to stimulate $POMC^{ARH}$ neuronal activity. This 27HC/ERα/POMC signaling pathway may serve as a critical defending mechanism against high-fat diet-induced obesity.

W14-5:

Investigating the Metabolic Function of an Orphan G Protein-coupled Receptor in the Intestinal Epithelium

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G protein-coupled receptors (GPCRs) in the gastrointestinal tract are involved in maintaining glucose and energy homeostasis by regulating the release of gut hormones in response to luminal dietary nutrients as well as microbial metabolites. We recently identified that an orphan GPCR, Gpr17, was co-expressed in glucagon-like peptide-1 (GLP-1)-expressing EECs in human and rodent intestinal epithelium. However, it is unknown how Gpr17 ablation in the intestinal epithelium affects feeding behavior and satiety regulation. To address this question, we used genetic knockout approach to generate intestinal Gpr17-deficient mice and analyzed their glucose metabolism and feeding behavior. We showed that intestinal Gpr17-deficient mice had similar growth curve, body composition, and *ad libitum* food intake compared with littermate controls. We found that acute genetic ablation of Gpr17 in intestinal epithelium (iKO) improved oral glucose tolerance and glucose-stimulated insulin secretion (GSIS) in female and male mice. iKO mice responded to glucose or lipid ingestion with increased secretion of GLP-1, but not the other incretin glucose-dependent insulinotropic polypeptide (GIP). Intestinal Gpr17-deficient mice responded to fasting-refeeding challenge with reduced fasting locomotor activity and less food intake after refeeding, suggesting increased satiety during the phase of rebound hyperphagia. Furthermore, comprehensive studies including gut morphology, gut epithelium heterogeneity by scRNA-seq, and microbiome analysis were examined but none of them was changed because of intestinal Gpr17 ablation. In addition, male Gpr17 whole body knockout mice have improved intraperitoneal glucose tolerance, and increased insulin sensitivity in hyperinsulinemic-euglycemic clamp studies. In conclusion, our work showed that ablation of intestinal Gpr17 signaling led to improved neurohormonal regulation to maintain metabolic homeostasis.

Workshop #15:

W15-1:

METTL3 regulates the homology-dependent DNA repair pathway choice

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Double strand breaks (DSBs) are the most toxic DNA damage lesions and infidelity of DSB repair often leads to genome instability, a hallmark across different cancer types. DSBs are repaired by either the homology-independent NHEJ pathway or the homology-dependent HR, SSA or MMEJ pathways. Much progress has been made toward understanding the choice between HR and NHEJ pathways through regulating the end resection at DSB. However, the mechanism that controls homology-dependent error-free HR and error-prone SSA repair pathway choice is still poorly defined. A defect in BRCA1-PALB2-BRCA2 axis suppresses HR and promotes SSA through an unknown mechanism. Here we show that METTL3, an RNA epigenetic modification protein, is a novel BRCA1-associated protein. BRCA1 is both necessary and sufficient for METTL3 recruitment to DSB sites to sustain HR. siRNA-mediated METTL3 depletion promotes HR and reduces SSA, a phenotype similar to the loss of PALB2 and BRCA2. Pathogenic mutations in BRCA1 coil-coil domain, which binds PALB2 and regulates the choice between HR and SSA, promote the METTL3 interaction while abolishing the interaction with PALB2. These results suggest that METTL3 is a new regulator of the HR and SSA pathway choice through the BRCA1-PALB2 axis.

W15-2:

Structural basis of DNA double-strand break repair by NHEJ

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As one of the most cytotoxic forms of DNA damage, DNA double-strand breaks (DSBs) can be detected and repaired by the highly regulated non-homologous end-joining (NHEJ) machinery. NHEJ is involved in multiple cancer therapeutics and versatility of antibody and receptor generations in immune cells. Core NHEJ factors (Ku70/80 heterodimer (Ku), catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), DNA ligase IV (LigIV), XRCC4 and XLF) form a long-range (LR) synaptic complex after NHEJ initiation, then transits into a short-range (SR) stage to align DSB ends. Using single-particle Cryo-Election Microscopy (Cryo-EM), we have visualized multiple DNA-protein complexes formed by NHEJ factors. Ku, DNA-PKcs, LigIV-XRCC4, and XLF form a Long-range synaptic complex, in which the DNA ends are held ~115 Å apart. Two DNA end-bound Ku-DNA-PKcs subcomplexes are linked by DNA-PKcs-DNA-PKcs interactions and a LigIV-XRCC4-XLF-XRCC4-LigIV scaffold. The relative orientation of the DNA-PKcs molecules suggests a mechanism for auto-phosphorylation in trans, leading to dissociation of DNA-PKcs and transition into the Short-range synaptic complex. Integrated modeling with both experimental reconstruction and in silico structural prediction reveals how an appendix NHEJ scaffolding factor, PAXX (paralog of XRCC4, XLF) stabilizes the LR complex during ATP-dependent DNA-PKcs activation. Upon DNA-PKcs auto-phosphorylation, the LR complex undergoes substantial conformational change, with both Ku and DNA-PKcs rotating outward to promote DNA double-strand break exposure and DNA-PKcs dissociation. In addition, we captured a dimeric state of catalytically inactive DNA-PKcs, which resembles previously determined structures of phosphatidyl inositol-3 kinase-like (PIKK) family kinases, revealing a model of the DNA-PKcs life cycle during NHEJ.

W15-3:

Inhibition is not the same as deletion – lessons from mouse models with catalytically inactive mutations in DNA damage response factors – ATM, ATR, DNA-PKcs and PARPs

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DNA strand breaks generated during normal development and cancer therapy activate the DNA damage response (DDR) that involves three large PI3-kinase-like protein kinases – ATM, ATR, DNA-PKcs, and two poly-ADP-ribose Polymerases (PARP1 and PARP2). Given their essential roles in response to oncogene-induced proliferation stress, specific inhibitors against each of those factors have been developed and advanced to clinical trials. Several ATR kinase inhibitors and four PARP1/2 dual inhibitors have been approved by FDA and shown promising anti-cancer effects. While the initial rationales for developing the specific inhibitors were based on their signaling role in the DDR, genetic and biochemical data suggest that those large DNA damage response kinases and PARP1/2 might have structural functions beyond their catalytic activities. To understand the physiological impact of the catalytically inactive DNA damage response factors, our lab generated a series of mouse models that carry knockin catalytically inactive point mutations in ATM, ATR, DNA-PKcs, as well as PARP1 and PARP2. In every case, the expression of catalytically inactive DNA damage response factors causes much more severe genomic instability than the corresponding null mutation. Specifically, while complete loss of ATM kinase is compatible with embryonic development in both patients and mouse models, expression of kinase-dead ATM leads to embryonic lethality, explaining why nearly all Ataxia-Telangiectasia patients with germline ATM deficiency carry truncating or frameshift mutations that confer little if any ATM protein expression. Moreover, we found that the presence of kinase-dead ATM causes hypersensitivity to Topoisomerase I inhibition, providing a potential avenue to selectively target some cancers based on distinct ATM mutation status. Mechanistically, ATM/ATR/DNA-PKcs, as well as PARP1/2, are recruited to sites of DNA damage, where they are activated. Using quantitative live-cell imaging, we showed that activation and completion of catalytic reactions are coupled with the rapid exchange of these DDR factors. Thus, the presence of inactivating mutations or kinase inhibitors prolong the presence of those large kinases or PARPs at DNA damage sites, where they dominant-negatively block subsequent DNA repair. Here we will discuss our new findings on the PARP1/2 catalytic inactive models and their implications for the on-target effect and toxicity of PARP inhibitors.

W15-4:

Defining the role of FANCA in breast cancer development and cell cycle progression

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FANCA is one of the 22 known Fanconi anemia (FA) pathway genes that are involved in interstrand crosslink (ICL) repair. We have also shown that and FANCA plays a role in double strand break repair by promoting strand annealing and exchange. In this report, we describe that FANCA expression in breast cancer is significantly elevated within TCGA databases and in patient tumors-derived sections on both transcription and translation levels. This elevation is epigenetically associated with the methylation status at the S-shore of CpG island ahead of the FANCA gene. Importantly, we demonstrate that FANCA inactivation by knocking out or knocking down inhibits growth of a breast cancer cell MDA-MB-231 *in vitro* and *in vivo* by causing cell cycle arrest at G1 phase. In addition, β-Galactosidase staining assay indicates an increase in senescent fraction in knockout cells. The cell cycle arrest is caused by hypophosphorylation of Rb protein resulting from CDK4 downregulation and PP2A upregulation. Our RNA-seq data has indicated that E2F targets are also downregulated in FANCA knockout cells compared to the WT MDA-MB-231 cells, which is consistent with the G1/S cell cycle arrest and Rb hypophosphorylation. Intriguingly, heterozygous FANCA KO in a breast cancer mouse model C3(1)-TAg model has shown reduction of breast tumor formation and volume. Homozygous FANCA knockout in the mouse model is currently ongoing. Our goal is to elucidate the role of FANCA in breast cancer development and to evaluate the potential of FANCA as a target for breast cancer treatment.

W15-5:
Radioresistant glioblastoma cells enhance fat burning with CD47 mediated anti-phagocytosis
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Metabolic reprogramming is a hallmark of cancers with a dominant glycolytic pathway, whereas increasing evidence suggests an adaptive tumor metabolism with reactivated mitochondrial functions for surviving genotoxic anti-cancer therapies including ionizing radiation. In normal cells, DNA repair and cell cycle progression can be accelerated by boosting mitochondrial energy output to meet the increased cellular energy demands. Such an adjustable metabolic pathway is implemented in a timely fashion by therapy resistant tumor cells to meet the rising energy demands associated with tumor radioresistance and metastasis. The current study identifies a glycolysis-to-fatty acid oxidation (FAO) metabolic shift in the radioresistant GBM cells surviving therapeutic regimen of radiation and in regrown GBM tumors after in vivo radiotherapy. CD47, an immune checkpoint protein signaling “do not eat me” against macrophage phagocytosis, is co-upregulated with FAO enzymes (CPT1A, CPT2 and ACAD9) in the radioresistant GBM cells and the regrown syngeneic tumors after radiation. Co-upregulation of the FAO enzymes and CD47 is correlated with a poor prognosis in GBM patients. Mechanistically, radiation-boosted FAO enhances mitochondrial ATP generation and upregulates CD47 transcription via acetyl-CoA mediated NF-κB/RelA acetylation. Inhibition of FAO by CPT1 inhibitor etomoxir or CRISPR-mediated deletion of CPT1A, CPT2, or ACAD9 reduces CD47 expression and re-sensitizes resistant GBM tumors to radiation accompanied by enhanced macrophage phagocytosis. These findings reveal a mechanism by which radioresistant cancer cells enhance fatty acid metabolism to fuel aggressive tumor proliferation and defend against macrophage clearance by upregulating CD47 expression. Modulating tumor lipid metabolism is a potential approach to eliminate resistant/recurrent cancers during combined radiation-immunotherapy.

Workshop #16:

W16-1:

KSHV hijacks CAD to promote metabolic reprogramming and cell proliferation

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent that causes Kaposi's Sarcoma (KS) and several lymphomas. Aerobic glycolysis, better known as "Warburg effect", preferentially converts glucose into lactate even with sufficient oxygen supply, which is a hallmark of cancer cells and normal proliferating cells. KSHV was previously reported to reprogram glycolysis, yet the mechanisms are not well understood. Our recent studies of herpesvirus immune evasion implicate cellular glutamine amidotransferases (GAT) in deamidating key signaling molecule. To probe the general role of protein deamidation, we performed a focused screen and identified Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, and Dihydroorotase (CAD), as a bona fide protein deamidase of the NF-kappaB transcription factor RelA. CAD catalyzes the first, rate-limiting step of the de novo pyrimidine biosynthesis. Remarkably, RelA deamidation downregulated NF-κB activation and the expression of oxidative phosphorylation genes, whereas potently promoted the expression of key glycolytic enzymes, thus fueling aerobic glycolysis and cell proliferation. During KSHV de novo infection in human oral keratinocytes (HOK) and hTert-immortalized microvascular endothelial cells (TIME), CAD activity was promoted, resulting in an upregulated pyrimidine biosynthesis and RelA deamidation-mediated glycolysis reprogramming. Mechanistically, KSHV deployed vCyclin to bind and activate CAD. As such, pharmacological inhibition or genetic depletion of vCyclin and CAD potently impeded KSHV lytic replication and thwarted proliferation of KSHV+ primary effusion lymphoma cells *in vivo*. Altogether, this work defines a novel function of CAD-RelA signaling axis in cellular metabolism, expanding the functional repertoire of protein deamidation in fundamental biological processes. Meanwhile, findings garnered from our study unveil a new viral mechanism underpinning KSHV oncogenesis and provide potential new means to treat KSHV-associated malignancies and diseases alike.
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W16-2:

New mechanistic insights into the host-virus interaction in EBV pathogenesis

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DNA damage response (DDR) and selective autophagy both can be activated by reactive oxygen/nitrogen species (ROS/RNS), and both are of paramount importance in cancer development. The selective autophagy receptor and ubiquitin (Ub) sensor p62 plays a key role in their crosstalk. ROS production has been well documented in latent infection of oncogenic viruses including Epstein-Barr Virus (EBV). However, p62- mediated selective autophagy and its interplay with DDR have not been investigated in these settings. In this study, we provide evidence that considerable levels of p62-mediated selective autophagy are constitutively induced, and correlates with ROS-Keap1-NRF2 pathway activity, in virus-transformed cells. Inhibition of autophagy results in p62 accumulation in the nucleus, and promotes ROS-induced DNA damage and cell death. We have further shown that p62 accumulation in the nucleus in response to autophagy inhibition promotes proteasome-mediated CHK1 and RAD51 protein instability. Furthermore, shRNA-mediated p62 depletion in EBV-transformed LCLs results in significant increases of endogenous RNF168-γH2AX damage foci and chromatin ubiquitination, indicative of activation of RNF168-mediated DNA repair mechanisms. Our results have unveiled a pivotal role for p62-mediated selective autophagy that governs DDR in the setting of oncogenic virus latent infection, and provide a novel insight into virus- mediated oncogenesis. (PLoS Pathogens, 2019. 15:e1007541)

W16-3:

A non-catalytic and intrinsically disordered herpesviral protein rewires the host cellular signaling by impacting protein phosphorylation, ubiquitination, and sumoylation through SLiMs

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As obligate intracellular parasites, viruses must modulate their hosts' cellular signaling pathways in order to modulate the cellular environment to their own advantages. The cellular signaling processes rely on reversible post-translational protein modifications (PTMs) to rapidly reconfigure protein functions and protein-protein interactions. Conceivably viruses have evolved diverse strategies to target the host PTMs. The most common and straightforward one is to genetically pirate the host enzymatic components, but non-catalytic viral proteins can also target the host PTMs, albeit more indirectly, by altering protein-protein interactions. We found a viral ORF45 of KSHV, which is non-catalytic and intrinsically disordered, can dramatically impact three major PTMs, phosphorylation, ubiquitination, and sumoylation. We demonstrate that ORF45 achieves its functions by acting as a scaffold to recruit proteins of interest into close proximity through multiple Short Linear Motifs (SLiMs).

W16-4:

Cholesterol-driven spontaneous morphagenesis of HBV subviral particles

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Chronic hepatitis B (CHB) remains a significant global health burden despite the availability of prophylactic vaccines against hepatitis B virus (HBV) for nearly 30 years. A defining feature of CHB is the persistence of high levels of circulating HBV surface antigen (HBsAg) in the form of subviral particles (SVPs) in the blood of CHB patients. Induction of HBsAg loss is considered to be essential for the restoration of host antiviral immune response and functional cure of CHB. Despite the pivotal importance of SVP in HBV infection and treatment, much remains unknown regarding its structure and morphogenesis. Through sequence analysis, we noticed significant similarities between HBsAg and tetraspanins—a group of cholesterol-modulated transmembrane (TM) proteins implicated in many cellular processes including the biogenesis of exosomes. We hypothesize that HBsAg is a structural homolog of tetraspanins and that the HBV SVP morphogenesis is more or less a spontaneous process driven by the cholesterol gradient in the cellular secretory pathway. In support of our hypothesis, a

cryoEM structure of the 24-kDa small (S) HBsAg shows as a V-shape, two-transmembrane protein with a planar density attributable to a binding cholesterol molecule in between the two TM helices. Inspired by the conformational change observed in structures of tetraspanin with or without cholesterol binding, we further propose that: 1. When HBsAg is translated and inserted in the ER membrane, where there is a very low level of cholesterol, they quickly dimerize to conceal the polar sidechains of Q16 and C100 on the two TM helices; 2. As they are transported to the ERGIC and encounter higher concentration of cholesterol, the HBsAg binds cholesterol, with polar sidechains of Q16 and C100 forming hydrogen bonds with the hydroxyl group of the cholesterol; cholesterol binding would trigger conformational change of the HBsAg TM helices from a straight bundle to the V-shape, inducing spontenous membrane curvature and eventually budding of the spherical SVP. This mechanism of cholesterol-driven spontaneous morphagenesis of SVP is consistent with previous EM observations in cells overexpressing HBsAg.

W16-5:

Tumor suppression of arginine sensor CASTOR1 in viral and nonviral cancers

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Cancer cells rewire metabolic pathways to support anabolic proliferation and survival. Kaposi's sarcoma-associated herpesvirus (KSHV) is the causal agent of Kaposi's sarcoma and several other malignancies commonly found in AIDS patients. Unlike most other types of cancer that are addicted to glucose and aerobic glycolysis, KSHV-transformed cells do not depend on glucose and have a reduced level of aerobic glycolysis; instead, they are addicted to glutamine. More surprisingly, glutamine is primarily shunted to nucleotides and amino acids in addition to TCA cycle. The glutamine provides the gamma-nitrogen to support the syntheses of nucleotides and induction of nitric oxide to activate the STAT3 pathway, both of which are essential for the proliferation of KSHV-transformed cells. Furthermore, KSHV hijacks numerous metabolic sensors to maintain the homeostasis of the transformed cells. In particular, downregulation of arginine metabolic sensor CASTOR1 by KSHV miRNAs is essential for activating the mTORC1 pathway, one of the most effective targets for KS therapy, while overexpression of CASTOR1 is sufficient to reverse the malignant phenotype of KSHV-transformed cells. In nonviral cancer such as breast cancer, AKT-mediated phosphorylation of CASTOR1 at S14 and RNF167 E3 ligase-mediated ubiquitination and degradation are essential for the activation of mTORC1, cell proliferation and tumor progression. In a K-Ras mouse cancer model, knockout of CASTOR1 significantly increases the incidence and progression of lung cancer. Analysis of TCGA database reveal that patients with lower expression levels of CASTOR1 have poor prognosis in ten types of cancer including lung and breast cancer. Together, these results demonstrate CASTOR1's tumor-suppressive function in both viral and nonviral cancers.

W16-6:

Chimeric Antigen Receptor (CAR)-Natural Killer (CAR-NK) Cells for Treatment of COVIDPatients with Cancer

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New SARS-CoV2 variants continue to emerge, imposing significant threats to the currently approved vaccines and therapeutics. Herein, we propose the use of CAR-NK cell-based immunotherapy for treatment of long COVID-19 patients with cancer. NK cells are known to provide the first line of defense against viral infections and tumor cells. Functional circulating NK cells are significantly reduced in severe COVID-19 patients, indicating the important role of NK cells in controlling the COVID-19 disease. Recent studies in CAR-NK cells also showed promising results in treating CD19-positive lymphoid tumors with reduced toxicity including graft versus host disease (GvHD) and cytokine release syndrome (CRS) compared to that of CAR-T cells in clinical trials. Additionally, CAR-NK cells are currently being investigated for treating solid malignanices. We designed the third-generation of CAR- NK cells expressing S309 single chain fragment variable (scFv) (hereinafter S309-CAR-NK cells), where S309 is a neutralizing antibody (NAb) that can neutralize both SARS-CoV-1 and SARS-CoV2. We demonstrated efficient binding of S309-CAR-NK cells to SARS-CoV2 wildtype pseudotyped virus as well as its variants of concern (VOCs), including the B.1.617.2 (Delta), B.1.621 (Mu), and B.1.1.529 (Omicron). We also showed the ability of S309-CAR-NK cells to neutralize different variants of SARS- CoV2 in addition to effectively killing target cells expressing spike mutations derived from SARS-CoV2 variants *in vitro*. To investigate the efficacy and safety of S309-CAR-NK *in vivo*, we challenged NOD scid gamma (NSG) mice expressing humanized angiotensin converting enzyme 2 (ACE2) receptor with SARS-CoV2 wildtype (strain USA/WA1/2020), followed treatments with S309-CAR-NK cells. Consistently, S309-CAR-NK cell-treated group exhibited decreased SARS-CoV2 viral loads compared to that of the untreated group. Our data collectively suggest the potential use of S309-CAR-NK cells for thetreatment of long COVID-19 patients, especially those who are unresponsive to currently available therapeutics due to VOCs.

Workshop #17:

W17-1:

Targeting Ferroptosis in Cancer Therapy

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Our lab has a long-standing interest in understanding nutrient signaling and metabolic stress response in both normal and cancer cells. Our recent work has studied the regulatory mechanisms of ferroptosis, a non- apoptotic cell death induced by lipid peroxidation resulting from metabolic imbalances, and its role in cellular metabolism, tumor suppression, and cancer therapy. In this presentation I will discuss the cross-talks between ferroptosis and cellular metabolism and therapeutic strategies to target ferroptosis as a vulnerability in cancer. Our studies link the tumor suppressor BAP1 to metabolic stress response and ferroptosis regulation. In addition, we revealed a critical role of ferroptosis in radiotherapy-induced cell death and tumor suppression and suggest to combine radiotherapy and ferroptosis inducers in cancer treatment. Finally, we recently uncovered a DHODH- mediated ferroptosis defense mechanism in mitochondria and identified DHODH inhibitors as ferroptosis inducers in cancer therapy. Together, our findings suggest that ferroptosis is an important tumor suppression mechanism and provide a broad framework for further understanding and targeting ferroptosis in cancer therapy.

W17-2:

Excess glucose reduces optimal mitochondrial function by reducing membrane fluidity

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TXNIP is an alpha arrestin protein that facilitates endocytosis of glucose transporters 1-4 via the clathrin- coated pathway. It also serves as the signaling hub where the cell controls acute glucose uptake. Both AMPK and Akt phosphorylate TXNIP on S308, leading to the dissociation of TXNIP from glucose transporters, ending their endocytosis and resulting in glucose influx. Cancer cells heavily utilize glucose as building blocks as well as an energy source. However, cancer cells often do not completely metabolize glucose to CO_2. Since mitochondrial defects are reported in patients with metabolic syndrome and diabetes, we wondered whether excess glucose detrimentally affects mitochondria in general, not just in cancer cells. Using TXNIP knockout animal as a model, we investigated excess glucose effect on mitochondria in brown adipose tissue during thermogenic response. We found that increased *in vivo* cellular glucose uptake results in increased amounts of short and saturated acyl-chains not only in storage lipids, but also in structural lipids. This includes the mitochondrial membranes which requires long polysaturated lipids to maintain their curvature and fluidity needed for their protein super complexes to function optimally. With rigidified membranes, TXNIP KO brown adipose tissue cannot generate sufficient heat during acute cold stress. This demonstrates a previously unidentified way glucose affects cellular function.

W17-3:

The context-dependent role of the TCA cycle in MYC-driven tumor development

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Despite its importance in normal cell function, the TCA cycle has emerged as a viable pathway for therapeutic intervention in certain types of cancer with minimal toxicity. MYC-driven cancers are addicted to glutamine, which can feed into the TCA cycle in the form of alpha- ketoglutarate. In this talk, I will discuss our recent findings on how a TCA cycle transferase functions differently to promote the development of cancers and its therapeutic implications.

W17-4:

Sustaining antitumor response of CD8+ T cells by distinct metabolic orchestration

Siqi Chen, Jie Fan, Ping Xie, Jihae Ahn, Michelle Fernandez, and **Bin Zhang**

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The $CD8^+$ T cells within tumor microenvironment are the most essential component in tumor immunity. Activation of $CD8^+$ T cells is coupled to various metabolic reprogrammings[1]. Glutathione (GSH) as the key role in oxidative metabolism to buffer increased reactive oxygen species (ROS) in activated T cells, is important for T cells effector functions in the context of inflammation[2,3]. The function and mechanism by GSH metabolism in regulating anti-tumor immunity of $CD8^+$ T cells remain unknown. Here, we show that GSH is dependent on glutathione peroxidase 4 (Gpx4) to maintain CD8+ T cell activity, and A2AR signaling pathway interacts with the GSH-Gpx4 axis to reprogram the metabolism and survival of functional $CD8^+$ T cells. Interestingly, A2AR signaling blockade increases effector function of antitumor $CD8^+$ T cells, but facilitates the assumption of intracellular GSH, leading to rapid ferroptosis. Notably, combination treatment with a potent ferroptosis inhibitor liproxstatin-1 (Lip-1) and A2AR antagonists elicits a synergistic antitumor efficacy and enhanced mitochondrial functionality of antitumor $CD8^+$ T cells in multiple mouse tumor models. Finally, we generate a gene expression signature for GSH metabolism in tumor-infiltrating CD8+ T cells positively correlating with favorable clinical outcomes. Our work demonstrate a critical role of GSH metabolism in modulating antitumor $CD8^+$ T cell survival and functionality, pointing to new strategies of targeting these cells for cancer immunotherapy.

W17-5:

Repurposing phenformin for the treatment of cancer

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Phenformin is a drug in the biguanide class that was used to treat type 2 diabetes, but withdrawn in the late 1970s in the United States due to the risk of lactic acidosis and replaced by metformin. Both phenformin and metformin inhibit mitochondrial complex I of the respiratory chain and increase the AMP to ATP ratio, resulting in AMP-activated protein kinase (AMPK) activation. Findings from retrospective population- based studies on metformin and preclinical studies using cultured cancer cells and mouse models have demonstrated that metformin and phenformin biguanides possess the antitumor activity and might

be repurposed for cancer prevention and treatment. However, no significant clinical benefit has yet been demonstrated for metformin monotherapy in various clinical trials in non-diabetic patients with multiple cancer types. Recent animal model studies from our laboratory have demonstrated that, compared to metformin, phenformin possesses more potent antitumor activity, due to its lipophilic nature that reduces its dependence on active transport for cellular uptake. We previously characterized a close cross-talk between AMPK and BRAF-MEK-ERK signaling pathways in melanoma. Importantly, we have demonstrated that phenformin enhances the antitumor activity of BRAF inhibitors (BRAFi) in cultured BRAF mutant melanoma cells and mouse models. These preclinical findings have led to an ongoing phase I clinical trial of phenformin with the dabrafenib/trametinib combination in patients with BRAF-mutated melanoma (NCT03026517), which represents the first clinical trial of phenformin in cancer. More recently, we have investigated the effects of phenformin on the tumor microenvironment. We demonstrated that phenformin selectively reduces the frequency and immunosuppressive function of myeloid-derived suppressor cells (MDSCs) and co-treatment with phenformin enhances the anti-tumor effect of anti-PD-1 in a melanoma mouse model. A clinical trial of phenformin and anti-PD-1 combination in anti-PD-1-refractory melanoma patients with BRAF mutations is warranted.

Workshop #18:

W18-1:

High-Throughput Synthetic Antibody Discovery in Metabolic Diseases

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Therapeutic monoclonal antibodies tend to be well-validated, while reagent antibodies typically are not tested to the same degree, leading to significant issues with quality, performance and reproducibility. There is a strong and increasing need to develop high-throughput technologies and platforms to speed antibody development expressly for the biomedical research community. IPI is a nonprofit research institute, dedicated to creating and distributing high quality, well characterized antibodies to accelerate research. Specifically, we have developed an antibody discovery platform that generates synthetic, recombinant antibodies against extracellular or secreted proteins. The antibody discovery is based on yeast display technology, circumventing hurdles encountered by traditional animal immunization
approaches. These often fail to produce antibodies against targets that are highly conserved across species. Our yeast display library was built from seven dominant human IgG heavy chain subfamilies and four human IgG light chains. Our library has variations in the CDR3 region of the heavy chains and achieves overall library diversity on par with a human B cell repertoire. We concurrently developed methods to recombinantly produce native human antigens and use them in a highly selective sorting protocol to identify strong binding candidates. We deployed next generation sequence analysis to further refine candidate selection for ultimate antibody production. Finally, we devised a series of high-throughput assays, using biophysical characterization and cell display technology to test antibody binding properties and poly specificity reactivity. The goal is to provide information about antibody fitness in broad applications in vivo, ex vivo and in vitro. We report the successful identification and characterization of high affinity binders for multiple targets in the immunoglobulin superfamily, including those critical for axon guidance. These confirm the feasibility of our approach and high-throughput platform to meet the growing demands of the biomedical research community.

W18-2:

Lineage tracing of origin and fate of beige adipocytes in adult mice

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Beige adipocytes hold therapeutic promise for obese and diabetic patients; however, enthusiasm for such a therapy is diminished because beige adipocytes are short-lived and rapidly convert to “white-like” adipocytes after discontinuing stimuli. Thus, there is a clear basic and clinical need to understand the origin and fate of beige adipocytes and their regulatory mechanisms. Remarkably, we have identified a subgroup of UCP1+ cells within white adipose tissue which are long-lived and display progenitor-like characteristics in response to β3-agonist. Herein, using lineage tracing and genetic mouse models, we demonstrated that a unique class of UCP1+ cells within white adipose tissue express proliferation markers and differentiate into new beige adipocytes in response to β3-agonist. When these cells were depleted or their cell division capability was blocked, β3-induced beige adipocyte formation was impaired; in contrast, when cell cycle inhibitor was removed in these cells, the mice generated more beige adipocytes. Furthermore, these newly generated beige adipocytes retained their life span with improved metabolic health even after removing the stimuli. Collectively, our findings provide new insights into the underlying mechanisms of beige adipocyte regulation and potentially therapeutic opportunities to increase the life span of beige adipocytes as a sustainable long-term therapy for the obese community.

W18-3:

Connexin43 in the adipose tissue

Yi Zhu[1]

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Obesity is a health risk for many diseases and is manifested by excess adipose tissue. Gap junctions are cellular structures that connect two cells and allow cellular exchanges of small hydrophilic molecules. In adipocytes, Connexin43 (Cx43) is the most abundant isoform in the protein family forming gap junctions. During adipocyte development, Cx43 expression rises upon forming cell-cell contacts and dwindles at the late stage of droplet formation. Our mouse study shows a dispensable role of Cx43 in mature adipocytes. However, when adipocytes undergo drastic morphological and functional remolding, for example, during cold-induced adipose tissue beiging, or lactation, Cx43 is upregulated to facilitate efficient signal dissemination. Breast cancer cells can also hijack the exact mechanism to promote lipolysis of adjacent adipocytes, probably using their lipids to meet the energy needs or for biosynthesis.
Many pharmacological agents have been developed to target adipose tissue to treat obesity. However, they frequently fail to achieve desired efficacy due to limited target engagement stemming from insufficient vascularization of the adipose tissue and extra-large adipocytes in obese subjects. We hypothesize that activating gap junctions can enhance the efficacy of those adipose-tissue-targeting pharmacological agents. Using an adipocyte-specific Cx43 overexpression mouse model, we demonstrated that connecting adipocytes via overexpressing Cx43 gap junctions enhanced the efficacy of Mirabegron and FGF21. We further showed that pharmacologically activating the Cx43 gap junction channels also enhanced the efficacy of Mirabegron and FGF21. And thus, Cx43 gap junction activators hold great potential to potentiate adipose-tissue targeting agents by connecting and priming adipocytes.

W18-4:

Mitokines-mediated adipose tissue crosstalk

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One third of American adults are suffering from obesity, which significantly contributes to the prevalence of many other life-

threatening diseases, such as type 2 diabetes, cardiovascular diseases, cancers, etc. Our lab has recently identified that overexpression of amyloid precursor protein (APP) in white fat and its subsequent mistargeting into mitochondria induces dramatic mitochondrial dysfunction, thereby promoting obesity and insulin resistance. Preliminary data obtained from this unique APP-induced mitochondrial distress model show that mitochondrial distress in white fat induces a "whitening" phenotype in brown fat and reduced heat production. After a series of screening, lipocalin-2 (LCN-2) is suggested to be a potential factor that drives the white to brown AT crosstalk to induce "whitening". We further hypothesized that lipocalin 2, a mitokine from the white AT, is induced by mitochondrial distress and promotes brown AT whitening. LCN-2 is observed to be significantly upregulated in the white adipose tissue upon high-fat diet feeding, and it is dramatically downregulated in humans post bariatric surgeries. Through in vitro and in vivo application, we found that recombinant LCN-2 protein is sufficient to induce whitening of brown fat and brown adipocytes. Upon LCN-2 treatment, mice were cold intolerant, and brown adipose tissues showed reduced uncoupling protein-1 (UCP-1) expression. We are currently deleting Lcn2 specifically in adipocytes to determine whether adipocyte-derived LCN-2 is required for the white to brown fat crosstalk and for brown fat whitening. Combined, our current study shed lights on novel mechanisms underlying brown adipose tissue whitening and novel mitokine targets that ameliorate brown AT whitening and improve obesity.

W18-5:

Adipose Tissue Specific Ces1d Mediates Whole-Body Metabolic Homeostasis

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Carboxylesterase 1d (Ces1d) is a crucial enzyme with a wide range of activities in multiple tissues in the body. It has been reported to localize predominantly in endoplasmic reticulum (ER) in the non-adipose tissue. Here, we discovered that Ces1d is also enriched in adipose tissue and its levels are significantly increased in obese patients with Type 2 diabetes. Intriguingly, a high level of Ces1d translocalizes onto lipid droplets where it digests the lipids to produce a unique set of fatty acids in adipose tissue. To investigate the metabolic consequences of loss of Ces1d, we generated an adipose tissue-specific Ces1d knockout mouse model (FKO). We found that FKO mice gained more body weight with increased fat mass during a high fat-diet (HFD) challenge. Furthermore, the FKO mice exhibited impaired glucose and lipid metabolism and developed exacerbated liver steatosis. Mechanistically, deficiency of Ces1d induced abnormally large lipid droplet deposition in the adipocytes, causing ectopic accumulation of triglycerides (TG) in other peripheral tissues, especially in the liver. Furthermore, loss of Ces1d diminished the circulating free fatty acids (FFAs) serving as signaling molecules to trigger the epigenetic regulations of energy metabolism via lipid-sensing transcriptional factors, such as HNF4α. The metabolic disorders induced an unhealthy microenvironment in the adipose tissue and liver of the mice, ultimately leading to systemic insulin resistance. In conclusion, our findings unveil a previously underappreciated lipolytic signaling pathway mediated by Ces1d which is essential for the systemic metabolic homeostasis.

W18-6:

Deficiency of lin28 induces senescence of activated hepatic stellate cells and limits liver fibrosis during alcohol-induced liver injury

Elise Slevin[1,2], Ying Wan[3], Sachiko Koyama[1,2], Wenjuan Xu[1,2], Xuedong Li[3], Tian Li[3], Yudian Zhang[3], Chiung-Kuei Huang[1,2] and **Fanyin Meng**[1,2]

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Background: Cellular senescence is a stress-responsive program limiting the proliferation of damaged cells and leading to stable cell-cycle arrest. Recent discoveries demonstrated that senescence program limits the fibrogenic response to acute tissue damage. The current study aimed to characterize the effect of lin28 regulated senescence of hepatic stellate cells on liver fibrosis during alcohol-induced liver injury. **Methods:** Senescence related gene expression was assessed using a Cellular Senescence PCR Array and/or real-time PCR analysis in LPS and TGF-β-treated normal human hepatic stellate cells (HHSCs), as well as in liver specimen from mice fed alcohol for 5 weeks relative to control liver tissue. HSCs were also isolated from control and TLR-4 knockout mice liver with ethanol feeding by laser capture microdissection (LCM). HSC senescence, proliferation, activation and transdifferentiation was evaluated by SA-β-gal Activity and MTS assay, Western blot and real-time PCR analysis through specific markers such as PAI-1, EGR1, α-SMA, collagen-A1 and MMPs. **Results:** We identified that 5 week of ethanol feeding significantly increased total liver histopathology score and hepatocellular senescence by PCR array and SA-β-gal assay. However, cellular senescence activity was significantly reduced in LCM isolated HSCs from ethanol feeding mice liver relative to controls. The down-regulation of hepatic senescence initiators in isolated HSCs, PAI-1 and EGR1, were further verified by real-time PCR assay. Treatment of HHSCs with LPS and TGF-β (20 ng/ml) for 72 hr significantly decreased PAI-1 and EGR1 expression, along with the reduced SA-β-gal activity and enhanced cellular proliferation detected by MTS assay. Overexpression of PAI-1 and EGR1 recovered LPS and TGF-β-reduced senescence and further decreased viability in HHSCs. Interestingly, overexpression of PAI-1 and EGR1 also reduced the pro-fibrotic markers, α-SMA, collagen-A1 and MMP-9 in HHSCs, whereas silencing of LPS receptor TLR4 decreased these markers in the same group of HHSCs. Furthermore, the expression of TLR4 and the verified LPS related senescence markers, including E2F1, ID1 and IGFBP3 were significantly altered in HSCs isolated by LCM from ethanol-fed mouse liver specimens compared to controls. Lin28 knockout mice displayed less sensitivity to alcoholic injury, and enhanced cellular senescence activity in LCM isolated HSCs along with the upregulated PAI-1 and EGR1 levels and reduced expressions of α-SMA, collagen-A1 and MMP-9. **Conclusion:** Our discoveries demonstrated that knockout lin28 induced senescent activated stellate cells that exhibit gene expression profile consistent with cell-cycle exit and reduced fibrogenic activity. The findings provide new insight into the therapeutic potentials of HSC senescence in alcohol induced liver injury and fibrosis.

Workshop #19:

W19-1:

Deciphering the mechanism of processive ssDNA digestion by the Dna2-RPA ensemble

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Single-stranded DNA (ssDNA) widely exists as the intermediates in various DNA metabolic pathways. Its integrity is guarded by single stranded DNA binding proteins, known as Replication Protein A (RPA) in eukaryotes. Besides ssDNA protection through its high-affinity binding, RPA also physically interacts with and directs the downstream factor that signals or repairs the ssDNA intermediate. However, it remains unclear how these enzymes/factors out-compete RPA and access to ssDNA. As one of the RPA interactors, Dna2 nuclease plays a unique role in eliminating RPA-bound ssDNA, an activity important for both lagging strand maturation and DNA double strand break (DSB) repair. Using Dna2 and RPA from the budding yeast, *Saccharomyces cerevisiae*, as a model system, we discovered that the bimodal interface between Dna2 and RPA allows Dna2 to act with RPA both *in cis* and *in trans*. The *cis*-action renders RPA a processive unit for Dna2-catalyzed ssDNA digestion, where RPA, instead of acting passively, delivers its bound ssDNA directly to Dna2. The *trans*-action mediated by an acidic patch at the amino-terminus of Dna2, on the other hand, is critical for Dna2 to maintain its full activity when the amount of RPA is sub-optimal or DNA secondary structures are encountered. Interestingly, inactivating the *trans*-acting element, while has no impact on cell viability, causes a major defect in DSB repair presumably due to the failure of resolving a critical recombination intermediate.

W19-2:

DNA capture and loop extrusion dynamics by cohesin-NIPBL at the single-molecule level

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3D chromatin organization plays a critical role in regulating gene expression, DNA replication, recombination, and repair. While initially discovered for its role in sister chromatid cohesion, emerging evidence suggests that the cohesin complex (SMC1, SMC3, RAD21, and SA1/SA2), facilitated by NIPBL, mediates topologically associating domains and chromatin loops through DNA loop extrusion. However, information on how conformational changes of cohesin-NIPBL drive its loading onto DNA, initiation, and growth of DNA loops is still lacking. Here, high-speed AFM (HS-AFM) imaging reveals that cohesin-NIPBL captures DNA through arm extension, followed by transfer of DNA to its globular domain and DNA loop initiation independent of ATPase hydrolysis. AFM imaging unexpectedly demonstrates additional shorter protrusions (feet) from cohesin-NIPBL that transiently bind to DNA facilitating DNA loading and distinct forward and reverse DNA loop extrusion steps by cohesin-NIPBL. These results provide critical missing links in our understanding of DNA binding and loop extrusion by cohesin-NIPBL.

W19-3:

The role of histidine phosphorylation in DNA alkylation damage repair

Yihan Peng and **Huadong Pei**

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Repair of alkylation damage to the genome is critical, because such damage is cytotoxic and potentially mutagenic. The oxidative demethylase ALKBH3 is a central component of a DNA alkylation damage repair (DAR) pathway. Along with the activating signal co-integrator complex (ASCC), ALKBH3 demethylates adenine methylated on the N1 position (1meA) and cytosine methylated on the N3 position (3meC) in single-strand DNA to maintain genomic integrity. We found that ASCC3, a subunit of ASCC, is phosphorylated at two histidine residues by the histidine kinase NME1/2. NME1/2-mediated phosphorylation of ASCC3 stimulates its activity through relief of an autoinhibitory function inherent to its amino terminus, which promoted ALKBH3 recruitment to DNA alkylation damage sites and DAR. Knock out of NME2 in neuroendocrine prostate cancer (NEPC) cells resulted in 3meC and 1meA accumulation in DNA, which sensitizes cancer cells to DNA-alkylating agents. Thus, histidine phosphorylation is a key regulator of DAR.

W19-4:

Understanding R-loop and mRNA dependent repair pathway in cancer therapy

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The repair of DNA double-stranded breaks (DSB) is crucial for genomic stability in both normal and cancer cells.In cancer cells under intrinsic oxidative stress or facing extrinsic DNA damage induced by radiation orchemotherapy, efficient DSB repair is essential for cell survival. Therefore, understanding the mechanisms of DSB repair is critical for exploiting the genomic vulnerabilities of cancer cells. Recent studies by us and others revealed that the DSB in transcribed regions of the genome are repaired through a novel mRNA-regulated mechanism. This mechanism uses DNA: RNA hybrids(or R-loops) to recruit DNA repair proteins to DSB, allowing efficient removal of DSB in active genes. We have identified a number of repair proteins that function in this repair pathway, including CSB, RAD52, and RAD51. Excitingly, our recent studies revealed that 5-methylcytosine (m5C), a specific modification of RNA, is induced in the DNA: RNA hybrids at sites of DSB, providing a unique “DNA damage code” to promote the recruitment ofrepair proteins. Furthermore, we found that the RNA methyltransferase TRDMT1 is the “writer” of m5C at DSB. Loss of TRDMT1 compromises the recruitment of RAD51 to DSB, impairs DSB repair, and renders cells sensitiveto radiation, PARP inhibitor, and cisplatin. Our study demonstrates that TRDMT-mediated m5C formation is a key regulator of the mRNA and R-loop dependent repair pathway for DSB repair in transcribed regions of the genome. We will discuss how to target this pathwayto selectively kill BRCA1/2-proficient and -deficient cancer cells as new therapeutic strategy to exploit the genomic instability and DNA repair defects of cancer cells.

W19-5:

TARGETING DNA DAMAGE RESPONSIVE PATHWAYS FOR CANCER THERAPY

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DNA double strand breaks (DSBs) result in activation of several key DNA damage response (DDR) kinases including ATM, ATR, and DNA-PK. These protein kinases not only promote DNA damage-induced checkpoint control, but also facilitate DSB repair in humans. Thus, these DDR kinases have become promising drug targets for cancer therapy. However, the benefits of targeting DDR kinases remain to be realized, in part due to the lack of predictive biomarkers. By undertaking CRISPR screens with inhibitors targeting key DDR kinases, we obtained a global and unbiased view of genetic interactions with DDR inhibition. Additionally, we compared the synergistic effects of combining different DDR inhibitors and found that an ATM inhibitor plus a PARP inhibitor induced dramatic levels of cell death, probably through promoting apoptosis. Our results provide a better understanding of DDR pathways, which may facilitate the use of these DDR-targeting agents in cancer therapy. Moreover, we also performed CRISPR screens to investigate DNA damage signaling as well as cellular response to PARPi in BRCA1 or BRCA2 deficient cells. These studies together reveal the interplays between DNA repair and cellular signaling pathways, which hopefully will improve therapeutic outcome for cancer patients.

W19-6:

RNA-dependent Homologous Recombination Repair DNA Double Strand Breaks

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Deficiency in DNA repair system leads to genomic instability, a hallmark of cancer. Researchers have been long interested in understanding the role of transcription in homologous recombination (HR) repair of DNA double-strand breaks (DSBs) but have been hindered by the lack of proper tools. Here we present our recent discovery of a novel RNA-dependent HR repair of DNA double-strand breaks. Using our newly established Tet-inducible HR repair reporter system, in which the local transcription can be tightly controlled, we find that local transcription significantly stimulated HR repair. By tethering RNA molecule to the close proximity of DSBs using nuclease-dead Cas9 when local transcription is off, we findthat the HR repair stimulated by transcription can be recapitulated by tethering RNA molecules to the vicinity of DSBs, suggesting transcription enhances HR through local RNA transcripts. Importantly, we have found that, the DNA repair factor RAD51AP1 is critical for the HR stimulation by local transcription or tethered RNAs, and is capable to catalyze the formation of DNA:RNA hybrid (R-loop) *in vitro*. Using DNA-RNA immunoprecipitation (DRIP) assay, we show that RAD51AP1 is necessary for the DSB- induced R-loop formation on donor DNA, suggesting the co-existence of R-loops and D-loops. Indeed, by *in vitro* reconstitution, we show that RAD51AP1-generated R-loops enhance the RAD51-mediated formation of D-loops locally and give rise to the intermediate molecule DR-loops, which contain both DNA:DNA and DNA:RNA hybrids. Thus, at DSBs in transcribed regions, RAD51AP1 promotes the invasion of RNA transcripts into template sister chromatid DNA to form R-loops, facilitates RAD51- mediated D-loop formation and/or subsequent steps of HR repair, results in increased HR efficiency in thetranscribed genome. This novel RNA-dependent DNA repair mechanism highlights the pivotal roles of transcription/RNA and the repair protein RAD51AP1, in protecting the transcribed genome.

Workshop #20:

W20-1:

gamma-Secretase Modulation: from Hypoxia to Neuroinflammation

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γ-Secretase is a macromolecular complex composed of four obligatory subunits: nicastrin (NCT), presenilin 1 (PS1) or presenilin 2 (PS2), Pen-2, and Aph-1. This protease is responsible for the final step of amyloid precursor protein (APP) processing to generate amyloid (Aβ) peptides, which have been proposed to play a causative role in AD, named the Aβ hypothesis. Mutations in PS1/PS2, and APP cause early onset AD (EOAD) and affect the production of Aβ, highlighting an essential role of γ-secretase in AD. γ-Secretase also cleaves Notch and other more than 100 type I transmembrane substrates. Despite its seemingly promiscuous enzymatic capacity, γ-secretase activity is tightly regulated. This regulation is a function of many cellular entities, including but not limited to the essential γ-secretase subunits, nonessential (modulatory) subunits, and γ-secretase substrates. We will discuss how γ-secretase is regulated by modulatory proteins under hypoxia and neuroinflammation. A better understanding of these mechanisms will aid in the development of effective therapeutics for γ-secretase-associated diseases like Alzheimer's disease and Notch-addicted cancer.

W20-2:

Pathogenic α-synuclein cell-to-cell transmission mechanism and related therapeutic development

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Emerging evidence indicates that pathogenesis of α-synucleinopathies may be due to cell-to-cell transmission of prion-like preformed fibrils (PFF) of α-syn. We identified Lymphocyte-activation gene-3 (Lag3) that exhibits the highest binding affinity with α-syn fibrils, and α-syn fibrils binding to Lag3 initiated pathogenic α-syn endocytosis, propagation, transmission, and toxicity. Furthermore, amyloid β precursor-like protein 1 (Aplp1) forms a complex with Lag3 that facilitates the binding, internalization, transmission, and toxicity of pathologic α-syn. Deletion of both Aplp1 and Lag3 eliminates the loss of dopaminergic neurons and the accompanying behavioral deficits induced by α-syn PFF. Anti-Lag3 prevents the internalization of α-syn PFF by disrupting the interaction of Aplp1 and Lag3, and blocks the neurodegeneration induced by α-syn PFF in vivo. The identification of Aplp1 and the interplay with Lag3 for α-syn PFF induced pathology advances our understanding of the molecular mechanism of cell-to-cell transmission of pathologic α-syn and provides additional targets for therapeutic strategies aimed at preventing neurodegeneration in Parkinson's disease and related α-synucleinopathies.

W20-3:

Abnormal triaging of misfolded proteins by adult neuronal ceroid lipofuscinosis-associated DNAJC5/CSPα mutants causes lipofuscin accumulation

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Mutations in *DNAJC5/CSPα* are associated with adult neuronal ceroid lipofuscinosis (ANCL), a dominant-inherited neurodegenerative disease featuring lysosome-derived autofluorescent storage materials (AFSMs) termed lipofuscin. Functionally, DNAJC5 has been implicated in chaperoning synaptic proteins and in misfolding-associated protein secretion (MAPS), but how DNAJC5 dysfunction causes lipofuscinosis and neurodegeneration is unclear. Here we report two functionally distinct but coupled chaperoning activities of DNAJC5, which jointly regulate lysosomal homeostasis: While endolysosome-associated DNAJC5 promotes ESCRT-dependent microautophagy, a fraction of perinuclear and non-lysosomal DNAJC5 mediates MAPS. Functional proteomics identifies a previously unknown DNAJC5 interactor SLC3A2/CD98hc that is essential for the perinuclear DNAJC5 localization and MAPS but dispensable for microautophagy. Importantly, uncoupling these two processes, as seen in cells lacking SLC3A2 or expressing ANCL-associated DNAJC5 mutants, generates DNAJC5-containing AFSMs resembling NCL patient-derived lipofuscin and induces neurodegeneration in a *Drosophila* ANCL model. These findings suggest that MAPS safeguards microautophagy to avoid DNAJC5-associated lipofuscinosis and neurodegeneration.

W20-4:

Proteomic landscape of Alzheimer's Disease: novel insights into pathogenesis

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Mass spectrometry-based proteomics empowers deep profiling of proteome and protein posttranslational modifications (PTMs) in Alzheimer's disease (AD). Here we present the advances and limitations in historic and recent AD proteomic research. Complementary to genetic mapping, proteomic studies not only validate canonical amyloid and tau pathways, but also uncover novel components in broad protein networks, such as RNA splicing, development, immunity, membrane transport, lipid metabolism, synaptic function, and mitochondrial activity. Meta-analysis of seven deep datasets reveals 2,698 differentially expressed (DE) proteins in the landscape of AD brain proteome (n = 12,017 proteins/genes), covering 35 reported AD genes and risk loci. The DE proteins contain cellular markers enriched in neurons, microglia, astrocytes, oligodendrocytes, and epithelial cells, supporting the involvement of diverse cell types in AD pathology. We discuss the hypothesized protective or detrimental roles of selected DE proteins, emphasizing top proteins in "amyloidome" (all biomolecules in amyloid plaques) and disease progression. Based on the identified aggregated proteome in AD, we introduce a novel disease model of dysfunctional U1 snRNP-mediated RNA splicing. Thus, proteomics-driven systems biology presents a new frontier to link genotype, proteotype, and phenotype, accelerating the development of improved AD models and treatment strategies.

W20-5:
In Situ Cell-Surface Proteomics: Method Development and Applications in Neurobiology

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Cell-surface molecules are direct executors for inter-cellular communications and thus regulate almost every aspect of the development and physiology of multicellular systems. We developed a method for quantitatively profiling cell-surface proteomes in native tissues with cell-type and spatiotemporal specificities. Applying this method to developing brains uncovers many novel molecules controlling neural circuit assembly, as well as their operating mechanisms. Here, I will share our discoveries on: 1) how unconventional wiring molecules control dendrite targeting; 2) how a lineage-defining transcription factor specifies a combinatorial cell-surface code for fly olfactory circuit assembly; and 3) how an endocytosis- associated cell-surface protein regulates mouse cerebellar Purkinje cell dendrite morphogenesis.

W20-6:
G3BP1 modulates SPOP to promote prostate tumorigenesis

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Speckle-type POZ protein (SPOP), a Cullin 3 based ubiquitin ligase ($CUL3^{SPOP}$), acts as a prostate-specific tumor suppressor. Loss-of-function mutations in SPOP occurs in 10% of primary prostate cancer with a high Gleason grade and poor prognosis. However, it is unclear how the ubiquitin ligase activity of SPOP is controlled, and how dysregulation of SPOP contributes to malignant transformation. Here, we identified GTPase Activating Protein (SH3 Domain) Binding Protein 1 (G3BP1) as an interactor and upstream regulator of $CUL3^{SPOP}$ and it functions as inhibitor of $CUL3^{SPOP}$ ubiquitin ligase. Transcriptomic analysis and functional studies reveal a G3BP1-SPOP ubiquitin signaling axis that promotes PCa progression through activating AR signaling. Moreover, AR directly upregulates G3BP1 transcription to further amplify G3BP1-SPOP signaling in a feed-forward manner. Our study supports a fundamental role of G3BP1 in disabling the tumor suppressive $Cul3^{SPOP}$, thus defining a PCa cohort independent of SPOP mutation, and suggesting a distinctive mode of $CUL3^{SPOP}$ inactivation that aggravates prostate cancer.

Workshop #21:

W21-1:

Memory-Like NK Cells Armed with Neoepitope-Specific CAR for Treating Acute Myeloid Leukemia

Jianzhu Chen[1], Han Dong[2], Guozhu Xie[1], James Ham[1], Guangan Hu[1], Jerome Ritz[2], and Laurie Glimcher[2], Rizwan Romee[2]

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Acute myeloid leukemia (AML) remains a therapeutic challenge, and a paucity of tumor specific targets has significantly hampered the development of effective immune based therapies. Recent paradigmchanging studies have shown that NK cells exhibit innate memory upon brief activation with IL-12 and IL- 18, leading to cytokine-induced memory-like (CIML) NK cell differentiation. CIML NK cells have enhanced anti-tumor activity and have shown promising results in early phase clinical trials in patients with relapsed/refractory AML. Here we show that arming CIML NK cells with a neoepitope specific chimeric antigen receptor (CAR) significantly enhances their anti-tumor responses to nucleophosphmin-1 (NPM1) mutated AML while avoiding off target toxicity. CIML NK cells differentiated from peripheral blood NK cells were efficiently transduced to express a TCR-like CAR that specifically recognizes a neoepitope derived from the cytosolic oncogenic NPM1 mutated protein presented by HLA-A2. These CAR CIML NK cells displayed enhanced activity against NPM1-mutated AML cell lines and patient-derived leukemic blast cells. CAR CIML NK cells persisted *in vivo* and significantly improved AML outcomes in xenograft models. Single-cell RNA-sequencing and mass cytometry analyses identified upregulation of cell proliferation, protein folding, immune responses and major metabolic pathways in CAR transduced CIMLNK cells, resulting in tumor-specific, CAR-dependent activation and function in response to AML target cells. Thus, efficient arming of CIML NK cells with an NPM1-mutation specific TCR-like CAR substantially improves their innate anti-tumor responses against an otherwise intracellular mutant protein. These pre-clinical findings justify evaluating this approach in clinical trials in HLA-A2$^+$ AML patients with NPM1c mutations

W21-2:

Reprogramming lipid metabolism to restore tumor-specific CD8$^+$ T cellfunction in tumor microenvironment

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Tumor-infiltrating T cells often lose their effector function; however, the mechanisms are incompletely understood. We previously showed that cholesterol in the tumor microenvironment (TME) induces CD8$^+$ T-cell expression of immune checkpoints and exhaustion. We found that tumor tissues enriched with cholesterol and cholesterol content in tumor-infiltrating CD8$^+$ T cellswas positively and progressively associated with upregulated T-cell expression of PD-1, 2B4, TIM-3, and LAG-3. Adoptively transferred CD8$^+$ T cells acquired cholesterol, expressed high levels of immune checkpoints, and became exhausted upon entering tumor. Tumor-culture supernatant or cholesterol induced immune checkpoint expression by increasing endoplasmic reticulum (ER) stress in CD8$^+$ T cells. Consequently, the ER-stress sensor XBP1 was activated and regulated PD-1 and 2B4 transcription. Inhibiting XBP1 or reducing cholesterol in CD8$^+$ T cells effectively restored antitumor activity. We further observed that increased CD36 expressionin tumor-infiltrating CD8$^+$ T cells, which was induced by TME-cholesterol, was associated with tumor progression and poor survival in human and murine cancers. Genetic ablation of Cd36 in effector CD8$^+$ T cells exhibited increased cytotoxic cytokine production and enhanced tumor eradication. CD36 mediated uptake of fatty acid by tumor-infiltrating CD8$^+$ T cells in TME, induced lipid peroxidation and ferroptosis, and led to reduced cytotoxic cytokine production and impaired antitumor ability. Blocking CD36 or inhibiting ferroptosis in CD8$^+$ T cells effectively restored their antitumor activity and more importantly, possessed greater antitumor efficacy in combination with anti-PD-1 antibodies. Thus, these studies uncover novel mechanisms underlying T cell exhaustion and dysfunction in TME and suggest a new strategy for restoring T cell function by reducing cholesterol or fatty acids to enhance T cell-based immunotherapy in human cancer.

W21-3:

Harnessing immunotherapy against neurodegeneration to restore vision

Dong Feng Chen

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Glaucoma, characterized by progressive optic nerve damage and retinal ganglion cell (RGC) death, is a leading cause of irreversible blindness worldwide and the most frequent age-related neurodegenerative disease. However, the mechanisms underlying neurodegeneration and vision loss in glaucoma are not fully understood. Our recent studies identified the bacteria-heat shock protein (HSP) primed T cell-response and autoimmune mechanism underlying neural loss in glaucoma and ischemic optic neuropathy, so called “optic nerve stroke”. Patients with primary open angle glaucoma show increased incidence of T cell-related autoimmune diseases and higher frequency of HSP-specific Th1 cells. Moreover, mice deficient in T cell functions displayed an attenuated optic nerve and RGC loss in glaucomatous mice. Importantly, mice raised in the absence of commensal microflora (germ-free) did not develop HSP-specific T cell responses, nor glaucomatous neurodegeneration under elevated intraocular pressure. These studies provide compelling evidence suggesting an essential role of adoptive immunity that are pre-sensitized by commensal bacteria in glaucomatous neurodegeneration and suggest new therapeutic interventions for glaucoma and age-related neurodegeneration.

W21-4:

Low disturbed flow induces dynamic immune compartment change in atherogenesis revealed by scRNA-seq

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Low disturbed blood flow (LDF) is a critical contributing factor to atherogenesis, but its direct impact on the immune compartment was not well-depict. To fill this knowledge gap, we adopted scRNA-seq to capture shear-stress-induced immune responses during atherogenesis. A partial carotid artery ligation (PCAL) model was selected for its paired comparison of carotid arteries

with the normal flow (NF) or LDF. Indeed, we observed drastic changes in both endothelial and immune compartments. Macrophages were the most significantly increased population induced by LDF (from 1% to 4.5% of CD45+ cells) with two well-separated subsets (Mac-c8, Mac-c9). MacSpectrum analyses revealed that Mac-c8 displayed higher inflammatory states than the lipid-laden Mac-c9. Interestingly, three T subtypes displayed unique flow-induced enrichment patterns that were selectively enriched in LDF but not in the NF condition. Furthermore, we created an original algorithm to evaluate the impact of shear stress on membrane protein-mediated cell-cell interaction among all cell types in the atheroma. Several pairs of molecule interactions were identified, including multiple APP-ligands interaction pairs and those in BAG2 Signaling. Moreover, signature genes identified in these LDF-induced T cells displayed a high correlation to the plaque severity in human artery-aorta samples. Collectively, our study provided a high-resolution and focused analysis of shear-stress-induced immune cell action during atherogenesis. This is also the first identification of unique T subsets, to our knowledge, that is enriched in arterial walls exposed to low and disturbed flow. Further characterization of these cells will provide valuable information to understand and treat atherogenesis.

W21-5:
Development of Innovative Nanotechnologies to Enhance Cancer Immunotherapy
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Immunostimulatory radiotherapy is believed to promote antigen presentation and T cell priming, and when used in combination with checkpoint blockade, can reactivate and sustain systemic anti-tumor immunity. In this talk, I will present recent breakthroughs in using nanotechnology to enhance radiotherapy for *in situ* cancer vaccination. These treatments are further combined with immune checkpoint inhibitors to promote immunostimulatory tumor microenvironments and potentiate cancer immunotherapy. I will also discuss the design of other immunostimulatory nanomedicines for synergistic combination with immune checkpoint blockade to treat immunologically cold tumors. Clinical translationof these innovative nanotechnologies will also be discussed.

Workshop #22:

W22-1:

The dynamic, motile and deformative properties of RNA nanoparticles lead to efficient tumor vasculature targeting, fast renal excretion and rapid body clearance.

Peixuan Guo

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A large number of noncoding RNAs have key roles in the regulation of cellular functions. The dynamic nature of RNA leads to its motion and deformation behavior. These conformational transitions, such as breathing within the complementary area, pseudoknot formation at the 2D level; induced fitting by substrate interaction, conformational capture by key and lock, and shifting in base pairing all are important for their biological functions including tissue binding, cell entry, gene regulation, and protein translation. Their dynamics, catalytic and motile features have led to the belief that RNA is the origin of life. We recently reported that the rubbery, amoeba and shape-shifting properties of RNA nanoparticles enhance their penetration through leaky blood capillary, leading to efficient accumulation in tumor vasculature. These dynamic, motile, and deformable properties of the RNA nanoparticles also enable the RNA nanoparticles to pass through the glomerulus, overcoming the filtration size limitation, resulting in rapid renal excretion and fast body clearance, therefore low or no toxicity. The performance of RNA nanoparticles can be further improved by incorporating cancer-targeting ligands. The inherent negative properties of RNA charge reduce nonspecific cell binding and organ retention due to the repulsion with the cell membrane which is also negatively charged. The multivalent nature of RNA nanoparticles allows for multi functionalities, which can be applied as an approach to overcome drug resistance. In addition to favorable biodistribution characteristics, RNA nanoparticles have other unique properties including self-assembly, programmable synthesis, advantageous size; antigenicity free, large volume distribution, CMC ease, solubilizing drugs, and high payload. All of these make them an excellent material for pharmaceutical applications. RNA drug has become the third milestone in pharmaceutical drug development!

W22-2:

Rational design for delivery and controlled release of siRNA from RNA nanoparticles via tumor targeting extracellular vesicles

Daniel Binzel

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Small interfering RNA (siRNA) for silencing genes and treating disease has been a dream since ranking as a top Breakthrough of the Year in 2002 by Science. However, the field of RNA interference (RNAi) therapeutics still faces challenges such as specificity in targeting, intracellular processing, and endosome trapping after targeted delivery. Dicer-substrate siRNAs included onto RNA nanoparticles may be able to overcome these challenges. Here, the structure optimization and chemical modification for controlled release of Dicer-substrate siRNAs in tumor cells were also evaluated through molecular beacon analysis. Extracellular vesicles were modified to carry the siRNA sequences while displaying RNA nanoparticle harboring cancer targeting aptamers and ligands. Extracellular vesicles loaded with pRNA-based nanoparticles with the Dicer- substrate siRNAs demonstrated efficient in vitro and in vivo delivery to the cytosol of tumor cells and release the siRNA. Taking advantage of the RNA ligand for specific targeting and extracellular vesicles for efficient membrane fusion, the resulting ligand-displaying extracellular vesicles were capable of specific delivery of siRNA to cells, and efficiently blocked tumor growth in three cancer models.

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W22-3:

Cryogenic Electron Microscopy of RNA Structures at Near Atomic Resolution

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Single particle Cryogenic electron microscopy (cryo-EM) is a mature methodology for routine structure determination with detailed features equivalent to those obtained by X-ray crystallography at comparable resolutions. However, majority of the PDB structures are protein containing macromolecules. RNA-only molecule is difficult to study structurally because of its inherent flexibility to make it less susceptible to be crystallized or to form a stable tertiary fold. Cryo-EM appears to be a structural tool readily useful to tackle RNA-only structure towards atomic resolution. We have used the latest data collection and image processing strategy in cryo-EM to study a number of RNA-only molecules from 28 kDa to 128 kDa at a resolution from 6.5 – 2.2 Å. The highest resolution RNA structure can reveal how the tertiary fold of the RNA are held together not only through base pairing and stacking but also networked with water and Mg^{+2} ions. A striking example is the intact tetrahymena ribozyme structure which reveals previously unexpected base interactions from distal nucleotides and the identification of the flexible subdomains of the molecule. This type of chemical information is critical to understand the basic principles of RNA structure but also to derive drug binding strategy.

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W22-4:

Predicting RNA-small molecule interactions

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The intricate 3D structures and their functional roles of RNAs make RNA molecules ideal targets for therapeutic drugs. The rational design of RNA-targeted drugs requires accurate computational modeling of RNA-small molecule interactions. Here using Flavin mononucleotide (FMN) docking to F. nucleatum FMN riboswitch as an example, we describe novel physics and deep learning-based approaches to the

computational prediction of RNA-small molecule binding. Furthermore, using the HIV-1 TAR system as an example, by combining the RNA-small molecule interaction model with cotranscriptional folding kinetics and the Vfold RNA 3D structure prediction models, we show the predicted parallel folding pathways during the transcriptional pause, the dynamics of the conformational switches, and an unconventional drug discovery strategy by targeting cotranscriptional folding pathways of nascent RNA structures during the transcription

W22-5:

Synthesis of RNA nanoparticles with cholesterol for ligand display on exosomes to target cancer cells for specific therapeutic drug delivery

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The dynamic, motile and deformative properties of RNA nanoparticles facilitate the third milestone of pharmaceutical drug development [(1)]. RNA nanoparticles with cholesterol were designed and synthesized for ligand display on exosomes to target cancer cells in specific therapeutic drug delivery. Exosomes are not only biological cargos carrying bioactive molecules (e.g., RNAs, proteins, and lipids), but also ideal for intracellular therapeutic agent delivery with advantages of high biocompatibility and low immunogenicity. Due to the enhanced targeting and delivery by cholesterol, the incorporation of cholesterol in RNA nanoparticles for ligand display on exosomes improves specific drug targeting and delivery efficacy and biodistribution to the cancer cells after systemic administration. Here we present the strategies to engineer exosomes for ligand display and therapeutics loading with RNA nanotechnology involving RNA aptamers, chemical ligands, RNA interference, drug conjugation, etc., and apply computational methods for feature selection to accelerate drug development.mRef. 1: Li X, Bhullar AS, Binzel DW, Guo P. (2022, https://doi.org/10.1016/j.addr.2022.114316).

W22-6:

Mannose decorated exosomes with RNA nanoparticles harboring miR-511-3p protects against allergicasthma

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Cockroaches are a potent source of allergen that is known to induce sensitization and drive allergic respiratory symptoms. However, no specific treatments have been developed to target cockroach allergen-induced allergic sensitization and asthma. We have recently demonstrated that over-expression of miR-511-3p can promote alternative (M2) macrophage polarization and protect against allergic airway inflammation. Here, we sought to further confirm the role of miR-511-3p in protecting against allergic airway inflammation by using miR-511-3p knockout mice (miR-511-3p$^{-/-}$). Indeed, our newly generated miR-511-3p$^{-/-}$ mice by CRISPR/Cas-mediated genome engineering exhibited enhanced airway inflammation with elevated levels of Th2 cytokines in the Bronchoalveolar lavage fluid (BALF) samples compared with wild-type (WT) mice. Further *in vivo* and *in vitro* analyses suggest that miR-511-3p can polarize lung macrophages into M2. Given that RNA nanotechnology has been shown to reprogram natural exosomes (EVs) for specific delivery of miRNAs to target recipient cells and mouse models, we thus created RNA nanoparticles with mannose decorated EV-miR-511-3p (Man-EV-miR-511- 3p) by loading miR-511-3p mimics into the decorated EVs with engineered RNA nanoparticle PRNA-3WJ. Inhaled Man-EV-miR-511-3p by *intra-tracheal* (i.t.) was confirmed to mainly target mouse lung macrophages. Importantly, these successfully delivered Man-EV-miR-511-3p reversed the increased airway inflammation andelevated BALF Th2 cytokines observed in miR-511-3p$^{-/-}$ mice. Further *in vitro* analysis demonstrated that Man-EV-miR-511-3p was uptake by macrophages and contributes to M2 macrophage polarization. Lastly, our gene expression profiling of macrophages identified *C3* as one of the miR-511-3p targets that was increased in M2 macrophages but antagonized by miR-511-3p. The interaction between miR-511-3p and C3 gene was further confirmed by C3 activity assay. Collectively, these findings suggest that the RNA nanotechnology reprogramed EVs are promising carriers for specific delivery of miRNAs and Man-EV-miR-511-3p has a great potential in preventing or treating allergic asthma.

Workshop #23:

W23-1:

TGFβ regulation of T-cell immunity

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Dr. Wanjun Chen's interest is elucidating mechanisms of immune tolerance, focusing on TGF-β regulation of T-cell immunity and tolerance. Dr. Chen also studies the development and function of regulatory T cells. Dr. Chen and his colleagues investigate the differences between T-cell immunity and tolerance in animal models to understand the pathogenesis of autoimmunity and inflammation, cancer, and infectious disease. Their studies aim to lead to the development of potential immunotherapies for relevant human diseases.

W23-2:

Preclinical advances in combined TGFβ/PD-L1 inhibition in head and neck cancer and the implementation to clinical studies

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Therapeutically targeting TGFβ has led to promising results in preclinical research, but has not led to successful clinical trials in cancer patients. This is likely due to a combination of the toxicity associated with the complexity of TGFβ functions and off-target effects of inhibiting this pathway, as well as the need for a combination treatment that can eradicate tumor cells. Currently, there are multiple therapeutic agents targeting both TGFβ/PD-L1 inhibition, which are undergoing clinical trials in multiple cancer types. The early reported clinical trials bintrafusp alfa, a bifunctional fusion protein targeting both of these pathways, have had mixed results. These mixed clinical trial results reflect the complex biology of the TME, and are limited by the cancer types and enrollment criteria in these clinical trials rather than an overall biological failure of targeting these signaling pathways. The most critical need of any successful clinical trial is patient selection. Notably, TGFβ overexpression is associated with both tumor initiation and progression in HNSCC, with the epithelial-specific loss of Smad4 or the TGFβ receptor resulting in spontaneous carcinomas in multiple mouse models. These observations are consistent with clinical data that TGFβ is overexpressed in HNSCC, and its level inversely correlated with SMAD4 chromosomal copy numbers. Because PD-L1 can be upregulated on both tumor cells and infiltrating immune cells, this creates a target-rich environment for TGFβ/PD-L1 dual inhibition and thus more opportunity to provide localized TGFβ inhibition in the tumor. Further, clinical trials for TGFβ/PD-L1 inhibition should also consider the temporal and spatial patterns of TGFβ and PD-L1 expression. For example, our recent study shows that cancer associated fibroblasts (CAFs) contribute more TGFβ to the TME than tumor cells. Because PD-L1 expression on CAFs is either low or a result of increased anti-tumor immunity, inhibiting TGFβ-mediated fibrosis followed by targeted TGFβ/PD-L1 inhibition to infiltrating $TGF\beta^{+}PD\text{-}L1^{+}$ myeloid cells may be more effective than concurrent dual inhibition. Therefore, preclinical studies that explore the combination therapeutic types and regimens will be essential to narrow down the optimal therapeutics before moving into clinic.

W23-3:

Targeting GARP to Thwart the TGFβ Pathway for Cancer Immunotherapy

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One of the key challenges in the field of immuno-oncology is both the primary and adaptive resistance to immune checkpoint blockade (ICB) against the PD-1/PD-L1 axis in a majority of patients with cancer such as pancreatic, ovarian, urothelial and most triple negative breast cancer. One underlying mechanism of ICB resistance relates to the accumulation of TGFβ in the tumor microenvironment (TME), which drives immune dysfunction by multiple mechanisms such as inducing Treg cells, excluding and inhibiting the function of effector $CD8^{+}$ T cells, and limiting effector T cell migration into the TME. However, targeting TGFβ, despite many decades of attempt, has proven to be difficult because of its pleotropic roles that are highly context dependent (Moreau *et al*., *Sci Immunol.* 2022 Mar 18;7(69):eabi4613). Glycoprotein-A repetitions predominant (GARP) is a cell surface docking receptor for activating latent TGFβ1, TGFβ2 and TGFβ3, with its expression restricted to effector Tregs, cancer cells as well as platelets in the TME. Herein my laboratory demonstrates that GARP overexpression in human cancers correlates with tolerogenic TME and poor clinical response to ICB. Importantly, we have generated, humanized and characterized a unique anti-human GARP antibody (named PIIO-1) that blocks activation of all isoforms of latent TGFβ in the TME. Using clinically relevant human *LRRC32* (encoding GARP) knockin mice and multiple preclinical tumor models, we showcased the potential drugability of the GARP-LTGFβ pathway for cancer immunotherapy. By doing so, we unraveled several novel aspects of the GARP biology in contributing to immune exclusion, ICB resistance, $CD8^{+}$ T cell exhaustion and their poor migration into the TME. We are actively developing PIIO-1 as a novel and promising clinical agent against a large array of ICB-resistant malignancies either as a monotherapy, or in combination with ICBs.

W23-4:

Exploiting Apoptotic Cell Induced TGFβ for Transplantation Tolerance

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Organ transplantation is the only cure for end stage organ diseases. A current major challenge for human allogeneic transplantation is the need for life-long immunosuppression, which has undesirable metabolic, hematological and infectious complications. Therefore, developing effective strategies to induce donor-specific tolerance to obviate the need for life-long immunosuppression is an unmet need. We developed an efficient donor-specific tolerance therapy that utilizes infusions of ethylene carbodiimide (ECDI)-treated donor splenic antigen-presenting cells that results in indefinite survival of allogeneic islet grafts in the absence of immunosuppression in murine models of transplantation. ECDI treatment leads to effective apoptosis of donor splenocytes. The apoptotic donor cells engage recipient phagocytes via the efferocytic receptor MerTK, and trigger downstream anti-inflammatory signaling. Furthermore, we show that induction of tolerance by this strategy is critically dependent on apoptotic cell induced TGF-β1 and expansion of CD4+CD25+Foxp3+ regulatory T cells. This strategy is widely applicable to a spectrum of allogeneic and xenogeneic transplantation, including allogeneic heart, kidney transplantation as well as xenogeneic islet cell

transplantation. It has further demonstrated efficacy in large animal models of allogeneic islet and kidney transplantation. When combined with additional costimulation blockade by anti-CD40L and a short course of the mTOR inhibitor rapamycin, this strategy is also effective in promoting long-term allograft survival in previously sensitized recipients. In summary, this highly efficient antigen-specific therapy has a significant therapeutic potential in human allogeneic transplantation.

W23-5:
STAT3: from fundamental discoveries to clinical translation
Hua Eleanor Yu, PhD.
Beckman Research Institute at City of Hope Comprehensive Cancer Center

STAT3 is persistently activated in many types of tumors and it plays a crucial role in tumor proliferation, invasion, resistance to therapy and immunosuppression. Inhibiting STAT3 can therefore have significant implications for improved cancer therapy. However, as a transcription factor lacking its own enzymatic activity, STAT3 is a challenging target for small-molecule drugs. We have recently developed two technology platforms to target STAT3. By covalently linking siRNA to the CpG moiety, we are able to silence STAT3 gene in those cells expressing Toll-like receptor 9 (TLR9), which is the receptor for CpG. These cells include immune cells such as dendritic cells, macrophages and B cells, and a variety of tumor cells. The CpG-*Stat3* siRNA can simultaneously inhibit cancer-promoting inflammation, activating immune cells and destroying tumor cells. Recently, we further show that CpGSTAT3siRNA can enhance the antitumor effects of anti-PD1 antibody. The CpGSTAT3siRNA has gained US FDA approval to test in lymphoma patients at City of Hope Comprehensive Cancer Center. Another exciting approach to inhibit STAT3 is by cell-penetrating antibodies/peptides. We demonstrate that using a specific approach to modify antibodies enable efficient antibody/peptide cellular internalization and target recognition. Both local and systemic deliveries of the modified antibodies/peptides effectively inhibit STAT3 activity in tumors, resulting in tumor cell apoptosis and antitumor immune responses in various models. We are improving this methodology for both research and clinical translation.

Workshop #24:

W24-1:

Structural basis of DNA polymerase catalyzed DNA synthesis, error incorporation, and double-strand break repair

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DNA polymerases catalyze template dependent DNA synthesis during DNA replication and repair. Error incorporations by DNA polymerases can result in human diseases and cancer, whereas targeting DNA polymerases have been proven effective in various cancer treatment. We have employed leading-edge structural techniques to investigate the mechanism of DNA polymerases. By observing DNA synthesis directly with time-resolved crystallography, we dissected the role of local conformational changes and metal ion cofactor binding in correct and incorrect DNA synthesis. In addition, we determined 2.4 Å cryo-electron microscope structures of DNA polymerase θ, which is an essential enzyme in DNA double strand break repair and a precision drug target for treating BRCA mutations related cancer. Our structural studies of DNA polymerases shed light on the general mechanism of DNA synthesis, error incorporation and repair, and provide valuable information for targeting polymerases for cancer treatment.

W24-2:

Molecular dissection of the evolutionarily diverse H2AX C-terminus in DNA repair

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H2AX plays a central role in the DNA damage response (DDR) pathway, including DNA double-strand breaks (DSBs) repair and replication stress. More specifically, it is phosphorylated at S139 (□H2AX) upon DNA damage. γH2AX triggers a signaling cascade via physically interacting with MDC1 and activating the RNF8-RNF168 ubiquitin pathway that is crucial for DDR proteins, including 53BP1 and BRCA1, accrual at damaged chromatin. Interestingly, the human H2AX C-terminal tail linker region (amino acid residues 120-138) is evolutionarily diverse and its function is largely unknown. We hypothesize that the unique H2AX C-terminal tail is involved in DNA repair. Using genetic and cell-based approaches, we define the minimal molecular requirement for the phospho-ubiquitin (□H2AX-MDC1-RNF8-RNF168) signaling pathway in the DDR through the interaction between the H2AX phosphorylated S139-Y142 residues and the MDC1 tandem BRCT domain. Strikingly, inactivation of the MDC1-H2AX Y142 binding retains a subset of 53BP1 damaged chromatin recruitment. Our data suggest that the H2AX linker region regulates the MDC1-independent 53BP1 damaged chromatin recruitment. This H2AX linker-mediated 53BP1 recruitment to DNA breaks is excluded in the S-phase, which confers resistance to S-phase specific DNA damaging agents. Furthermore, we found that 53BP1 interacts with the H2AX linker region-pS139 besides the H2A K15ub and H4K20me2 marks. Collectively, our findings provide novel insights into the molecular role of H2AX in 53BP1 regulations in response to genotoxic insults during the cell cycle.

W24-3:

Molecular mechanism of DNA single-strand break repair and signaling

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Genomes of all organisms are exposed to constant stress or insults from endogenous or exogenous resources, leading to DNA lesions in the nuclear and mitochondrial genome. Cells have evolved a variety of different DNA repair and DNA damage response pathways to mitigate the vulnerabilities, and deficiencies of these pathways are often implicated in the pathogenesis of human genetic syndromes and diseases such as cancer. Although PARP1 and XRCC1 are implicated in the SSB repair pathway, it remains incomplete how SSB repair and SSB signaling pathways are coordinated and regulated. Using *Xenopus* egg extract and *in vitro* reconstitution systems, we have demonstrated that both APE1 and APE2 play important role in the SSB-induced ATR-Chk1 DDR pathway activation. Further mechanistic studies have revealed that the defined SSBs are first sensed by APE1 to initiate 3'-5' SSB end resection and followed by APE2 recruitment and activation to continue SSB end resection. Notably, the 3'-5' exonuclease activity of APE1 and APE2 is critical for SSB signaling and SSB repair. We also first reported the first APE2 small molecule compound that inhibits APE2 association with ssDNA and its exonuclease activity, sensitizing pancreatic cancer cells to chemotherapy drugs. Due to the synthetic lethality of APE1 and APE2 in BRCA1/2-deficient cancer cells, our mechanistic studies of APE1 and APE2 in genome integrity and cancer etiology will provide novel insight into future potential cancer therapeutic strategies.

W24-4:

Antioxidant system Trx1/Trx1R is a novel determinant of CHK1 inhibitor sensitivity in treating NSCLC via regulation of RRM1 oxidation/reduction

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The cell cycle checkpoint protein CHK1 is critical to cell survival under replication stress (RS). CHK1 inhibitors in combination with unspecific chemotherapy have shown promising results in preclinical studies. However, this approach has achieved minimal efficacy with substantial toxicity in clinical trials, which significantly limits the benefit of these agents. Thus, identifying novel combinational conditions that can specifically sensitize cancer cells by targeting modern signaling regulators to CHK1 inhibitor might be the key to improving the efficacy of those agents. To explore novel combinational strategies, we performed high-throughput genome-wide loss-of-function screening using the Decode Pooled shRNA library with a non-small cell lung cancer (NSCLC) cell line. Thioredoxin1 (Trx1), a major antioxidant system component, was identified as a top candidate. A synergistic interaction between knockdown (KD) of Trx1 or thioredoxin reductase1 (TrxR1) and CHK1 inhibitor in suppressing cell growth of NSCLC was observed in both in vitro and in vivo assays. Trx1 and TrxR KD lead to defective DNA replication elongation due to dysregulation of the dNTP pool. CHK1 inhibitor further increases Trx1/TrxR1 KD-induced RS, DNA pool scarcity, and cell death. Surprisingly, the oxidation of RRM1, the larger subunit of ribonucleotide reductase (RNR), is important for Trx1/TrxR1 KD-induced RS and the synergistic interaction between Trx1/TrxR KD and CHK1 inhibitor, whereas ROS is not required. A pharmaceutical TrxR inhibitor (auranofin) that has been used clinically to treat rheumatoid arthritis has a similar synergistic interaction with CHK1 inhibitor. In summary, Trx1/TrxR1 inhibition leads to DNA replication

defect via RRM1 oxidation and acts synergistically with CHK1 inhibitor in suppressing cancer cell growth. Our study reveals a new combinational approach to treat NSCLC by identifying the unrecognized link between Trx1/TrxR inhibition-induced RRM1 oxidation and CHK1 inhibitor-associated cancer therapy.

W24-5:
A novel tumor suppressor of colorectal cancer
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Genomic instability is a key molecular and pathogenic factor during the initiation process of cancer. Defects in genome stability maintenance genes such as BRCA genes are often associated with predisposition of cancer. The mammalian ssDNA-binding protein complex CTC1-STN1-TEN1 (CST) has emerged as a key player in protecting genome stability. Our lab has demonstrated that CST protects stalled fork stability by antagonizing unscheduled nuclease attack of nascent strand DNA. However, the potential role of CST in carcinogenesis is unknown. In this study, we analyzed The Cancer Genome Atlas (TCGA) Pan Cancer data and found that reduced STN1/CTC1 expression is common in colorectal cancers (CRCs) and associated with poor survival in advanced CRC. Furthermore, CRC tumors with altered STN1/CTC1 display a higher mutation burden than those without. To investigate the effect of STN1 deficiency in CRC tumorigenesis, we generated an inducible STN1 knockout mouse model and found that STN1 deficiency increased CRC incidence and tumor volume, along with elevated malignancy in the azoxymethane (AOM)-induced CRC model. *Stn1*$^{-/-}$ tumors showed enhanced proliferation, reduced apoptosis, elevated DNA damage, and harbored mutational signatures representing defective DNA repair. STN1 depletion in cells led to a high level of replication stress and DNA damage that were further elevated by AOM. In addition, STN1 deficiency caused an accumulation of oxidative DNA damage in colon tissues. Collectively, our study reveals an unexpected role of mammalian STN1 in regulating the base excision repair pathway and protecting cells and colon tissues from oxidative damage, thereby identifying STN1 as a novel suppressor of CRC.

W24-6:
NT-seq: a Chemical-based Sequencing Method for Genomic Methylome Profiling
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Three forms of DNA methylation, N6-methyladenine (6mA), N4-methylcytosine (4mC), and 5-methylcytosine (5mC), are prevalent in prokaryotic genomes and play vital functional roles. However, few technologies can obtain single-base resolution genomic maps of the full methylome. Recently, 6mA has been reported to be present and play vital roles in a variety of eukaryotic species, including mammals. Yet, the bottleneck of current 6mA research in eukaryotes is lacking an unbiased single-base resolution sequencing method for 6mA profiling. Here, we present NT-seq (nitrite treatment followed by next-generation sequencing), a sequencing method to simultaneously detect all three types of methylation genome-wide. NT-seq reliably detects all known 6mA, 4mC, and 5mC motifs, and accurately maps 6mA and 4mC at single-base resolution in bacterial genomes. In contrast to previously reported nitrite sequencing, which detects 6mA by inducing A-to-G transition in unmethylated adenine sites but not 6mA sites, the NT-seq can read 6mA by inducing A-to-T conversion at 6mA sites by coupling with specific enzymes. This unique workflow of NT-seq tremendously improved the performance in mapping genomic 6mA at single-base resolution by increasing both the sensitivity and specificity. We demonstrated NT-seq can accurately detect 6mA at single-base resolution in eukaryotic genomes, such as *Chlamydomonas reinhardtii* and *Oxytricha trifallax*. Overall, NT-seq provides a simple, chemical-based, and efficient solution for detecting multiple types of DNA methylation in bacteria genomes and an unbiased single-base resolution sequencing method for 6mA profiling in eukaryotic genomes. This method overcomes the challenge in the DNA 6mA field and paves the way for exploring new biology of the epigenetic DNA modifications.

Workshop #25:

W25-1:

Pharmacological modulation of bile acid pool and its impact on non-alcoholic steatohepatitis and cholestasis in mice

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Intrahepatic bile acid accumulation is a major contributor to cholestatic liver injury. Emerging evidence also suggests that bile acids may promote injury and inflammation in non-alcoholic steatohepatitis (NASH), and reducing the bile acid pool may be desirable in NASH treatments. Pharmacological inhibition of bile acid synthesis promotes intestinal bile acid preservation, while inhibition of intestinal bile acid re-uptake induces bile acid synthesis. These compensatory mechanisms limit the degree of bile acid pool reduction by pharmacological interventions. In this study, the effects of combining an apical sodium-bile acid transporter (ASBT) inhibitor GSK2330672 and AAV-mediated fibroblast growth factor-15 (FGF15) overexpression are investigated in high fat/cholesterol/fructose diet-induced NASH mouse model and Cyp2c70 knockout mice exhibiting human-like bile acid pool and hepatobiliary injury. In the NASH model, the combined intervention demonstrates higher efficacy against NASH and fibrosis than either single treatment. Mechanistically, the combined intervention markedly reduces the bile acid pool that not only decreases hepatic bile acid burden but also limits intestinal lipid absorption, which, together with FGF15 signaling activation, ameliorates obesity, hepatic lipotoxicity, and organelle stress. In Cyp2c70 knockout mice, the combined intervention reduces portal fibrosis in female mice but worsens portal fibrosis in male mice despite markedly reducing the bile acid pool in both sexes. In conclusion, concomitant ASBT inhibition and FGF15 signaling activation are effective in reducing bile acid pool and hepatic bile acid burden in diseased livers. In addition, the combined intervention produces metabolic changes that partially mimic the bariatric surgery condition, whereby lipid malabsorption and increased FGF15/19 signaling synergistically mediate weight loss and metabolic improvement in obese mice. However, the sex-dependent differential outcomes of the combined intervention in Cyp2c70 knockout mice suggest that altered gut-liver bile acid signaling independent of reduced tissue bile acid load may play a significant role in modulating hepatic injury and inflammatory response.

W25-2:

Effects of Overexpression of Fibroblast Growth Factor 15/19 on Hepatic Drug Metabolizing Enzymes

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Fibroblast growth factors 15 (FGF15) and 19 (FGF19) are endocrine growth factors that play an important role in maintaining bile acid homeostasis. FGF15/19-based therapies are currently being tested in clinical trials for the treatment of nonalcoholic steatohepatitis and cholestatic liver diseases. To determine the physiologic impact of long-term elevations of FGF15/19, a transgenic mouse model with overexpression of *Fgf15* (*Fgf15* Tg) was used in the current study. The RNA sequencing (RNA-seq) analysis revealed elevations in the expression of several genes encoding phase I drug-metabolizing enzymes (DMEs), including *Cyp2b10* and *Cyp3a11*, in *Fgf15* Tg mice. We found that the induction of several *Cyp2b* isoforms resulted in the increased function of CYP2B in microsomal metabolism and pharmacokinetics studies. Because the CYP2B family is known to be induced by constitutive androstane receptor (CAR), to determine the role of CAR in the observed inductions, we crossed *Fgf15* Tg mice with CAR knockout mice and found that CAR played a minor role in the observed alterations in DME expression. Interestingly, we found that the overexpression of *Fgf15* in male mice resulted in a phenotypical switch from the male hepatic expression pattern of DMEs to that of female mice. Differences in the secretion of growth hormone (GH) between male and female mice are known to drive sexually dimorphic, STAT5b-dependent expression patterns of hepatic genes. We found that male *Fgf15* Tg mice presented with many features similar to GH deficiency, including lowered body length and weight, *Igf-1* and *Igfals* expression, and STAT5 signaling. SIGNIFICANCE STATEMENT: The overexpression of *Fgf15* in mice causes an alteration in DMEs at the mRNA, protein, and functional levels, which is not entirely due to CAR activation but is associated with lower GH signaling.

W25-3:

Inhibition of endothelin receptor A (ET-A) reduces biliary damage, angiogenesis and liver fibrosis in cholestasis

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Endothelin (ET)-1, ET-2 and ET-3 modulate various mechanisms via activation of their receptors, ET-A and ET-B. ET-A promotes, whereas ET-B reduces, inflammation in cardiovascular disease. ET-1 is synthesized by cholangiocytes in cholestatic rats, and ET-A induces fibrosis. Primary sclerosing cholangitis (PSC) is a cholangiopathy complicated by microcirculatory dysfunction and portal hypertension associated with angiocrine signaling. The biliary tree promotes vascular

expansion through the release of growth factors, and the vasculature supports biliary growth. We aimed to understand ET-A mediated crosstalk between cholangiocytes and vascular endothelial cells (VECs) in PSC. Aging multidrug resistance 2 knockout ($Mdr2^{-/-}$, PSC model) mice had ductular reaction occurring at 4 wks of age that expanded from 8-12 wks of age, occurring prior to angiogenesis seen at 8-12 wks of age. $Mdr2^{-/-}$ mice at 12 wks of age and human PSC samples had increased ET-1, ET-2 and ET-A biliary and VEC expression. We treated $Mdr2^{-/-}$ mice at 12 wks of age with Ambrisentan (ET-A antagonist, 5 mg/kg BW/day) or vehicle for 1 wk. Ambrisentan treatment reduced ductular reaction, biliary senescence, immune cell infiltration and liver fibrosis in $Mdr2^{-/-}$ mice. Importantly, angiogenesis was decreased in $Mdr2^{-/-}$ mice treated with Ambrisentan. Biliary tree and vascular expansion were visualized in $Mdr2^{-/-}$ mice by ink injection, but this was regressed with Ambrisentan treatment. TEM imaging demonstrated that $Mdr2^{-/-}$ mice had squamous cholangiocytes with shortened microvilli, and dilatated arterioles lacking cilia (indicative of inflammatory signaling); these changes were reversed with Ambrisentan treatment. $Mdr2^{-/-}$ mice and human PSC had increased expression, and cholangiocytes had increased secretion, of pro-angiogenic transforming growth factor (TGF)-β1 that was reduced with Ambrisentan treatment, and Ingenuity Pathway Analysis confirmed ET-A was linked with TGF-β1. ET-A promotes biliary secretion of TGF-β1 that induces angiogenesis, suggesting that biliary-vascular crosstalk influences PSC pathogenesis. Inhibition of ET-A may prove therapeutic for PSC patients.

W25-4:

Regulation of Liver Cell Remodeling and Ductular Reaction by mTOR Activation in Alcoholic Hepatitis

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Conflict of Interest: These authors declare that they have nothing to disclose.

Alcohol-associated liver disease (ALD) is a major cause of liver disease-related mortality. Increased hepatocyte degeneration, infiltrated immune cells, ductular reaction(DR) and fibrosis are key features of human alcoholic hepatitis (AH). However, no current ALD animal models can phenocopy these phenotypes. We generated liver-specific *Tsc1* KO (*Tsc1*$^{flox/flox}$, Albumin-Cre+, L-*Tsc1* KO) mice and subjected these mice and their matched wild type (WT) littermates (*Tsc1*$^{flox/flox}$, Albumin-Cre-) to Gao-binge alcohol protocol. To delete Tsc1 more specifically in hepatocytes and cholangiocytes, *Tsc1*$^{flox/flox}$ mice were injected with hepatocyte-targeting AAV-TBG-Cre and inducible cholangiocyte-targeting Ad-CK19-CreERt2 followed by tamoxifen injection, respectively. We found Gao-binge alcohol markedly increased liver/body weight ratio and serum alanine aminotransferase (ALT) levels in L-*Tsc1* KO mice, suggesting increased hepatomegaly and liver injury in L-*Tsc1* KO mice compared to either Gao-binge alcohol-fed WT mice or control diet-fed L-*Tsc1* KO mice. Remarkably, alcohol significantly increased CK19 and SOX9 positive ductular structures as well as increased hepatic F4/80 positive macrophages and myeloperoxidase (MPO) positive neutrophils infiltration. Furthermore, qPCR and Sirius Red staining showed significantly increased expression of fibrotic genes and fibrosis, respectively, in alcohol-fed L-*Tsc1* KO mice. RNAseq and qPCR data showed gene signature of hepatocytes decreased, while gene signature of cholangiocytes, hepatic stellate cells (HSCs) and Kupffer cells (KCs) increased, in alcohol-fed L-*Tsc1* KO mice. These results indicate that Gao-binge alcohol induces typical pathological features of human ASH in L-*Tsc1* KO mice. Mechanistic studies revealed increased YAP and Notch activation as well as endoplasmic reticulum (ER) stress markers in alcohol-fed L-*Tsc1* KO mice. Moreover, deleting *Tsc1* in cholangiocytes but not hepatocytes aggravated alcohol-induced liver injury, hepatomegaly, DR, fibrosis, and inflammation in mouse livers. Interestingly, administration of torin1 partially reversed hepatic *Tsc1* deletion-induced liver injury and hepatomegaly, DR, fibrosis, inflammatory cells infiltration in alcohol-fed mouse livers. In conclusion, our findings indicate that persistent activation of mTORC1 due to the loss of hepatic TSC1 promotes liver injury, hepatomegaly, DR, inflammation, fibrosis, and liver cell repopulation in Gao-binge alcohol-fed liver-specific Tsc1 KO mice, which phenocopy human AH.

W25-5:

EPP-related cholestasis: mechanism and novel therapy

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Erythropoietic protoporphyria (EPP) is an inherited disease caused by loss-of-function mutations of ferrochelatase, an enzyme in the heme biosynthesis pathway that converts protoporphyrin IX (PPIX) into heme, leading to PPIX accumulation in the body. The liver is responsible for PPIX elimination through the hepatobiliary system. Because PPIX is highly hydrophobic, excessive PPIX in bile will precipitate and result in bile duct blockage and cholestatic liver injury. Unfortunately, there is no cure for PPIX-mediated hepatotoxicity in EPP patients. Our work revealed that the PPIX efflux transporter ABCG2 determines EPP-associated hepatotoxicity. We found that ABCG2 deficiency protects against EPP-associated hepatotoxicity by modulating PPIX distribution, metabolism, and excretion. In summary, our work has uncovered an essential role of ABCG2 in EPP-related cholestasis, which provides a novel strategy for EPP therapy by targeting ABCG2.

W25-6:

Modulating gut microbiota to improve intrahepatic immunity against hepatocellular cancer

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Gut microbiota plays a pivotal role in the pathogenesis and treatment effect of hepatocellular cancer (HCC), it has been reported that the HCC patients' response to immunotherapy of PD-1/PD-L1 blockade is associated with their gut microbiota

profiles. However, the impact of gut microbiota on anti-HCC immunityand its underlying mechanism are poorly understood. Treating HCC-bearing mice with a specific and non-hepatotoxic antibiotic cocktail causes the increased relative abundance of certain commensal bacteria suchas *Bacteroides*, resulting in suppression of HCC growth and development which is involved in activation of intrahepatic antitumor immune responses. Gut microbiota repopulation of gut-sterilized HCC-bearing mice with *Bacteroides thetaiotaomicron (B. th)* significantly improves therapeutic efficacy of anti-PD-1 antibody (αPD-1 Ab) against HCC. Transplantation of human gut microbiota from the HCC patients who received and responded to αPD-1 Ab treatment improves αPD-1 Ab efficacy against HCC in mice model. Mechanistic studies demonstrate that *Bacteroides'* CpG-enriched genomic DNAs accumulate in livers/tumors as a messenger to activate tumor antigen specific $CD8^+$ T cells through dentritic cell modulation in a TLR9-dependent manner. In conclusion, gut microbiota as a causative factor can be targeted to improve anti-HCC immunotherapy.

Workshop #26:

W26-1:

Widespread RNA hypoediting in schizophrenia and its molecular impact on mitochondrial function

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Dysregulated RNA editing, the endogenous modification of nucleic acids, has been previously implicated in a number of neurodevelopmental disorders, including schizophrenia (SCZ), It was reported that aberrant RNA editing in SCZ occurs in genes with important roles in brain function, such as those encoding serotonin and glutamate receptors. However, our understanding of the global profile of RNA editing and the molecular function of differentially edited loci in SCZ remains limited. Here, we generated global profiles of RNA editing sites in postmortem brains of 4 SCZ cohorts of the PsychENCODE consortium. We uncovered more than 26,000 unique differential editing sites comparing SCZ and controls. Importantly, we observed a significant trend of hypoediting in SCZ, which is reproduced in 3 of the 4 cohorts. Single cell analysis revealed that the differential editing patterns in SCZ likely reflect editing differences occurring in each cell type, especially neurons, rather than cell compositional differences in SCZ brains relative to controls. We next examined the potential molecular functions of differentially edited sites in SCZ, which revealed a close relevance of RNA editing to mitochondria function. Specifically, using massively parallel report assays and bioinformatic analysis, we observed that differentially edited sites in the 3'UTR affecting host gene expression were enriched in mitochondrial processes. In addition, we identified two recoding editing sites in the mitofusin 1 (MFN1) gene, one of which being hypoedited in SCZ. We showed that these editing sites alter MFN1's function in regulating mitochondrial fission and fusion, balancing mitochondrial cytochrome C release, and maintaining stress tolerance. To our knowledge, this study is the first global analysis of RNA editing in SCZ to show replicable reduced editing in the disease and investigate the synergistic effects of dysregulated RNA editing on mitochondrial function.

W26-2:

The long noncoding RNA lnc-FANCI-2 restricts RAS signaling but maintains constitutive IFN response via TLR3 and MCAM in HPV-infected cervical cancer cells

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We recently discovered increased expression of cytoplasmic lnc-FANCI-2 coinciding 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 promoting lnc-FANCI-2 expression (Liu H., et al. PNAS PNAS 118 (3): e2014195118, 2021). To explore possible roles of lnc-FANCI-2 in HPV-induced cervical carcinogenesis, we knocked out lnc-FANCI-2 in the HPV16-positive cervical cancer cell line, CaSki, by using CRISPR-Cas9 technology. Knock-out (KO) single cell clones expressed HPV16 oncogenes normally but displayed altered cell morphology and reduced proliferation and migration when compared with wild-type (WT) parental cells. RNA-seq analyses revealed significantly increased expression of genes involved in RAS signaling and decreases in IFN pathways in KO cells over WT parental cells. Proteome profiling of cytosolic and secreted proteins from WT cells and KO cells further identified that lnc-FANCI-2 regulates expression of a subset of cell surface or adhesion-related proteins, including decreased MCAM, PODXL2 and ECM1 and increased ADAM8. Phosphorylated Akt and Erk, two important RAS pathway effectors , were increased more than 3-fold, accompanied by ~2.9-fold increase of MCAM, a possible PI3K/AKT activator, in KO cells over WT cells. Simultaneous siRNA knockdown of MCAM in the lnc-FANCI-2 silenced cells blocked the increased phosphorylation of Akt and slightly reduced p-Erk2. Interestingly, high-level lnc-FANCI-2 expression and lower MCAM expression in cervical cancer patients are associated with improved survival ($P<0.001$). Moreover, lnc-FANCI-2 has been found to maintain the expression of Toll-like receptor 3 (TLR3), a dsRNA-binding protein, and constitutive IFN genes, such as IRF1, IFIT2 and OAS3. We found that lnc-FANCI-2-mediated IRF1 expression requires TLR3, and knockdown of TLR3 led to increased levels of MCAM and Akt phosphorylation. Altogether, we show that lnc-FANCI-2, a host long noncoding RNA, restricts RAS signaling and maintains constitutive IFN response via TLR3 and MCAM, thereby affecting cervical cancer outcome.

W26-3:

TENT2, TUT4, and TUT7 selectively regulate miRNA sequence and abundance

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TENTs generate miRNA isoforms by 3' tailing. However, little is known about how tailing regulates miRNA function. Here, we generated isogenic HEK293T cell lines in which TENT2, TUT4 and TUT7 were knocked out individually or in combination. Together with rescue experiments, we characterized TENT-specific effects by deep sequencing, Northern blot and *in vitro* assays. We found that 3' tailing is not random but highly specific. In addition to its known adenylation, TENT2 contributes to guanylation and uridylation on mature miRNAs. TUT4 uridylates most miRNAs whereas TUT7 is dispensable. Removing adenylation has a marginal impact on miRNA levels. By contrast, abolishing uridylation leads to dysregulation of a set of miRNAs. Besides let-7, miR-181b and miR-222 are negatively regulated by TUT4/7 via distinct mechanisms while the miR-888 cluster is upregulated specifically by TUT7. Our results uncover the selective actions of TENTs in generating 3' isomiRs and pave the way to investigate their functions.

W26-4:

METTL16 plays oncogenic roles in liver cancer and leukemia through distinct mechanisms

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Beckman Research Institute of City of Hope

As the most prevailing internal decoration of mammalian mRNA, N^6-methyladenosine (m^6A) plays essential roles in normal biological processes and development. The m^6A methyltransferase complex (MTC), composed of METTL3 and METTL14, has been thought to be the main m^6A writer. Nevertheless, depletion of METTL3 and METTL14 is known to cause only ~60% decrease of m^6A modification in some cell types, with a good portion of m^6A marks in transcriptome do not map well with the binding sites of METTL3-METTL14. Such observations imply the existence of additional m^6A

methyltransferase. Human methyltransferase like proteins (METTL) belong to a superfamily composed of over 30 members, and the functions of most METTL proteins remain elusive. METTL16 was recently identified as a new m^{6}A methyltransferase to add m^{6}A to several non-coding RNAs including U6 snRNA, *MALAT1* and *XIST*, and one mRNA target (*MAT2A*). However, the biological functions of METTL16 in normal development and diseases (e.g., cancers) have yet to be investigated. We recently showed that METTL16 exerts both methyltransferase activity-dependent and -independent functions in gene regulation. Besides acting as a m^{6}A writer to add m^{6}A to hundreds target transcripts, METTL16 directly interacts with eukaryotic initiation factor 3a/b (eIF3a/b) and ribosomal RNAs (rRNAs) through its Mtase domain, thereby facilitating the assembly of the translation initiation complex (TIC) and promoting translation of thousands of transcripts. In liver cancer, FTO plays a critical oncogenic role largely independent of its catalytic activity. More recently, we found that METTL16 is also overexpressed in human acute myeloid leukemia (AML), especially in leukemia stem cell (LSC) population. METTL16 is required for AML development/maintenance and LSC self-renewal. However, METTL16's oncogenic role in AML is highly dependent on its m^{6}A methyltransferase activity. Thus, while METTL16 plays oncogenic roles in both liver cancer and AML, the functional mechanisms are somehow different.

W26-5:
RNA methylation in gene expression regulation
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Over 170 types of post-transcriptional RNA modifications have been identified in all kingdoms of life. We have previously discovered two RNA demethylases, FTO and ALKBH5, which catalyze oxidative demethylation of the most prevalent modifications of mammalian messenger RNA (mRNA) and other nuclear RNA, *N*^{6}-methyladenosine (m^{6}A). We have recently discovered m^{6}A methylation of chromatin-associated regulatory RNAs (carRNAs), which modulates chromatin state and transcription in mouse embryonic stem cells (mESCs). These findings suggest that reversible RNA modification could impact biological regulation analogous to the well-known reversible DNA and histone chemical modifications. I will present recent mechanistic investigation of chromatin and transcription regulation through m^{6}A methylation and demethylation, and impacts on mammalian early development as well as plant growth.

W26-6:
Abundant m6A modification on RNA signifies strong expression maneuverability of tumor suppressors
Xinlei Gao[1,2,3,6], Yang Yi[4,6], Jie Lv[3,6], Yanqiang Li[1,2,3], Sahana Suresh Babu[3], Ivone Bruno[3], Lili Zhang[1], Qi Cao[4], Kaifu Chen[1,2,3,5]
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Cancer genes were known to display unique epigenetic features on chromatin of benign cells. Investigations into these features are making it increasingly clear that cancer genes differ from other genes regarding the mechanisms regulating their transcription. It is yet unknown whether cancer genes have a unique epitranscriptomic feature on RNAs and thus differ from other genes in post-transcriptional regulation of their RNA expression. Here we found RNAs of tumor suppressor genes tended to decay fast in multiple benign cell types when compared with other RNAs. Consistent with a negative effect of m6A modification on RNA stability, we observed preferential deposition of m6A on tumor suppressor RNAs. With frequent transcription, the fast RNA decay of tumor suppressors did not lead to low expression in benign cells. However, abundant m6A and fast decay of tumor suppressor RNAs both tended to be further enhanced in prostate cancer cells relative to benign prostate epithelial cells. This enhancement correlated with a down regulation of tumor suppressor expression. Further, knockdown of m6A methyltransferase METTL3 and reader protein YTHDF2 in prostate cancer cells posed stronger effect on tumor suppressor RNAs than on other RNAs. These results indicated a strong expression maneuverability of tumor suppressors mediated by abundant m6A modification on RNAs.

Workshop #27:

W27-1:

Dissecting Shp2 function in hepatocytes unveils a new cell-cell communication mechanism under stress

Kota Kaneko, Yan Liang, Qing Liu, Wendy Chen, and **Gen-Sheng Feng**

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Background. Cell proliferation is driven by proliferative signaling pathways activated by receptor tyrosine kinases (RTKs). It has also become clear that cells, such as hepatocytes, possess a robust capacity to overcome inhibitory signals and struggle to proliferate under signal deficit. However, how cells respond to and overcome proliferative signal deficit is unclear.

Methods. We examined hepatocyte proliferation patterns and dynamics in mouse lines with hepatocyte-specific deletion of Shp2 or Met following partial hepatectomy (PHx). We also interrogated the CD133 expression and functions in mutant mouse lines in vivo and in a variety of cancer cell lines in vitro.

Results. While deletion of Shp2, a cytoplasmic PTPase that acts downstream of RTKS to promote Ras-Erk signaling, attenuated the overall proliferation of hepatocytes during liver regeneration, some of them gained proliferative capacity and managed to divide. Interestingly, the actively dividing Shp2-deficient hepatocytes formed distinctive colonies with high CD133 expression. Although CD133 is widely recognized as a stem cell marker, the colonized $CD133^+$ cells were negative for other stem cell markers. The $CD133^+$ cell colonies appeared transiently following PHx and disappeared after completion of liver regeneration. This phenotype was also observed in MET-deficient livers following liver injuries. Furthermore, CD133 expression was upregulated in a variety of cancer cell lines from different organs under proliferative signal inhibition, which indicates a commonly shared mechanism likely involved in drug resistance and cancer recurrence. Remarkably, we identified and isolated $CD133^+$ intracellular vesicles, which were enriched with transcripts of immediate early responsive genes and trafficking directly between tightly contacting cells in colonies. Thus, we give a name “intercellsome” here, for vesicle that mediates direct cell-cell communication. scRNA-seq demonstrated that cells defective for proliferative signaling exhibited lower intracellular transcriptional diversity (entropy), which was remedied in $CD133^+$ cell clusters, apparently through intercellular sharing/exchange. ***Conclusion.*** These data reveal a long-sought CD133 function in normal and cancer cells and elucidate a mechanism by which cells strive to proliferate under proliferative signal deficit.

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W27-2:

Generation of a novel mature liver organoid

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Organoids are widely used to model and investigate tissue development, regeneration, physiological functions. They also serve as useful disease models for mechanistic discovery and drug development. Recent studies have generated hepatocyte organoids that recapitulate some aspects of liver regeneration. However, these hepatocyte organoids are fetal liver like and express very low levels of critical hepatic metabolic genes characteristic adult liver, hampering their application in studies of liver metabolic functions.

In the present study, we generated a novel hepatocyte organoid culture system that mimic critical aspects of hepatic functions in adult liver such as bile acid production and urea generation. Compared with freshly isolated hepatocytes, which loses hepatic phenotypes quickly in in vitro culture, our hepatocyte organoids recapitulated distinct liver metabolic function during long term culture in different medium at both transcriptomic and functional levels. We will provide evidence that the mature hepatic organoid culture can be used to studies liver disorders including alcoholic and non-alcoholic fatty liver.

W27-3:

Spatial single-cell dissection of tumor and immune cells reveals stable lock-and-key features of a malignant ecosystem in liver cancer

Lichun Ma[1], Sophia Heinrich[1], Limin Wang[1], Friederike L. Keggenhoff[2], Subreen Khatib[1], Marshonna Forgues[1], Michael Kelly[3], Stephen M. Hewitt[4], Areeba Saif[5], Jonathan M. Hernandez[5], Donna Mabry[6], Roman Kloeckner[2], Tim F. Greten[6,7], Jittiporn Chaisaingmongkol[8,9], Mathuros Ruchirawat[8,9], Jens U. Marquardt[2], Xin Wei Wang[1,7]

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Intratumor heterogeneity may result from the evolution of tumor cells and their continuous interactions with the tumor microenvironment which collectively drives tumorigenesis. However, an appearance of cellular and molecular heterogeneity creates a challenge to define molecular features linked to tumor malignancy. We aimed to identify a stable molecular map underlying tumor heterogeneity that reflects intrinsic tumor biology. We performed multiregional single-cell transcriptome profiling of 80 liver tumor specimens derived from 29 hepatocellular carcinoma (HCC) and 15 intrahepatic cholangiocarcinoma patients. We identified unique cellular dynamics of malignant cells and their communication networks with tumor-associated immune cells as a stable fingerprint embedded in a malignant ecosystem representing features of tumor aggressiveness. Specifically, ligand-receptor interactions via the LGALS9-SLC1A5 and SPP1-PTGER4 pairs among tumor cells and macrophages were associated with unique transcriptome and a stable landscape of HCC malignancy, which was validated among additional 542 HCC patients with diverse race/ethnicity and etiologies. Our results uncovered a specific molecular network of tumor cells and macrophages that may represent a stable hub of HCC malignancy, opening a path for therapeutic exploration.

W27-4:

Androgen receptor and AR variant 7 exacerbate the c-Myc-mediated hepatocarcinogenesis

Tatsuo Kido and **Yun-Fai Chris Lau**

Department of Medicine, VA Medical Center, the Liver Center and the Institute for Human Genetics, University of California, San Francisco, USA

Hepatocellular carcinoma (HCC) is a sexually dimorphic cancer affecting more men than women among the patient populations. The exact etiologies of such sex differences are unknown, although genes on the sex chromosomes, particularly those on the Y chromosome, as well as sex hormones and their receptors have been postulated to play important roles in modifying the oncogenic processes in sex-biased manners. In particular, the roles of the male sex hormone receptor, androgen receptor (AR), could exert male-biased actions on hepatocarcinogenesis. Using various expression analytic approaches, we demonstrated that AR and AR variants, particularly AR-V7, are heterogeneously expressed in selected HCC clinical specimens. To evaluate the potential actions of AR and AR-V7 in liver cancer, we have utilized the hydrodynamic tail vein injection procedure to specifically express various combinations of AR, AR-V7 and different oncogenes, such as constitutively active Akt+Ras and c-Myc, *in vivo* in the hepatocytes of the FVB mice. Our results showed that expression of Akt+Ras or c-Myc induced HCC development in the livers as previously demonstrated, while over-expression of AR or AR-V7 alone in the livers have no significant effects in the host animals. Importantly, co-expression of AR-V7 significantly exacerbated the c-Myc oncogenesis in the livers of both male and female recipients. Similar co-expression of AR with c-Myc produced intermediate levels of enhancement of the oncogenic process in male animals only. Our findings suggest that AR-V7 exacerbates c-Myc-mediated hepatocarcinogenesis in a ligand-independent manner while AR exerts milder effects on a likely ligand-dependent manner. Comparative transcriptome analysis showed that AR-V7 amplifies c-Myc oncogenic actions in cell cycle, ribosomal biosynthesis, metabolism, and ribonucleoprotein complex biosynthesis among the top differentially affected signaling pathways. Importantly, various genes up-regulated by AR-V7 showed poor prognoses in HCC patients with respective high-level expression as compared to those patients with low-level expression. Summary: AR and AR variants are heterogeneously expression in selected HCC patients. Experimental evaluation shows that co-expression of AR or AR-V7 promote c-Myc-mediated hepatocarcinogenic processes in a moderate ligand-dependent and a high ligand-independent manner respectively, thereby potentially contributing to the sex differences in liver cancer among the HCC patients.

W27-5:

Engineering CAR T cells Targeting GPC3 for liver cancer immunotherapy

Aarti Kolluri, Shaoli Lin, Dan Li, Nan Li, and **Mitchell Ho**

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CAR-T cell therapy shows promising potency against hematological malignancies, but it remains challenging for treating solid tumors including liver cancer. We and others have shown that glypican 3 (GPC3) is an oncofetal antigen that is highly expressed in many hepatocellular carcinoma (HCC) patients and that GPC3 modulates Wnt/β-catenin and Yap signaling in HCC growth. Using proteomics and cell biology techniques, we recently further analyzed the role of GPC3 in Yap signaling and identified FAT1 as a novel GPC3-associated protein in HCC cells. To establish GPC3 as a target of cell-based immunotherapy in HCC, we have made the hYP7 scFv (membrane-proximal epitope) and the HN3 human V_H nanobody (membrane-distal epitope) as GPC3 targeting elements. Using the conventional CAR format, we have shown that hYP7 scFv is more potent than HN3 V_H for treating HCC in mice, highlighting the importance of targeting a membrane-proximal epitope, a current dogmatic hotspot in the CAR-T field. In the present study, we sought to determine how hinges and transmembrane portions of varying structures and sizes affect CAR T cell function. We re-engineered HN3 nanobody CAR-T and generated multiple permutations of CAR T cells containing CD8, CD28, IgG4, and Fc domains. We have found that newly engineered HN3 CAR T cells can be improved by two independent, synergistic changes in the hinge and transmembrane domains. The T cells expressing the HN3 CAR which contains the hinge region of IgG4 and in the CD28 transmembrane domain (HN3-IgG4H-CD28TM) exhibit high cytotoxic activity and cause complete HCC tumor eradication in mice. HN3-IgG4H-CD28TM CAR T cells avert exhaustion, enrich for cytotoxic-memory CD8+ T cells and NFAT TCR signals, and reduce β-catenin levels in HCC cells. Our new study provides strategies for engineering nanobody-based CARs and targeting a functional membrane-distal epitope for clinical development.

W27-6:

Novel intermediate-avidity glypican-3 specific CARTs resist exhaustion and mediate durable antitumor effects against human hepatocellular carcinoma

Leidy D Caraballo Galva[1,†], Xiaotao Jiang[1,†,‡] , Mohamed S Hussein[1, †], Huajun Zhang[3, †, ‖], Rui Mao[1], Pierce Brody[1], Yibing Peng[1], Aiwu Ruth He[4], Mercy Kehinde-Ige[1], Ramses Sadek[1], Xiangguo Qiu[3], Huidong Shi[1], , and **Yukai He**[1, 2,* †]These authors contributed equally to this work.

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Background and Aims: The chimeric antigen receptor engineered T cells (CARTs) for hepatocellular carcinoma (HCC) and other solid tumors are not as effective as they are for blood cancers. CARTs may lose their function inside tumor lesions due to persistent strong antigen engagement. The aims of this study is to develop intermediate-affinity monoclonal antibodies (mAb) and intermediate-avidity CARTs for HCC and to test the hypothesis that intermediate-avidity CARTs can resist exhaustion and apoptosis and maintaineffector functions in solid tumors, generating durable antitumor effects. **Methods and results:** We developed 3 new human glypican-3 (hGPC3)-specific mAbs from immunized mice. The mAbs only stained HCC tumors, but not the adjacent normal liver tissues. One of them, 8F8, bound an epitope close to that of GC33, the frequently used high-affinity mAb, but with ~17 fold lower affinity. We then built and compared the intermediate-avidity 8F8 CARTs to high-avidity GC33 CARTs for their *in vitro* function and antitumor effects. *In vitro*, 8F8 CARTs killed both $hGPC3^{high}$ and $hGPC3^{low}$ HCC tumor cells to the same extent as GC33 CARTs. However, the expansion of 8F8 CARTs was 3-5 folds more than GC33 CARTs after engaging with target tumor cells. 8F8 CARTs were less exhausted andless apoptotic than high-avidity GC33 CARTs. The expansion advantage of 8F8 CARts was maintained under hypoxia culture condition. Importantly, the 8F8 CARTs also expanded and persisted to a greater extent than GC33 CARTs *in vivo,* resulting in durable responses against HCC xenografts. Compared to GC33 CARTs, there were 5 folds

more 8F8-BBz CARTs in the tumor mass for a longer period of time. Remarkably, the tumor infiltrating 8F8 CARTs were less exhausted and apoptotic, and more functional than GC33 CARTs. **Conclusion**: The novel intermediate-avidity 8F8-BBz CART resists exhaustion and apoptosis inside solid tumors, demonstrating a greater therapeutic potential than high-avidity CARTs.

Workshop #28:

W28-1:

Microglial Mechanism of interneuron development

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Cortical interneurons provide the main cortical inhibitory input and play a critical role in maintaining circuit rhythm and excitation-inhibition balance in the brain. Cortical interneuron deficits have been implicated in multiple neurological disorders including autism spectrum disorders. Microglia, the tissue macrophage in the brain, regulate interneuron development and presumably serve as cellular target of maternal immune activation (MIA) and interneuron deficit. However, the underlying molecular mechanisms remain largely unknown. Here, we investigate how microglia regulate interneuron formation during normal development and in setting of MIA. In mouse model, we demonstrate that MIA induce microglial *Gpr56* downregulation and that conditional deletion of microglial *Gpr56* (*Gpr56* cKO) results in similar parvalbumin positive (PV^+) interneuron deficits and autism-like behaviors as observed in MIA offspring. Genetically restoring *Gpr56* expression in microglia ameliorates PV^+ interneuron deficits and autism-like behaviors in MIA offspring. To study how microglia regulate human interneuron development, we performed single nucleus RNA sequencing (snRNAseq) using four human postmortem brains ranging from third trimester to 16 weeks of age. Eleven unique cell clusters were identified. Among those, we performed a comprehensive CellChat ligand-receptor interaction study to identify potential communications from microglia to interneurons. Interestingly, Insulin-like growth factor 1 (IGF1) and IGF1R pathway was among the most probable communications between microglia and interneurons. Epidemiological studies in human revealed that IGF1 has been implicated in ASD and epilepsy. Further expression analysis showed that IGF1 was expressed by a subset of microglia, whereas IGF1R was highly expresses in interneuron progenitors and young interneuron. Ongoing studies are carried out to further characterize this pathway in human interneuron generation using human induced pluripotent stem cells (iPSC)-derived neuroimmune.

W28-2:

Modeling microglia pathology in Down syndrome and Alzheimer's disease using human iPSC models

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My lab focuses on studying the cellular and molecular basis of human neural development and pathogenesis of neurological disorders by using human patient-derived induced pluripotent stem cells (iPSCs). In my talk, I will introduce our recent work on using human iPSC-based cerebral organoid and human-mouse chimeric brain models to study Down syndrome (DS) microglia. Microglia are critical for brain development and play a central role in Alzheimer's disease (AD) etiology. DS, caused by trisomy of human chromosome 12 (Hsa21), is the most common genetic developmental disorder and the most common risk factor for AD. Surprisingly, little information is available on the impact of trisomy of Hsa21 on microglial functions during DS brain development and in AD in DS. We find that DS microglia exhibit enhanced synaptic pruning function, which alters neuronal synaptic functions. In response to human brain tissue-derived pathological tau, DS microglia undergo cellular senescence and exhibit elevated type I interferon signaling. Mechanistically, knockdown of Hsa21-encoded type I interferon receptors, *IFNARs*, rescues the DS microglial phenotypes both during brain development and in response to pathological tau. Our findings provide *in vivo* evidence that human microglia respond to pathological tau by exhibiting dystrophic phenotypes. Targeting IFNARs may improve DS microglial functions and prevent senescence.

W28-3:

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

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In the developing central nervous system, oligodendrocyte precursor cells (OPCs) differentiate into oligodendrocytes, which form myelin around axons. Oligodendrocytes and myelin are essential for the function of the central nervous system, as evidenced by the severe neurological symptoms that arise in demyelinating diseases such as multiple sclerosis and leukodystrophy. Although many cell-intrinsic mechanisms that regulate oligodendrocyte development and myelination have been reported, it remains unclear whether interactions among oligodendrocyte-lineage cells (OPCs and oligodendrocytes) affect oligodendrocyte development and myelination. Here, we show that blocking vesicle-associated membrane protein (VAMP) 1/2/3-dependent exocytosis from oligodendrocyte-lineage cells impairs oligodendrocyte development, myelination, and motor behavior in mice. Adding oligodendrocyte-lineage cell-secreted molecules to secretion-deficient OPC cultures partially restores the morphological maturation of oligodendrocytes. Moreover, we identified L-type prostaglandin D synthase as an oligodendrocyte-lineage cell-secreted protein that promotes oligodendrocyte development and myelination *in vivo*. These findings reveal a novel autocrine/paracrine loop

model for the regulation of oligodendrocyte and myelin development.

W28-4:
In vivo multilineage reprogramming of adult astrocytes
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Neural injury or neurodegeneration frequently leads to irreversible loss of neurons; however, the adult mammalian central nervous system (CNS) has largely lost the ability to produce new neurons. A key question in the regeneration field is how to generate new neurons for functional reconstruction in the adult CNS. Our lab has taken an in vivo reprogramming approach, which is to engineer the fate of resident glial cells for neural regeneration. Our recent in vivo screens identified that the transcription factor DLX2 can reprogram adult striatal astrocytes to become ASCL1+ induced neural progenitor cells (iNPCs). Single-cell RNA-seq and pseudotime trajectories reveal a neural stem cell-like behavior of the reprogrammed astrocytes, transitioning from quiescence to activation, proliferation, and neurogenesis. The reprogramming process also induces endogenous DLX family transcription factors and requires suppression of Notch signaling. Most interestingly, genetic lineage tracing showed that iNPCs can give rise to multiple cell types including neurons, astrocytes and oligodendrocytes. Such multilineage reprogramming of resident astrocytes may be further exploited for regenerative medicine.

W28-5:
HAP40 in Huntingtin Regulation and Huntington's disease Pathogenesis
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Perturbation of huntingtin (HTT)'s physiological function is one postulated pathogenic factor in Huntington's disease (HD). However, little is known how HTT is regulated *in vivo*. In a proteomic study, we isolated a novel ~40kDa protein as a strong binding partner of *Drosophila* HTT and demonstrated it was the functional ortholog of HAP40, an HTT associated protein shown recently to modulate HTT's conformation but with unclear physiological and pathologic roles. We showed that in both flies and human cells, HAP40 maintained conserved physical and functional interactions with HTT. Additionally, loss of HAP40 resulted in similar phenotypes as HTT knockout. More strikingly, HAP40 strongly affected HTT's stability, as depletion of HAP40 significantly reduced the levels of endogenous HTT protein while HAP40 overexpression markedly extended its half-life. Conversely, in the absence of HTT, the majority of HAP40 protein were degraded, likely through the proteasome. Further, the affinity between HTT and HAP40 was not significantly affected by polyglutamine expansion in HTT, and contrary to an early report, there were no abnormal accumulations of endogenous HAP40 protein in HD cells from mouse HD models or human patients. Lastly, when tested in *Drosophila* models of HD, HAP40 partially modulated the neurodegeneration induced by full-length mutant HTT while showed no apparent effect on the toxicity of mutant HTT exon 1 fragment. Together, our study uncovers a conserved mechanism governing the stability and *in vivo* functions of HTT and demonstrates that HAP40 is a central and positive regulator of endogenous HTT. Further, our results support that mutant HTT is toxic regardless of the presence of its partner HAP40, and implicate HAP40 as a potential modulator of HD pathogenesis through its multiplex effect on HTT's function, stability and the potency of mutant HTT's toxicity.

W28-6:
Phase separation and zinc-induced liquid-to gel phase transition modulate synaptic distribution and association of autism-linked CTTNBP2 and SHANK3
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Many synaptic proteins form biological condensates via liquid-liquid phase separation (LLPS). Synaptopathy is a key feature of autism spectrum disorders (ASD), partly attributed to the phase separation and transition properties of ASD-linked synaptic proteins. Here, we report that LLPS and zinc-induced liquid-to-gel phase transition regulate the synaptic distribution and protein-protein interaction of cortactin-binding protein 2 (CTTNBP2), an ASD-linked protein. CTTNBP2 is a brain-specific cytoskeleton associated protein and highly enriched at dendritic spines in mature neurons. It forms self-assembled condensates through its C-terminal intrinsically disordered region and facilitates SHANK3 co-condensation at dendritic spines. Zinc binds the N-terminal coiled-coil region of CTTNBP2, promoting higher-order assemblies. Consequently, it leads to reduce CTTNBP2 mobility and enhance the stability and synaptic retention of CTTNBP2 condensates. Moreover, ASD-linked mutations alter condensate formation and synaptic retention of CTTNBP2 and impair mouse social behaviors, which are all ameliorated by zinc supplementation. Our study suggests the relevance of condensate formation and zinc-induced phase transition to the synaptic distribution and function of ASD-linked proteins.
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Shih, P.-Y., Hsieh, B.-Y., Tsai, C.-Y., Lo, C.-A., Chen, B. E., **Hsueh Y.-P.*** (2020) Autism-linked mutations of CTTNBP2 reduce social interaction and impair dendritic spine formation via diverse mechanisms. ***Acta Neuropathologica Communications*** 8:185.

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Workshop #29:

W29-1:

Reprogramming tumor microenvironment by a second-generation recombinant modified vaccinia virus Ankara

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Immune checkpoint blockade (ICB) therapy has brought hope to many cancer patients, but the response rate is low in many cancer types and acquired resistance to ICB can develop over time. Oncolytic or non-oncolytic viruses are promising therapeutic agents for advanced cancers. Modified vaccinia virus Ankara (MVA) is an attenuated, replication-deficient poxvirus safe for human use, making it a favorable platform for cancer immunotherapy. Our first-generation recombinant MVA has shown promising antitumor efficacy in multiple murine tumor models due to the deletion of the E5R gene (encoding an inhibitor of the DNA sensor cGAS) from the MVA genome and the insertion of two membrane-anchored transgenes – Flt3L and OX40L, which leads to the activation of the host innate and adaptive antitumor immunity. Here in this study, we engineered our second-generation recombinant MVA (MQ833) with the deletion of two more viral immune evasion genes – E3L and WR199, and the insertion of IL12 anchored to the extracellular matrix to mitigate toxicity. Intratumoral (IT) delivery of MQ833 resulted in an 80-100% cure in the mouse B16-F10 melanoma model, which is dependent on both the cytosolic DNA and dsRNA-sensing pathways mediated by STING and MDA5, and the IFN signaling pathway mediated by STAT2. Single-cell RNA sequencing analysis revealed that IT MQ833 injection reprogrammed the tumor microenvironment into an immune-stimulating state, by activating $CD8^+$ and $CD4^+$ T cells, depleting regulatory T cells and M2 macrophages, recruiting and activating neutrophils, and polarizing M1 macrophages. Interestingly, IT MQ833 treatment cured 70% of B2M knock-out melanomas likely due to combined effects of IL-12 and type I and II IFN. Loss of MHC-I is the most common mechanism of tumor resistance to ICB. Hence, our results support the use of MQ833 for ICB-resistant tumors.

W29-2:

Overcoming tumor immune resistance by targeting tumor intrinsic pathways

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Background: Despite approval of immunotherapy for wide ranges of cancers, the majority of patients fail to respond to immunotherapy which is partially attributed to immunosuppression co-opted by tumor cells. However, it is challenging to utilize conventional methods to systematically evaluate the potential of tumor intrinsic factors to act as immune regulators in cancer patients.
Methods: An unbiased integrative strategy were designed to leverage the complementary strength of in vitro functional genomic screens and multi-omics clinical dataset to assess roles of individual tumor-intrinsic factors in regulating T cell tumor infiltration and T cell-mediated tumor killing, the two most important rate-limiting steps of cancer immunotherapy. Initially, a genome-wide CRISPR-Cas9 screening system using paired murine tumors and tumor-reactive T cells was employed to globally screen tumor intrinsic factors modulating the tumor sensitivity to T cell-mediated killing. Then, findings from the screening were integrated with the bioinformatics analysis of clinical datasets to further evaluate roles of each tumor intrinsic factor in governing antitumor immunity.
Results: The integrative analysis successfully identified several novel tumor intrinsic factors as effectors of immune resistance, but also demonstrated distinct roles of these factors in controlling immune cell trafficking and tumor sensitivity to T cell-mediated killing. Among these factors, candidates controlling both rate-limiting steps of T cell tumor infiltration and T cell-mediated tumor killing were termed as "Dual immune resistance regulators" and the remaining factors whose expression levels were not associated with tumor immune infiltration were termed as "Cytotoxicity resistance regulators". By selecting PRMT1 and RIPK1 as the representatives of these two groups respectively, we confirmed that genetically depletion of PRMT1 and RIPK1sensitized tumors to T-cell mediated killing via two independent experimental approaches. Furthermore, Type I PRMT inhibitor combined with anti-PD1 treatment significantly extended the survival of tumor-bearing mice and delayed tumor growth in a panel of immunocompetent mouse models. Mechanistically, Type I PRMT inhibitor significantly increased the apoptotic sensitivity of tumor cells to autologous tumor-reactive T cells *in vitro* and the infiltration of total T cells and cytotoxic T cells in multiple murine tumor models.
Conclusions: Collectively, our data not only demonstrate distinct immunoregulatory roles and therapeutic potentials of PRMT1 and RIPK1 in T cell-mediated antitumor activity, but also provide a rich resource of novel targets for rational immuno-oncology combinations.

W29-3:

Rational design of vaccinia virus for cancer therapy

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Oncolytic vaccinia virus (VV) is promising anti-cancer agents owing to their ability to infect, replicate in, and lyse tumor cells, and spread to other tumor cells in successive rounds of replication. VV therapy has shown promise in clinical studies. Complete clinical responses, however, have been rarely observed, highlighting the need for further improvement. The effectiveness of oncolytic viruses is limited by several factors. First, the use of viruses as the cancer treatment delivery vectors is significantly limited by the strong immune response induced by the virus. Immune factors such as antibodies neutralize the virus by binding to it directly and preventing a successful infection of the cells or by marking it for destruction either by complement or by other immune cells. Second, virus infiltration into and spread within the tumor is restricted by the dense extracellular matrix (ECM) or stroma around tumor cells, which is primarily composed of cancer-associated fibroblasts (CAFs) that are present in the majority of common epithelial cancers, sarcomas, and melanomas. In this study, we developed a T-cell

Engager-Armed Neutralization Escape Variant of Oncolytic Vaccinia Virus (TEA-NEV) platform technology. Our studies have demonstrated that our lead product, CAF-targeted TEA-NEV, would overcome the limitations by escaping Nabs and inducing T-cell mediated destruction of tumor stroma. Our objective is two-fold: 1) to generate and characterize a next-generation oncolytic VV with the ability of escaping the host's neutralization antibody response, thus allowing a systemic delivery of the recombinant virus to the tumor cells; 2) to combine the T-cell engager (TE), CD3-scFv with FAP-scFv to create the bispecific FAP-TE antibody expressed by VV that binds to both the human T-cell CD3 antigens and tumor and CAF stromal surface FAP antigens resulting in enhanced specific tumor infiltration, spread and lysis activity as well as the by-stander killing of tumor cells.

W29-4:

Th9 cells represent a new paradigm of CD4+ T cells endowed with the ability to eradicate advanced tumors

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Resistance can occur in patients receiving adoptive cell therapy (ACT) due to cancer cells that escape the immune attack by becoming antigen-loss variant cancer cells (ALVs). Here we demonstrated that murine and human Th9 cells, but not Th1, Tc1, and Th17 cells, expressing tumor-specific T-cell-receptors (TCR) or chimeric antigen receptors (CAR), eradicate advanced tumors that contain 10% ALVs. This unprecedented antitumor capacity of Th9 cells is attributed to both enhanced direct killing of tumor cells and bystander antitumor effects promoted by the intratumor release of IFNα/β. Mechanistically, tumor-specific Th9 cells increased the intratumor accumulation of extracellular ATP (eATP, released from dying tumor cells), because of a unique feature of Th9 cells that lack the expression of the ATP degrading ectoenzyme CD39. Intratumor enrichment of eATP promoted the infiltration of monocytes and stimulated their production of IFNα/β by inducing the activation of an eATP-endogenous retrovirus-TLR3/Mavs. These results identify tumor-specific Th9 cells as a unique T cell subset endowed with the unprecedented capacity to eliminate ALVs for curative responses and hold promise to significantly advance the therapeutic index of ACT.

W29-5:

Delving into proteostasis network reveals an immunogenic vulnerability in mismatch repair deficient cancer

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Proteostasis network is central in maintaining genome and proteome stability in the cell. Deficient DNA mismatch repair (dMMR) induces a hypermutator phenotype, leaving a genomic scar known as microsatellite instability (MSI). MSI is observed in approximately 30% of endometrial cancers, 20% of gastric cancers, 15% of colorectal cancers, and in a smaller fraction of other tumor types. This hypermutator phenotype is thought to produce large numbers of immunogenic neoantigens, leading to the approval of MSI status as a clinical biomarker for immunotherapy. However, more than 60% of patients with MSI tumors fail to respond to immune checkpoint therapy. To uncover alternative therapeutic vulnerabilities for these patients, we use transcriptome signature-guided approaches to identify MLN4924 (pevonedistat), a Nedd8-activating enzyme inhibitor, as a potential therapy for dMMR/MSI cancers. We discover that destabilizing mutations from the dMMR mutation process lead to rampant proteome instability in MSI tumors, resulting in an abundance of misfolded protein aggregates. To compensate, MSI cancer cells activate a Nedd8-mediated degradation pathway to facilitate clearance of misfolded proteins, which is blocked by treatment with MLN4924. The accumulation of misfolded proteins in MSI cancer cells following MLN4924 treatment activates the unfolded protein response, promotes immune cell migration, and induces immunogenic cell death. Antitumor vaccination with MLN4924-treated cells stimulates the generation of endogenous tumor antibodies and prevented tumor incidence upon re-challenge. Based on this immunostimulation, we combine MLN4924 with PD1 blockade, finding that the combination increases recruitment of CD8+ lymphocytes and improves therapeutic efficacy beyond either treatment alone. Taken together, our results indicate that targeting proteome instability is a novel therapeutic avenue for MSI patients and may potentiate immune checkpoint blockade, potentially increasing the depth and duration of response, as well as the fraction of dMMR/MSI patients who can benefit.

W29-6:

Cancer mutation landscape reveals marker genes and molecular regulators of immunotherapy efficacy

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High tumor mutation burden has emerged as a predictive biomarker for efficacy of immunotherapy. However, accurately defining a high tumor mutation burden across the whole genome for prediction of immunotherapy efficacy is still quite challenging. Therefore, identifying robust biomarkers of tumor mutation burden would be a key solution to guide cancer immunotherapy decision. We hereby performed an integrative analysis of TCGA datasets from nearly 10,000 patients to investigate the cancer mutation landscape and identified a panel of mutator genes, of which the functions are to protect the genome from mutations. The deficiency of these newly identified mutator genes is associated with severe mutation load and strong activation of immune response. Integrating the deficiency of mutation regulators and clinical response of patients treated with immune checkpoint blockade, we demonstrated that these cancer mutation regulators are robust prognostic biomarkers for strong positive effect of immunotherapy. Therefore, the mutator genes identified by our novel computation strategy will be a useful resource to improve efficiency of immune checkpoint blockade in cancer immunotherapy.

Workshop #30:

W30-1:

The role of VMP1 in regulating hepatic lipoprotein secretion and NASH

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Hepatic lipid accumulation is a hallmark of nonalcoholic fatty liver disease (NAFLD), which may progress to the more severe nonalcoholic steatohepatitis (NASH). Hepatic lipid accumulation is due to increased hepatic lipid input (such as increased uptake of lipids from circulation and de novo synthesis) that exceeds hepatic lipid output (such as impaired mitochondrial oxidation and secretion of very-low-density lipoprotein (VLDL)). Deciphering novel pathways regulating liver lipid content has profound implications for understanding the pathophysiology of NAFLD and NASH. Vacuole membrane protein 1 (VMP1) is an endoplasmic reticulum (ER) transmembrane protein that regulates the formation of autophagosomes and lipid droplets. Recent evidence suggests that VMP1 plays a critical role in lipoprotein secretion in zebra fish and cultured cells. However, the pathophysiological roles and mechanisms by which VMP1 regulates lipoprotein secretion and lipid accumulation in NAFLD and NASH are unknown. Hepatic VMP1 KO mice were generated and characterized for lipid and energy metabolism, metabolomics and transcriptome analyses. Hepatic VMP1 overexpressed mice fed on a NASH diet were also characterized. We found that hepatic deletion of VMP1 severely impaired VLDL secretion resulting in massive hepatic steatosis, hepatocyte death, inflammation and fibrosis, which are hallmarks of NASH. Mechanistically, loss of VMP1 led to decreased hepatic levels of phosphatidylcholine and phosphatidylethanolamine as well as the changes of phospholipid composition. Deletion of Vmp1 in mouse liver also led to neutral lipids accumulated in ER bilayer and impaired mitochondrial beta-oxidation. Hepatic deletion of Vmp1 also altered ER-mitochondrial contact site. Overexpression of VMP1 ameliorated steatosis in diet-induced NASH by improving VLDL secretion. We also found that decreased liver VMP1 is associated with NAFLD/NASH in humans. Our results provide novel insights on the role of VMP1 in regulating hepatic phospholipid synthesis and lipoprotein secretion in the pathogenesis of NAFLD/NASH.

W30-2:

Guarding against fatty liver disease by sirtuin 6

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Liver plays a critical role in metabolic homeostasis. Overnutrition often causes triglyceride accumulation in the liver and leads to the development of nonalcoholic fatty liver disease (NAFLD) over time. However, the underlying molecular mechanisms remain incompletely understood. Sirtuin 6 (Sirt6) is a key epigenetic factor that has been implicated in the regulation of glucose and lipid metabolism. We used both cell and animal models to investigate the function of Sirt6 in the hepatic lipid homeostasis and NAFLD pathogenesis. Our data have revealed that Sirt6 inhibits hepatic lipogenesis by suppression of several major lipogenic transcription factors including liver X receptor, sterol regulatory element binding protein 1, and carbohydrate response element binding protein. Sirt6 directly interacts and deacetylates these transcription factors in hepatocytes. In addition, Sirt6 also plays a critical role in hepatic stellate cells, which are a predominate cell population contributing to liver fibrosis. Our data have illustrated that Sirt6 restraints the hepatic stellate cell activation by suppression of the two major fibrogenic pathways – transforming growth factor beta and Hippo signaling. Specifically, the downstream transcription factors SMAD family member 3 and Yes-associated protein 1 are direct substrates of Sirt6. Overall, our data suggest that Sirt6 is a crucial epigenetic regulator for maintaining hepatic function and protecting against NAFLD.

W30-3:

Histone deacetylase-11 (HDAC11) as a novel therapeutic target for non-alcoholic steatohapatitis (NASH)

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HDAC11 is the newest member of the HDAC family and the only Class-IV HDAC. HDAC11 deficiency decreases high-fat diet-induced hepatic steatosis, suggesting HDAC11 may play an important role in NASH development. This study investigated whether LP110, a novel small molecule HDAC11 inhibitor with high selectivity and potency, could be used for NASH treatment and its underlying mechanisms of action. Mice were fed a Western diet (WD) with high-fat, high-fructose, and high-cholesterol for up to 8wks. LP110 or vehicle were given during the last 2wks of WD-feeding. WD feeding markedly increased hepatic HDAC11 expression. After 6wk-WD, overt steatosis with large numbers of macrovesicular fat droplets, ballooning degeneration, leukocyte infiltration and necroinflammatory foci formation were observed, indicating occurrence of NASH at 6wk. These pathological events continued to increase slightly at 8wk. LP110 decreased all pathological changes to even lower than that in 6wk, indicating that LP110 not only blocked the progression but even reversed the pathological changes of NASH. LP110 also markedly decreased the liver weight/body weight ratio, apoptosis (cleaved caspase-3), inflammation (MPO, NLRP3, IL-1β), and profibrotic responses (TGFβ1, αSMA) after 8wk-WD. Hepatic ATP level decreased after 8 wk-WD feeding, and mitochondrial depolarization occurred in living mice; both were blunted by LP110. PGC-1α, the master regulator of mitochondrial biogenesis and energy metabolism, and Cpt1, the enzyme that carries fatty acyl-CoA into mitochondria for β-oxidation, decreased by 8wk-WD. LP110 blunted the changes in PGC-1α and Cpt1. After WD-feeding, LP110 also increases brown adipose tissue mass and PGC-1α and UCP-1 expression, decreases blood glucose and blunted increase of serum insulin. In conclusion, HDAC11 is a novel therapeutic target and our new HDAC11 inhibitor is a promising therapy for NASH. LP110 works intrahepatically and extrahepatically and most likely, at least in part, through promoting energy metabolism and fatty acid catabolism (NIDDK, NIGMS & NIAAA).

W30-4:

Role of Fatty Acid Desaturase 1 (FADS1) in Non-alcoholic Fatty Liver Disease (NAFLD)

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Background & aims: Imbalanced n-3 and n-6 polyunsaturated fatty acids (PUFAs) levels in both blood and the liver is associated with NAFLD. Fatty acid desaturase 1 (FADS1, encoding delta 5 desaturase) is one of the key rate-limiting enzymes in the production of n-6 and n-3 long-chain PUFAs (LC-PUFAs), i.e., arachidonic acid (AA, n-6) and eicosapentaenoic/docosahexaenoic acids (EPA/DHA, n-3). Our previous studies have demonstrated that reduced function of FADS1 due to genetic polymorphisms in the human population is associated with NAFLD. In this study, we used both *in vitro* and *in vivo* models to explore the causal mechanism by which FADS1 ameliorates NAFLD as well as the impact of FADS1 status on the therapeutic efficacy of n-3 PUFA supplementation.
Methods: Mice with three Fads1 genotypes [wide-type (WT), heterozygous (HET), homozygous null (KO)] were fed a high-fat, high fructose, high cholesterol diet (AMLN diet) for 32 weeks to induce steatohepatitis (NASH). After 24 weeks of feeding, mice were either supplemented with fish oil containing EPA/DHA or continued on the AMLN diet for additional 8 weeks. Liver metabolomics and transcriptomics were performed. Primary hepatocytes isolated from low-density lipoprotein receptor (Ldlr)-KO mice as well as HepG2 and LX-2 cells were used to conduct mechanistic analyses.
Results: Fads1-KO mice developed significantly more severe NASH phenotypes after being fed the AMLN diet for 32 weeks compared to WT mice. Supplementation with n-3 LC-PUFAs largely ameliorated the severity of NASH in KO mice. Omics analyses revealed that Fads1-KO altered the composition of phospholipids as well as cholesterol homeostasis. Fads1-KO mice exhibited an increased hepatic but a decreased plasma cholesterol level as compared to WT mice. From a mechanistic perspective, inhibition or knockdown of FADS1 significantly increased Ldlr-mediated internalization of cholesterol into both HepG2 and LX-2 cells, resulting in the accumulation of lipid droplets and stellate cell activation, respectively. The increased intracellular level of cholesterol significantly activated mTOR signaling pathway in HepG2 cells and primary mouse hepatocytes.
Conclusion: Reduced FADS1 function contributes to the progression of diet-induced NASH via the remodeling of phospholipids profiles and the upregulation of cholesterol-modulated mTOR signaling axis. Therapeutic dietary supplementation with n-3 LC-PUFAs attenuated FADS1-associated NASH.

W30-5:
Role of microbiota metabolite indole in NAFLD/NASH
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As a microbiota metabolite that is anti-inflammatory, indole, along with its derivatives, has been increasingly shown to be inversely corelated with the degrees of systemic insulin resistance and non-alcoholic fatty liver disease (NAFLD). To gain the mechanistic insights of indole actions, the present study examined the extent to which indole supplementation alleviates the severities of NAFLD and non-alcoholic steatohepatitis (NASH), the advanced form of NAFLD, using various mouse models. In C57BL/6J mice with high-fat diet (HFD)-induced NAFLD, hepatic indole levels were decreased relative to those in control mice, accompanied with increased severity of NAFLD phenotype and altered composition of gut microbiota. These aspects of NAFLD were significantly alleviated in response to indole supplementation. Furthermore, the effect of indole on alleviating HFD-induced NAFLD phenotype in mice was nearly blunted by the lack of PFKFB3, a master regulator of glycolysis, in myeloid cells. In a mouse model of NASH, indole supplementation alleviated the severity of methionine- and choline-deficient diet (MCD)-induced hepatic steatosis and inflammation, as well as liver fibrosis. In the *in vitro* systems, indole treatment significantly decreased lipopolysaccharide (LPS)-induced proinflammatory responses in hepatocytes incubated with either basal or MCD-mimicking media. However, indole treatment only decreased LPS-induced proinflammatory responses in bone marrow-derived macrophages incubated with basal, but not MCD-mimicking media. While indole supplementation decreased liver expression of desmin in MCD-fed mice, treatment of LX2 cells (a line of hepatic stellate cells (HSC)) with indole decreased the expression of various markers of HSC fibrogenic activation. Lastly, indole supplementation decreased intestinal inflammation in MCD-fed mice, evidenced by decreased intestinal accumulation of CD68 cells and CD11c expression. Collectively, these results demonstrate that indole supplementation alleviates NAFLD/NASH phenotypes via suppressing the proinflammatory responses in multiple cell types and through decreasing HSC fibrogenic activation. Additionally, indole mimetics may offer a therapeutic approach for managing NAFLD/NASH.

W30-6:
The Requirement and Sufficiency of Hepatocyte Toll-like Receptor 4 (TLR4) in Alcohol-associated Insulin Resistance
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Excessive alcohol drinking is associated with dysregulated insuln response in both human subjects and rodent models. However, the underlying mechanisms and critical factors that impair insulin signaling remain unclear. Previously it has been report that hepatocyte toll-like receptor 4 (TLR4) plays an importantrole in regulating obesity-associated hepatic steatosis, chronic inflammation and insuln resistance. In addition, hepatocyte TLR4 deficient mice ($Tlr4^{\Delta Hep}$) are protected from alcohol-induced fatty liver disease. These findings led us to investigate the requirement of hepatocyte TLR4 in alcohol overconsumption- associated insulin resistance. To answer this question, both $Tlr4^{\Delta Hep}$ mice and their littermate controls ($Tlr4^{fl/fl}$) were fed either control or alcohol-containing liquid diet chronically for 8 weeks. Glucose tolerancetest (GTT), insulin tolerance test (ITT) and insulin signaling experiments were performed to examine systemic and tissue-specific insulin sensitivity in mice. We found that alcohol-fed $Tlr4^{\Delta Hep}$ mice, comparedwith littermate $Tlr4^{fl/fl}$ mice, had lower blood glucose levels in response to intraperitoneal injection of insulin, indicating enhanced whole-body insulin sensitivity. Moreover, increased protein expression of phosphorylated glycogen synthase kinase-3β (GSK3β) were observed in the liver and epididymal white adipose tissue (eWAT) of $Tlr4^{\Delta Hep}$ mice after chronic alcohol intake compared with that of $Tlr4^{fl/fl}$ mice. Next, to explore the sufficiency of hepatocyte TLR4 in alcohol-induced metabolic abnormality, we

took advantage of the TLR4 reactivatiable mouse model ($Tlr4^{LoxTB}$), which allows us to restore the endogenous TLR4 expression only in hepaotocytes ($Tlr4^{LoxTB}$ x Alb-Cre). GTTs showed that $Tlr4^{LoxTB}$ x Alb-Cre mice had elevated blood glucose levels compared with littemate control $Tlr4^{LoxTB}$ mice after long-term alcohol- containg liquid diet feeding. In addition, dramatically reduced phosphorylated Akt protein expression wereobserved in the liver and eWAT of alcohol-fed $Tlr4^{LoxTB}$ x Alb-Cre mice. Taken together, these findings suggest that hepatocyte TLR4 is both required and sufficient in the development of insulin resistance induced by alcohol overconsumption.

Workshop #31:

W31-1:

anti-cancer compound JTE-607-mediated inhibition of pre-mRNA 3' processing is sequence-dependent

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Mammalian pre-mRNA 3' processing takes place in two steps, cleavage and polyadenylation. The cleavage step is catalyzed by CPSF73. JTE-607, a small molecule with anti-cancer activities, has recently been shown to block pre-mRNA 3' processing. JTE-607 is a pro-drug and is hydrolyzed to Compound 2 (Cmp 2) in cells, which binds directly to CPSF73 and inhibits its nuclease activity. As CPSF73 is a general pre-mRNA 3' processing factor, JTE-607 is expected to block the cleavage of all pre-mRNAs. Surprisingly, however, our nascent RNA sequencing and 3' end sequencing analyses of JTE607-treated cells revealed a sequence-specific inhibition of pre-mRNA cleavage.
To characterize the molecular basis of the sequence-specific effect of JTE-607, we tested the effect of Cmp2 on pre-mRNA cleavage in vitro using over 30 different poly(A) site RNAs. Our results confirmed that the inhibitory effect of Cmp2 is sequence-dependent and showed that the sensitivities of different poly(A) site sequences to Cmp2 can differ by over a hundred-fold. Through mutational analyses, we have identified a specific region within poly(A) sites that determine Cmp2 sensitivity. Next, we have performed a massively parallel screen of over 2 million random sequences and identified specific sequence motifs that render a poly(A) site sensitive or resistant to Cmp2. Finally we studied how these sequence motifs are detected by the pre-mRNA 3' processing machinery to influence the inhibitory effect of Cmp2. Together these results not only revealed the molecular basis of the sequence-specific effect of JTE-607, but also provided novel insights into the fundamental mechanisms of pre-mRNA 3' processing.

W31-2:

Human PRPF39 is a new alternative splicing factor affecting weak 5' splice sites

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Splicing, an essential process required for eukaryotic gene expression, is catalyzed by the spliceosome, which is composed of U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins (snRNPs) and other non-snRNP associated proteins. Each splicing event follows a stepwise splicing cycle to remove introns and ligate exons of a pre-mRNA to assemble a mature mRNA. U1 snRNP recognizes the 5' splice site (ss) of the pre-mRNA through U1 snRNA base pairing and is a critical component to begin this process. Therefore, U1 snRNP is a common target for alternative splicing factors which can alter the binding of this complex to the pre-mRNA 5'ss. Due to the transient nature of the interaction between these alternative splicing factors and U1 snRNP, it has been difficult to capture these interactions structurally. On the other hand, the yeast homologs of many human alternative splicing factors stably associate with the yeast U1 snRNP. We therefore determined the yeast U1 snRNP cryo-EM structure to use as a model in understanding the mechanism of human alternative splicing. The structure demonstrates that the yeast U1 snRNP core is structurally similar to the human U1 snRNP with seven additional proteins (including the Prp39/Prp42 paralogs) stably associated with the yeast U1 snRNP core. Using the yeast U1 snRNP structure as a model, we demonstrated that human PRPF39 acts as a homodimer, serving as the counterpart of the yeast Prp39/Prp42 heterodimer. We next showed that human PRPF39 is a previously unrecognized alternative splicing factor that affects weak 5' splice sites. We further characterized the interaction between human PRPF39 and several other alternative splicing factors with U1 snRNP, revealing significant parallels to the yeast U1 snRNP structure and supporting the yeast U1 snRNP as a valuable model for understanding the mechanism of human alternative splicing.

W31-3:

Alternative polyadenylation-wide association study (3′aTWAS) identifies novel APA-linked susceptibility genes in human disease.

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Despite the emerging evidence that alternative polyadenylation (APA) plays an essential role in disease progression and that risk SNPs identified in many disorder genome-wide association studies (GWAS) are enriched near 3′UTRs, current transcriptome-wide association studies (TWAS) largely overlook APA in nominating susceptibility genes. Here, we performed the first TWAS study of 3′UTR usage (3′aTWAS) for 11 human genetic disorders by combining their GWAS data with 17,300 RNA-seq samples across 49 tissues from 2,937 individuals. We identified 367 3′aTWAS-significant genes, including previously reported APA risk genes, such as SNCA in Parkinson's disease. Among these susceptibility genes, ~55% are overlooked by conventional expression TWAS and splicing TWAS analyses. APA may regulate the translation, localization and protein-protein interaction (PPI) of the target susceptibility genes independent of mRNA level expression or splicing. We found that modulation of ataxin-3 (ATXN3), a novel 3′aTWAS susceptibility gene for amyotrophic lateral sclerosis (ALS), substantially impacted pathological hallmarks of ALS in vitro. Furthermore, global analyses highlight the convergence of known and novel susceptibility genes in PPI networks and pathways related to disease risk, including autophagy and membrane trafficking pathways. Together, this study suggests that 3′aTWAS is a powerful strategy to nominate novel and important APA-linked disease susceptibility genes for many human diseases.

W31-4:

MAPT splicing, tauopathies and lineage-specific regulation in the primate brain

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Divergence of pre-mRNA alternative splicing (AS) is widespread in mammals including primates, but the underlying mechanisms and functional impact is poorly understood. Here we modeled cassette exon inclusion in primate brains as a quantitative trait and identified 1,170 (~3%) exons with lineage-specific splicing shifts under stabilizing selection. Among them, microtubule associated protein tau (*MAPT*) exon 10 underwent a two-step

evolutionary shift in the Catarrhine and hominoid lineages that led to its present inclusion level in humans, which if disrupted, causes frontotemporal lobar degeneration. We found that this divergence is developmental stage-specific and mediated by divergent distal intronic binding sites recognized by MBNL proteins. Competitive binding of these sites by guide RNAs in complex with dCas13d effectively reduces exon 10 inclusion, potentially providing a therapeutically compatible approach to modulating tau isoform expression. Our data suggests adaptation of *MAPT* function and more generally a role for AS in the evolutionary expansion of the primate brain.

W31-5:
A new function of ribosome: guiding piRNA formation
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Eukaryotic ribosomes generally do not assemble downstream of long open reading frames (ORFs). Here we showed that 80S ribosomes guide the piRNA formation from mRNA 3´UTRs after pioneer rounds of translation in mice and chickens. Rather than a conventional initiation or an 80S readthrough, 80S ribosomes are assembled on 3´UTRs from unrecycled 40S ribosomes from upstream ORFs. This co-translational process also fine-tunes protein production from the precursor mRNAs. Thus, we reveal an unconventional mechanism for ribosomes to assemble on the non-protein coding regions of RNA precursors of piRNAs during germ cell development that is evolutionarily conserved in amniotes.

W31-6:
Circular RNA Microarray Analysis in Mdr2$^{-/-}$ mice
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Chronic cholestatic liver diseases, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), often lead to end-stage liver diseases. No therapeutic agent is available due to a limited understanding of pathogenesis. Circular RNAs (circRNA) are covalently closed natural RNA circles. Recent advances in high-throughput RNA sequencing (RNAseq) and circRNA-specific bioinformatics algorithms have identified thousands of circRNAs with tissue-specific expression patterns. As a large emerging class of ncRNAs, circRNAs have been recently identified as important regulators of various physiological and pathological processes by functioning as "sponges" of miRNAs and RNA binding proteins (RBPs) to sequester them away from their targets. However, the role of circRNAs in liver diseases, especially in cholestatic liver diseases, remains largely unknown. In this study, Arraystar Mouse circRNA Array V2 analysis was used to detect the circRNA expression in wild type and Mdr2$^{-/-}$ mice (C57BL6 background, male, 10-week old, n = 3). The results showed that the majority circRNAs are exonic (74%). We identified 201 upregulated and 43 downregulated circRNAs (fold change ≥1.3, $P < 0.05$) in Mdr2$^{-/-}$mice compared to WT controls. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis further showed that these differentially expressed circRNAs were linked to multiple signaling pathways, including mTOR and Notch signaling pathways, oxidative phosphorylation, and inflammatory process, PI3K and phospholipase pathways. By using Arraystar's prediction software togetherwith TargetScan and mirnet2.0 databases, we identified potential miRNAs which may be involved in disease progression. This study identified potential circRNAs and miRNAs involved in cholestatic liver injury. Compared to other members of ncRNAs and mRNA, circRNAs are remarkably stable. Understanding the regulation of tissue- or cell-specific expression of circRNAs will provide critical information for potential applications of circRNAs as novel diagnostic and prognostic markers and therapeutic targets for various diseases, including cholestatic liver diseases.
Keywords: circular RNA, cholestatic liver injury, miRNAs, expression profile

Workshop #32:

W32-1:

Outcomes of liver transplantation for combined hepatocellular cholangiocarcinoma: a single-center experience and literature review

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Combined hepatocellular cholangiocarcinoma (cHCC-CCA) is a rare, aggressive hepatic malignancy that exhibits histological features of both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). cHCC-CCA is often diagnosed incidentally on liver explant pathology in patients undergoing liver transplantation for the indication of HCC with or without cirrhosis.

We retrospectively reviewed 303 patients who underwent orthotopic liver transplantation for the indication of HCC with or without cirrhosis at Keck Hospital of USC from 2010 to 2020. Patients with cHCC–CCA or both HCC and CCA were identified and were grouped into three phenotypes based on the types of tumor nodules present on explant: cHCC-CCA with the presence of other HCC or CCA nodules, cHCC-CCA alone, and those with both discrete HCC and CCA nodules. Ten patients identified to have cHCC–CCA or both HCC and CCA. The average age of transplantation is 62 years old. In these ten patients, 80% of those transplanted are male and the predominate etiology of cirrhosis was split between alcohol and hepatitis C. Four patients had recurrence of malignancy, all of which occurred within one year after transplant; average time to recurrence was 6.23 months. The average survival time of those deceased was 14 months (median 13.8), and recurrence was the cause of death in all three patients. Three patients in the recurrence group have the phenotype of cHCC-CCA with HCC or CCA on explant. Two patients had cHCC-CCA on recurrence biopsy and concurrently had the two shortest survival times post-transplant. One patient had HCC on biopsy of recurrence site and has the longest survival time of the recurrence group (46.5 months, still alive). No major difference in tumor burden nor tumor differentiation was observed between those with and without hepatic cancer recurrence. Combined hepatocellular cholangiocarcinoma is associated with high post-transplant recurrence rates and poor overall survival. Further investigation should be conducted to aid identification of cHCC-CCA at early stages prior to the liver transplantation.

W32-2:

Role of Eosinophils in Acute Liver Injury

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Eosinophils are a subset of bone marrow-derived granular myeloid cells. For a long time, they were regarded as cytotoxic cells involved in fighting against parasite infections and causing allergic diseases. Emerging evidence expands this view and suggests a critical role of eosinophils in resolving inflammation and promoting tissue remodeling. However, the role of eosinophils in liver injury had not been investigated. Unexpectedly, we detected the recruitment of eosinophils into the liver in several models of acute liver injury caused by ischemia reperfusion (IR) or treatment with acetaminophen, concanavalin (Con)-A, or CCl4. Mice deficient of macrophages or IL-33 exhibited impaired hepatic eosinophil recruitment during acute liver injury. CCL24, but not CCL11, was increased after liver injury in an IL-33 and macrophage-dependent manner. *In vitro* experiments demonstrated that IL-33, through stimulating IL-4 release from eosinophils, promoted macrophages to produce CCL24. Importantly, studies with genetic models of eosinophil deficiency (PHIL or ΔdblGata1 mice) or antibody-mediated eosinophil-depletion revealed exacerbated injury following hepatic IR or treatment of hepatotoxins. In contrast, adoptive transfer of bone marrow-derived eosinophils attenuated liver injury in these models. Mechanistic studies identified a critical role of IL-33, through its receptor tumorigenicity (ST2), in inducing IL-13 production by eosinophils. Together, these studies provide new insight into a novel mechanism of eosinophil-mediated liver protection that could serve as a therapeutic target to improve outcomes of acute liver injury.

W32-3:

Immune response mediated by extracellular matrix in liver fibrosis

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Liver Fibrosis is a consequence of chronic liver inflammation caused by immune cell response and characterized by excessive deposition of extracellular matrix, which can progress to cirrhosis. Cirrhosis is associated with significant liver dysfunction, portal hypertension, ascites, and the development of hepatocellular carcinoma. Although clinical trials for treating fibrosis caused by non-alcoholic fatty liver disease are currently very active, several clinical trials have discontinued. Therefore, we have to understand more precise mechanism of developing liver fibrosis. Recently, we discovered that hyaluronan (HA) is overproduced from hepatic steallte cells (HSCs) by overexpressing hyaluronan synthesis 2 (HAS2). HSC-derived HAS2-mediated HA stimulates both Kupffer cells and HSCs, enhancing liver inflammation and fibrosis. Mechanistically, HA induces migration of Kupffer cells and production of chemokines. HA also contributes to HSC invasion and collagen production, which is mediated by binding to TLR4 and CD44, and upregulation of Notch1 in HSCs. Overexpressed Notch1 in HSCs is stimulated by its ligand Jagged1 expressed in Kupffer cells and HSCs. We also found that HAS2 expression in HSCs is transcriptionally regulated by Wilms Tumor 1 (WT1) induced by TGF-b stimulation. Interestingly, WT1 not only induces HAS2, but also CSF1 and IL-11 to infiltrate myeloid cells. These findings indicate that HSC-derived HA has the bi-directional effects between immune cells and HSCs, which promotes liver fibrosis progression. Lastly, we validated that inhibition of HA production by 4-MU reduced liver fibrosis progression along with reduced HSC activation and liver macrophage infiltration. Thus, HAS2 and HA can be a target for treating liver fibrosis by modurating both immune cell and myfibroblastic cell activities.

W32-4:

Steatosis Promote Liver Cancer Development by Inducing Chemokine Production From Kupffer Cells

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Chronic inflammation is a hallmark for liver cancer where liver steatosis underlies the development of liver cancer. To explore this tumor microenvironment, we employed a model where steatosis drives the development of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) due to loss of tumor suppressor PTEN (phosphatase and tensin homologue deleted

on chromosome 10). In this model, we showed that attenuating steatosis and depletion of macrophages leads to inhibition of liver cancer development. To investigate how these immune cells are recruited to the cancer site and their function, we identified ae chemokine that was commonly upregulated in all tumor model and attenuated in non-tumor models. CXCL5 is a member of the neutrophil-activating chemokines. Analysis of patient proteomic data detected CXCL5 as the primary chemokine that is induced in liver tumors and is significantly upregulated in the Pten deleted tumor mice whereas its expression is inhibited when steatosis is attenuated via caloric restriction or deletion of a metabolic kinase, Akt2. Our analysis further identified Kupffer cells as the source for CXCL5 in tumors. We explored the hypothesis that macrophages secrets CXCL5 to establish the tumor environment and promote tumor growth. This induction of CXCL5 in Kupffer cells appears to be a unique function of Kupffer cells and not macrophages as neither murine macrophage cell lines nor primary peritoneal macrophages displayed induced CXCL5 expression in response to LPS. We showed that CXCL5 induces the proliferation of hepatocytes and liver cancer cells. This effect is further blocked by the inhibition of CXCR2, the receptor of CXCL5, demonstrating the specificity for CXCL5 mediated effects. In summary, our data identified CXCL5 as a novel chemokine produced by Kupffer cells that plays key roles in steatosis driven liver cancer development. Chronic liver diseases such as steatohepatitis can establish tumorigenesis environment through stimulating CXCL5 release from Kupffer cells.

W32-5:

Gasdermin B is an RNA sensor that promotes interferon response and airway inflammation

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Respiratory virus-induced inflammation is the leading cause of asthma exacerbation, often due to over- activated interferon response. How viral infection-induced interferon response subsequently leads to exuberant airway inflammation in genetically susceptible asthmatics remains largely unknown. Here, we demonstrate that gasdermin B encoded by GSDMB, one of the most significant asthma susceptible genes at 17q21, acts as a novel RNA sensor, promoting interferon response and subsequent inflammation. In airway epithelium, GSDMB is induced by respiratory viral infections. Higher expression of GSDMB and interferon-stimulated genes showed significant association in respiratory epithelium from two independent asthma cohorts. Notably, inducible and conditional expression of human GSDMB gene in mouse airway epithelium leads to enhanced interferon response, increased airway inflammation with mucus hyper-secretion upon neonatal respiratory syncytial virus infection. In summary, GSDMB, a novel RNA sensor, promotes interferon response and enhances airway inflammation upon respiratory virus infection in asthmatics carrying the risk genotype at 17q21.

W32-6:

Eradication of Gastrointestinal Cancers and Neuroendocrine Tumors by CDH17CAR T Cells Without Toxicity to Healthy Tissues

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Gastrointestinal cancers (GICs) and neuroendocrine tumors (NETs) are common solid tumors, and often fatal andrefractory to therapy after metastasis. Adoptive cell therapy using chimeric antigen receptor (CAR) T cells are remarkably efficient for treating leukemia and lymphoma, but not yet developed for GICs and NETs. This is a keenly unmet need. Via an unbiased screening for GI-NET cell-selective nanobodies, we isolated a llama-derived nanobody, VHH1, that specifically bound NET cells. Screening of the VHH1-bound cell surface antigens identified CDH17, a cell surface adhesion protein that is upregulated in NETs and GICs. VHH1-CAR T cells targeting CDH17 (CDH17CARTs) specifically killed both human and mouse tumor cells in CDH17-dependent manner. CDH17CARTs eradicated CDH17-expressing NETs in several preclinical models. Moreover, CDH17CARTs also suppressed gastric, pancreatic, and colorectal cancers (GC, PC, and CRC) in either tumor xenografts or autochthonous primary mouse models. While CDH17 is also expressed in normal intestinal epithelial cells, the CDH17CARTs do not attack the normal cells, showing no detectable toxicity *in vivo*. Notably, treating normal mice with chemicals that damage the integrity of intestinal epithelium increased T cell infiltration into the intestinal tissue. Histological studies indicate that CDH17 is only localized at the tight junction between the normal intestinal epithelial cells, but not at the luminal or basal cell membrane. In contrast, NET cells lost polarity of CDH17 distribution, allowing CDH17 displayed all over the tumor cell surface, attracting potent CART attack. Thus, CDH17 represents a new class of tumor-associated antigens (TAAs) that is highly susceptible to CART attack on tumor cells, yet "masked" from attack by the CARTs in normal tissues, suggesting that CDH17CARTs are promising for safely treating GICs and NETs.

Workshop #33:

W33-1:

MutaGene links deficiency of mutator genes to immunotherapy efficacy

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Genomic instability is a hallmark of most cancers and presumably results from dysfunction of mutator genes. Yet methods to identify the complete catalogue of mutator genes in human and systematic investigations of their roles in cancer are lacking. Here we developed MutaGene to define genes associated with suppression of genomic instability and identified 1534 putative mutator genes based on the TCGA dataset. We observed that cancer patients with deficiency of mutator genes, in contrast to other genes in cancer pathways or DNA repair pathways, were coupled with strong immunogenicity, e.g., elevated neoantigen burden, immune infiltration, and expression of immune checkpoint inhibitors. The results suggest that patients with deficiency of mutator genes may be favorable candidates for immunotherapy due to their elevated mutation burden. We thus integrated the mutator gene catalogue with data of cancer patients post immune checkpoint blockade treatment in the MSK-IMPACT cohort. We observed that deficiency of mutator genes, particularly the ones suppressing insertion-deletion mutations, predicted strong anti-tumor effects of the immunotherapy and positive clinical outcomes. Taken together, the mutator genes identified in this study enabled fresh insights into genome instability and serve as robust biomarkers for cancer immunotherapy.

W33-2:

Mathematical Models of Cancer Therapies

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The mathematical theory of phylogenetic spaces can help evolutionary biologists unveil hidden information in evolutionary history. My talk will describe some of these tools. To start, I will introduce phylogenetic spaces that represent evolutionary histories. I will then describe embeddings between spaces of phylogenetic trees and networks. Secondly, I will introduce deep neural networks that model genetic mutations that may lead to fitness advantages. Then I will report our results in terms of accuracy in predicting genetic mutations via cross-validation and compare our method with baseline models.

W33-3:

CRISRP screens reveal genetic determinants of PARP inhibitor sensitivity and resistance in prostate cancer

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Prostate cancer (PCa) patients harboring BRCA1/2 mutations are often exquisitely sensitive to PARP inhibition. However, genomic alterations in other DNA damage response (DDR) genes have not been consistently predictive of clinical response to PARP inhibition. Importantly, current targeted cancer therapies are largely guided by mutations of a single gene, which overlooks concurrent genomic alterations. Using genome-wide CRISPR/Cas9 loss-of-function screens, we discovered genes that mediate cellular response and resistance to PARP inhibitors. Specifically, we found that RNASEH2B and MMS22L are frequently deleted on chromosomes 13q and 6q respectively in PCa, loss of which confers BRCA-like synthetic lethality to PARP inhibition. On the other hand, inactivation of TP53 and RB1 may compromise PARP inhibitor response, which can be overcome by combining ATR inhibition. Our findings may inform the use of PARP inhibitors beyond BRCA1/2-deficient tumors and support reevaluation of currently used biomarkers for PARP inhibitor treatment in PCa.

W33-4:

Managing acquired resistance to third generation EGFR inhibitors in lung cancer

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Osimertinib (AZD9291) and other third generation EGFR-tyrosine kinase inhibitors (EGFR-TKIs) such as almonertinib (ameile) selectively and irreversibly inhibits EGFR activating and T790M resistant mutants while sparing wild-type EGFR. Osimertinib and almonertinib are now approved therapeutic options for non-small cell lung cancer (NSCLC) patients with EGFR activating mutations (first-line) or those who have become resistant to the 1st generation EGFR-TKIs through the T790M mutation (second-line). These drugs have achieved remarkable and impressive outcomes in improving patient overall survival. Unfortunately, all patients eventually relapsed and developed resistance, limiting their lone-term efficacies. Hence, the effective strategies that can delay or overcome the emergency of the acquired resistance are urgently needed in the clinic. In this direction, fully understanding the biology or action mechanisms of these EGFR-TKIs and the underlying resistance mechanisms certainly warrants development of effective strategies for delaying or overcoming acquired resistance to 3rd generation EGFR-TKIs. Thus, my lab has made a great effort toward this direction for the past 6 years. Our preclinical findings strongly suggested that blockage of the MEK/ERK survival signaling and targeting induction of apoptotic cell death are all effectively strategies not only for overcoming acquired resistance to third generation of EGFR-TKIs, but also for delaying or preventing emergence of acquired resistance to these EGFR-TKIs.

W33-5:

Nano-optogenetic Cellular immunotherapy

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Chimeric antigen receptor (CAR) T cell-based immunotherapyhas shown curative potential in patients. However, owing to the lack of control over the location and duration of the anti-tumour immune response, CAR T cell therapy still faces safety challenges arising from cytokine release syndrome and on-target, off-tumour toxicity. Herein, we

will discuss the design of light-switchable CAR (designated LiCAR) T cells that allow real-time phototunable activation of therapeutic T cells to precisely induce tumour cell killing. When coupled with imaging-guided, surgically removable upconversion nanoparticles that have enhanced near-infrared-to-blue upconversion luminescence as miniature deep-tissue photon transducers, LiCAR T cells enable both spatial and temporal control over T cell-mediated anti-tumour therapeutic activity in vivo with greatly mitigated side effects. Our nano-optogenetic immunomodulation platform not only provides a unique approach to interrogate celluar anti-tumour immunity, but also sets the stage for developing precision medicine to deliver personalized anticancer therapy.

Workshop #34:

W34-1:

An Integrated Structure-based Approach for the Development of MDM2 inhibitors

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MDM2 is an oncogenic E3 ligase found to be overexpressed in a number of human cancers associated with poor prognosis. MDM2 overexpression inhibits the function of the tumor suppressor p53, which plays a critical role in safeguarding the integrity of the human genome. MDM2 ubiquitinates p53 and targets it for proteasomal degradation. MDM2 also exhibits p53-independent oncogenic activities through targeting other tumour suppressor proteins, such as FOXO3a and Rb. Thus aberrant regulation of MDM2 is a key factor in promotion of tumor formation and progression and represents an important cancer therapeutic target. Here, we describe an integrated structure-based approach to develop potential lead compounds for inhibition of MDM2 oncogenic activity. We established a structure-based virtual screening strategy and used the crystal structure of the MDM2:MDMX RING domain heterodimer to predict the potential "druggable" pocket on MDM2. An in silico screening of a small molecule compound library was employed to identify the candidate compounds that could interact with the MDM2:MDMX heterodimer RING domain. Additionally, using biochemical and cellular assays, the candidate compounds were examined for their ability to inhibit MDM2 E3 ligase activity, to induce apoptosis and to inhibit cell proliferation in cancer cell lines. This study reveals that inhibition of the MDM2:MDMX RING domain heterodimer could be a plausible approach for the development of MDM2 inhibitors as potential anti-cancer therapeutic agents.

W34-2:

Targeted protein degradation by ubiquitin variant induced proximity

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In recent years, researchers have leveraged the ubiquitin-proteasome system (UPS) to induce se-lective degradation of proteins by E3 ubiquitin ligases, which has great potential as novel thera-peutics for human diseases, including cancer and neurodegenerative disorders. However, despite extensive efforts, only a handful of ~600 human E3 ligases were utilized, and numerous protein–protein interaction surfaces on E3 ligases were not explored. To tackle these problems, we leveraged a structure-based protein engineering technology to develop a multi-domain fusion protein bringing functional E3 ligases to the proximity of a target protein to trigger its proteasomal deg-radation, which we termed Ubiquitin Variant Induced Proximity (UbVIP). We first generated non-inhibitory synthetic UbV binders for a selected group of human E3 ligases. With these UbVs employed as E3 ligase engagers, we designed a library of UbVIPs targeting a DNA damage re-sponse protein 53BP1. We observed that two UbVIPs recruiting RFWD3 and NEDD4L could ef-fectively induce proteasome degradation of 53BP1 in human cell lines. This provides a proof-of-principle that UbVs can act as a means of targeted degradation for nucleus-localized proteins. Our work demonstrated that UbV technology is suitable to develop protein-based molecules for targeted degradation and can help identify novel E3 ligases for future therapeutic development.

W34-3:

Engineering ubiquitin transfer in the cell – the orthogonal way

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E3 UB ligases regulate diverse processes in the cell, including protein quality control and degradation, gene activation and repair, and cell cycle and apoptosis, etc. The multifaceted roles of E3s are underpinned by their diverse substrate specificities, yet it has been a challenge to profile E3 substrates due to the transient interactions of the E3-substrate pairs. To overcome such a challenge, we developed a method to enable the direct tracking of UB transfer from an E3 to its substrate proteins through an engineered orthogonal UB transfer cascade (OUT). The OUT cascade is constituted by an engineered set of xE1, xE2 and xE3 that would enable the exclusive transfer of an engineered xUB to the substrates of a specific E3. xUB shares no cross reactivities with the native UB transferring enzymes, so its labeling of the E3 substrates through the OUT cascade would enable the unambiguous identification of the ubiquitination targets of an E3. So far, we have used phage and yeast cell surface display to engineer OUT cascades with various classes of E3s and used them to profile substrate specificities of HECT E3s E6AP and Rsp5, RBR E3s Parkin and HHARI, and U-box E3s E4B and CHIP. Based on the newly generated substrate profiles of various E3s, we have discovered new pathways of cell regulation through E3 catalyzed ubiquitination of kinases, phosphatases, transcription factors, and chaperon proteins that suggested essential roles of the E3s in regulating cell senescence, metastasis, mitophagy, and stress response. Overall, our work has established OUT as an empowering proteomics platform for deconvoluting E3-substrate relationships and deciphering the role of E3s in cell biology.

W34-4:

UBXN3B Controls B Lymphopoiesis via BLNK

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Hematopoiesis is finely regulated to enable timely production of the right numbers and types of immune cells tomaintain tissue homeostasis. Herein, we report a crucial role of a UBX domain-containing protein UBXN3B in maintenance of hematopoietic homeostasis. *Ubxn3b*$^{-/-}$ mice have significantly fewer B cells

(~10-fold), while more myeloid cells, than *Ubxn3b*$^{+/+}$ littermates. Transfer of wild type bone marrow to *Ubxn3b*$^{-/-}$ mice corrects the B cell deficiency, while reverse transplantation does not. B cell specific *Ubxn3b* knockout mice present a tenfoldreduction of B cells. Mechanistically, UBXN3B is essential for pre-BI transition to pre-BII and proliferation of smallpre-BII by regulating pre-B cell receptor signaling and B cell linker (BLNK) protein stability. This dysregulated immune compartmentalization renders mice highly vulnerable to SARS-CoV-2, typified by reduced production of virus-specific antibodies, increased lung immunopathology and delayed resolution of disease. These results reveal a B cell-intrinsic role of UBXN3B in early B cell development and control of immunopathogenesis of respiratory viruses. **Significance Statement:** Hematopoiesis is finely regulated to enable timely production of the right numbers and types of immune cells to maintain tissue homeostasis. Our results demonstrate a new function of UBXN3B in the maintenance of hematopoietic homeostasis and in particular B lymphopoiesis and antibody response during steady state and viral infection. Aberrant immune compartmentalization associated with UBXN3B deficiency may predispose an individual to persistently heightened immunopathology during viral infection.

W34-5:

CD44 is a checkpoint blockade for dendritic cell maturation in tumor immune suppression

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Dendritic cells (DC) are essential to adaptive tumor immunity. Mature conventional type I dendritic cells (cDC1) are uniquely specialized for presenting tumor antigens to $CD8^+$ T-cells, thereby stimulating their proliferation and cytolytic activity. Immature DCs also present antigens to $CD8^+$ T-cells but trigger immune tolerance instead. The processes of DC maturation and antigen presentation are frequently hijacked by tumor cells to evade immunosurveillance. However, the mechanisms underlying DC maturation remain elusive. Here, we report that the cell surface glycoprotein CD44 represses DC maturation through activation of the IL-6-STAT3 signaling pathway. Using the syngeneic B16.SIY melanoma mouse model, we found that ablation of host CD44 expression inhibits tumor growth. Depletion of cytotoxic $CD8^+$ T-cells in CD44 knockout mice abrogates the tumor inhibitory effect, indicating that host CD44 expression promotes tumor progression through obstructing anti-tumor immunity. Through high parameter mass cytometry (CyTOF) and flow cytometry, we identified cDC1 maturation as the key process that is significantly enhanced in tumor-bearing CD44 knockout mice, leading to increased $CD8^+$ T-cell proliferation and cytotoxicity. Adoptive transfer of bone-marrow DC from CD44 knockout mice to WT tumor-bearing mice significantlyincreases the abundance of activated $CD8^+$ T-cells in the tumor microenvironment. Furthermore, treatmentof WT tumor-bearing mice with an anti-CD44 blocking antibody promotes cDC1 maturation, $CD8^+$ T-cell activation, and ultimately reduced tumor volumes. Mechanistically, we demonstrate that CD44 hyperactivates the IL-6-STAT3 signaling cascade, thereby inhibiting DC maturation and downstream $CD8^+$ T-cell activity. Overall, our results provide the mechanistic groundwork for utilizing CD44 blockade to promote DC maturation and tumor immunosurveillance, establishing a novel class of checkpoint inhibitors that can be used alone or in combination with currently available therapeutics to benefit patients with advanced or treatment refractory disease.

Workshop #35:

W35-1:

A Yellow Fever Virus NS4B-Targeting Antiviral Compound Functions Through Disruption of the ER Membrane-Derived Replication Organelles and Stimulation of dsRNA-Mediated Immune Response

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We previously reported a benzodiazepine compound BDAA that specifically inhibits yellow fever virus (YFV) replication *in vitro* and in *vivo*, with resistant mutation mapped to nonstructural protein 4B (NS4B). Extensive structure-activity-relationship study has led to the identification of lead candidates, which showed good druggable properties and 100% protection against lethal YFV infection in hamster model. In support of preclinical development of this family of antiviral compounds, we performed mechanistic studies and found that BDAA enhances YFV-induced inflammatory cytokine response in association with the induction of dramatic ultrastructure alteration of viral replication organelles (ROs) and exposure of dsRNA in virus-infected cells using transmission electron and fluorescent microscope, respectively. These results support a model that BDAA interaction with NS4B may impair the integrity of YFV RO, which not only directly abrogates viral genome replication, but also promotes viral replication intermediates releasing from RO to activate cytosolic RNA sensing pathways. Indeed, BDAA directly inhibits nascent YFV RNA synthesis in cell culture in a 5-ethynyl uridine incorporation experiment as well as in an endogenous polymerase reaction system. Furthermore, we demonstrated that BDAA treatment activates three dsRNA recognizing pathways, RLR, PKR and OAS-RNase L, in YFV-infected cells. While activation of RLR pathway depends on both RIG-I and MDA5, activation of OAS-RNase L pathway is mediated by OAS-3, but not OAS-1 or 2. In addition, we observed that BDAA treatment significantly accelerated YFV-induced cell death via caspase 8/9/3 mediated-apoptosis in a variety of cell types including primary human fibroblast cells. Taken together, BDAA primarily hits the YFV RO and executes multi-mode antiviral action including direct disturbing of viral replication, enhancing antiviral cytokine response, and premature killing of infected cells, which may collectively lead to an unprecedented rapid-acting and potent inhibition of virus replication and spreading *in vivo*.

W35-2:

Some Interesting Chemistry Involving the SARS-CoV-2 Main Protease

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The SARS-CoV-2 main protease (Mpro) is a demonstrated target for the development of COVID-19 antivirals. During the structural and mechanistic investigation of Mpro, we noticed that Mpro is functionally regulated by posttranslational modifications that involve C22, C44 and K61. These modifications lead to large structural variations around the active site that inactivate the enzyme and provide unique implications in designing new inhibitors. Mpro is a cysteine protease. A high-throughput screening analysis revealed multiple inhibitors that use the nucleophilic aromatic substitution (SnAr) reaction to covalently link to the catalytic cysteine. SnAr represents a new reaction type for the development of covalent inhibitors for Mpro.

W35-3:

Rational design of SARS-CoV-2 main protease inhibitors with novel warheads and improved selectivity

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My lab is interested in developing antivirals targeting emerging and re-emerging viruses, including influenza A and B viruses, enterovirus D68 (EV-D68), EV-A71, coxsackievirus, poliovirus, and the coronaviruses such as SARS-CoV-2. The central themes are the identification of new drug targets and the development of novel small molecules for use as chemical probes for target validation as well as drug candidates for translational research. In this talk, I will introduce our efforts in developing antivirals targeting SARS-CoV-2 main protease (Mpro or 3CLpro). Mpro is involved in the cleavage the viral polyproteins during the viral replication and is a validated antiviral drug target. Through high-throughput screening and structure-based drug design, we have discovered covalent and non-covalent Mpro inhibitors with potent enzymatic inhibition and cellular antiviral activity against SARS-CoV-2. The talk will focus on target selectivity, new cysteine reactive warheads, assay development, and pharmacology of Mpro inhibitors.

W35-4:

Targeting human cytomegalovirus (HCMV) pUL89 endonuclease for drug discovery

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Human cytomegalovirus (HCMV) infects the majority of the global population, and causes substantial morbidities and mortalities in immmunoincompetent individuals, including newborns, HIV-1 co-infected patients, and organ transplant recipients. Current direct-acting antivirals (DAAs) approved for treating HCMV infections are often plagued by drug resistance and / or dose-related adverse effects. Towards developing mechanistically novel HCMV antivirals, we have been exploring a unique metal-binding chemical space for targeting the endonuclease activity of HCMV pUL89, a key component of the viral terminase complex required for viral genome packaging and cleavage. The medicinal chemistry of a few distinct metal-binding chemotypes will be presented, highlighting both the successes and challenges.

W35-5:

Screening of an epigenetic compound library identifies BRD4 as a potential antiviral target for hepatitis B virus covalently closed circular DNA transcription

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HBV cccDNA is the persistent form of viral genome, which exists in host cell nucleus as an episomal minichromosome decorated

with histone and non-histone proteins. cccDNA is the authentic viral transcript template and resistant to current antivirals. Growing evidence shows that the transcriptional activity of cccDNA minichromosome undergoes epigenetic regulations, suggesting a new perspective for anti-cccDNA drug development through targeting histone modifications. In this study, we screened an epigenetic compound library in the cccDNA reportercell line HepBHAe82, which produces the HA-tagged HBeAg in a cccDNA-dependent manner. The compounds were added to the cells harboring established cccDNA pool in the presence of 3TC, the supernatant HA-HBeAg signal was measured by chemiluminescence ELISA and compared to 3TC treatment alone. For the initial hits that reduced HA-HBeAg by more than 50%, intracellular HBV cccDNA and precore mRNA were measured by qPCR and those hits significantly reduced precore mRNA without affecting cccDNA copy numbers were prioritized tofurther study. Among them, a histone acetyltransferase inhibitor MS436 exhibited marked inhibition of cccDNA transcription in both HBV stable cell line HepAD38 and HepG2-NTCP or primary human hepatocytes infection system under noncytotoxic concentrations. Chromatin immunoprecipitation (ChIP) assay demonstrated that MS436 dramatically reduced the enrichment of H3K27ac, an activate histone modification pattern, on cccDNA minichromosome. Interestingly,the MS436-mediated inhibition of cccDNA transcription is accompanying with cccDNA destabilization in HBV infection and Cre/LoxP-based recombinant cccDNA systems, indicating that BRD4 activity may also play a role in cccDNA maintenance. Furthermore, depletion of BRD4by siRNA knockdown or PROTAC degrader resulted in cccDNA inhibition in HBV-infected HepG2-NTCP cells, further validating BRD4 as an antiviral target. Taken together, our study hasdemonstrated the practicality of HepBHAe82-based anti-HBV drug screening system and provided a proof-of-concept for targeting HBV cccDNA with epigenetic compounds.

W35-6:

Identification of host factors limiting SARS-CoV-2 cytopathic effects by genome-wide CRISPR drop-off screens

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The ongoing global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) hascaused a major public health crisis and led over five million death worldwide. Although several vaccines and therapeutic strategies approved by FDA greatly slowed down the process of the pandemic, the death rate of COVID patients worldwide still remains around 1.8% which is almost10 times higher than the seasonal flu, highlighting an unmet need to develop more effective treatments. Given that the pathogenesis of SARS-CoV-2 is highly dependent on the host machinery, it is critical to develop novel therapeutics that take advantage of the host factors essential for SARS-CoV-2 infection. To achieve this goal, a genome-scale CRISPR loss-of- function screen was performed to identify critical host factors in governing sensitivity of host cellsto SARS-CoV-2 infection. In the initial screen, A549, which is a lung carcinoma epithelial cancercell line and often served as investigational models of alveolar type II pulmonary epithelium, wasgenetically modified to overexpress ACE2 and Cas-9 for viral entry and gene editing. Then, overexpressing of ACE2- and Cas9-A549 cells (A549-AC) were transduced with lentiviralgenome-scale human gRNA library that cover 18,436 human genes (5 gRNAs/each gene). PooledgRNA expressing A549-AC cells were co-cultured with infectious SARS-CoV-2 at the MOI of 5for 44 hours. The screen is based on the premise that the cells whose essential gene for SARS- CoV-2 infection is knocked down after gRNA integration will become more resistant to SARS- CoV-2 infection and will thus be enriched after computational analysis of the sequencing data. While the gRNAs that target genes protecting host cell from viral infection will be depleted in infected samples. By analyzing the distribution of each gRNA in samples with and without SARS-CoV-2 infection,a total of 147 genes ($|\log_2 \text{Fold Change}| \geq 0.5$ and $P<0.05$) whose gRNA distributions were significantly changed in samples with SARS-CoV-2 infection were identified. This list includes several host factors that have been reported to be involvement of SARS-CoV-2 infection, including ACE2, TMEM41B, Cathepsin L and LY6E. More importantly, several new factors that could playcritical role in controlling the life cycle of SARS-CoV-2 were successfully identified. Subsequently, findings from the screening were integrated with the comprehensive bioinformatics analysis including interactome datasets based on SARS-CoV-2 viral-human protein and mRNA interactions, genome-wide association study (GWAS) of Covid-19 hospitalization and severity, and single cell RNA sequence (scRNA) profile obtained from the subpopulation of epithelial cellswhich were isolated from bronchoalveolar lavage fluid of COVID-19 patients to further evaluate the role of each host factor in governing antiviral response. Afterwards, referring to the relative fold change and statistical significance, total 31 candidates that have never been reported as host factors for SARS-CoV-2 (4 for pro-viral factors and 27 for anti-viral factors) were selected for the following validation at single gene level. Two rounds of validation assays were performed independently. 24 of selected candidates (~78%) were successfully validated at either round of the validation. Among candidates which can be validated in both rounds, integrating the screening index including fold change, MAGeCK score and together with the clinical relevance from the analysis of GWAS and scRNA datasets, two pro-viral candidates (ATP6V0D1 and DPAGT1) andthree anti-viral factors (DAZAP2, VTA1 and KLF5) were selected for further mechanistic studies. Following studies to dissect roles of selected host factors in modulating sensitivity of host cells to SARS-CoV-2 infection indicated that knocking down of pro-viral candidates ATP6V0D1 and DPAGT1 dramatically inhibited virus replication and virus-induced cytopathic effect, while knocking down of anti-viral candidates DAZAP2, VTA1 and KLF5 strikingly enhanced virus-induced cytopathic effect and suppression of DAZAP2 dramatically increased virus replication. *Vice versa*, overexpression of DAZAP2 significantly attenuated virus-induced cytopathic effect. Collectively, our data not only provides

a genome-scale resource of critical first responders of host factors participating to SARS-CoV-2 infection response but also represents a rich resource to explore biomarkers of identifying potential increased risk for severe COVID-19 illness. Moreover, these findings offer important insights for the understanding of the coronavirus life cycle and the development of novel host-directed therapeutic strategies for acute COVID-19 and potential futurecoronavirus pandemics.

Workshop #36:

W36-1:

Broad antiviral agents based on innate immune response to viral infection

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The global pandemic of COVID-19 reminds us the urgent needs for broad spectrum of antiviral agents against emerging viruses such as SARS-CoV-2. We have known for a long time that Type I interferon (IFN-I) is the major host defense weapon with broad antiviral activities against different types of viruses. We have performed unbiased screens of over 300 interferon stimulatory genes (ISGs) and identified overlapping but distinct subsets of ISGs that can strongly suppress different types of viruses. Among these antiviral ISGs, we identified cholesterol 25-hydroxylase (CH25H), a metabolic gene encoding the enzyme that converts cholesterol to 25-hydroxy-cholesterol (25HC), as a novel ISG with a broad antiviral activity. We further demonstrated that 25HC can block viral entry by preventing the fusion between the viral envelope and cell membrane, and have shown that it strongly suppresses infection of every enveloped virus that have tested so far including SARS-CoV-2. In addition, our recent studies on gene expression profiles regulated by IFN-I revealed that IFN suppresses viral infection not only by upregulating ISGs, but also by downregulating many host metabolic genes involved in the synthesis of fatty acids, nucleotides and amino acids. In particular, we found that IFN-I strongly downregulates fatty acid synthase (FASN), a key enzyme involved in the synthesis of long-chain fatty acids. Importantly we found many FASN inhibitors to have strong antiviral effects against different types of viruses including SARS-CoV-2. Therefore, understanding host immune response to different viral infection may provide insights to the development of novel broad antiviral agents against unpredictable emerging viruses.

W36-2:

SUMV-2, but not SUMV-1, mediates RNAi-independent antiviral immunity against Orsay virus during natural infection in *C. elegans*

Rui Lu

To look for *C. elegans* genes that mediate RNAi-independent antiviral immunity, we carried out a biased genetic screen that utilizes a flock house virus-derived replicon transgene as reporter. This genetic screen, combined with whole genome sequencing, identified *sumv-1* and *sumv-2* as key genes mediating RNAi-independent antiviral immunity. Both SUMV-1 and SUMV-2 are known to function with MYS-2, an ortholog of human lysine acetyltransferase 8 (KAT8), and predicted to regulate transcription through chromatin modification. By targeting and silencing *mys-2* through feeding RNAi we confirmed that *mys-2* also contribute to RNAi-independent immunity against transgene-introduced virus. To rule out the possibility that the enhanced viral replication in *sumv* mutants is a result of enhanced transcription of the viral transgene we checked the transcription of the viral transgene in worm mutants containing *sumv-1* or *sumv-2* null allele. We detected enhanced transcription in *sumv-1*, but not in *sumv-2*, mutants. To reconfirm that RNAi activity is not compromised in the absence of *sumv-1* or *sumv-2* we performed small RNA deep sequencing and northern blot analyses to detect biogenesis of virus-derived siRNAs in corresponding mutants. We found that function loss for sumv-1 or sumv-2 does not compromise viral siRNA production and the virus siRNAs produced in *sumv-1* or *sumv-2* mutants are functionally active. Consistently our RNA-seq analyses confirmed that the transcription of all known RNAi genes are not affected by the loss of *sumv-1* or *sumv-2* function. Intriguingly, we found that although both are required for antiviral defense against Orsay virus introduced as transgene it is *sumv-2,* but not *sumv-1,* that mediates anti-Orsay virus defense during natural infection. Since SUMV-1, SUMV-2 and MYS-2 are known to function in nucleus whereas the life cycle of transgene-introduced viruses involves a nuclear phase we believe that these tree proteins may mediate the modification of viral RNA thereby to suppress viral genome replication.

W36-3:

Precision in plant immune regulation

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Since plants are sessile organisms without specialized immune cells, plant defense occurs in coordination with growth-related activities within the organism and with environmental conditions, such as light, temperature and humidity. Therefore, we cannot fully understand plant immunity by studying it in isolation. Besides the cell-autonomous pattern-triggered immunity mediated by surface-localized pattern recognition receptors, and effector-triggered immunity mediated by intracellular nucleotide-binding leucine-rich repeat immune receptors, plants can also mount systemic acquired resistance through production of the immune hormone, salicylic acid. A major gap in our knowledge of the plant defense response is an understanding of how these three distinct types of defense responses are interrelated in coordination with the environment. In my talk, I will present our discoveries on the interplay between plant immune responses, the redox rhythm and the circadian clock; and explain how transcription and translation of defense genes are regulated to mount effective immune responses with minimal fitness cost. I will also show how information obtained from such systems studies can lead to the development of new strategies in engineering disease resistance in plants.

W36-4:

Influenza pathogenesis and cross-species infection

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Influenza is an important zoonotic disease that causes significant threats to human and animal health. Annual influenza epidemics result in 250 to 500 million human infections, which cause 250,000 to 500,000 fatalities worldwide. Especially, influenza pandemic may lead to millions of deaths people, which is caused by influenza A virus belonging to the *Orthomyxoviridae* family. This family also contains influenza B, C and D viruses. Four types of influenza viruses display different features in their genomes, host ranges, receptors used and pathogenesis in humans and animals. In this presentation, I will combine our research to discuss pathogenesis and cross-species infections of influenza viruses, and challenge on preventing influenza.

W36-5:

VASA helicase promote the phase separation of germ granules to ensure proper recognition of self and non-self nucleic acids

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The ability to distinguish non-self from self is the key characteristic for any defense system. piRNAs function as guardians of the genome by silencing non-self nucleic acids and transposable elements in animals. Many piRNA factors are enriched in perinuclear germ granules, but whether their localization is required for piRNA biogenesis or function is not known. Here we show that GLH/VASA helicase mutants exhibit defects in forming perinuclear condensates containing PIWI and other small RNA cofactors. These mutant animals produce largely normal levels of piRNA but are defective in triggering piRNA silencing. Strikingly, while many piRNA targets are activated in GLH mutants, we observed that hundreds of endogenous genes are aberrantly silenced by piRNAs. This defect in self versus non-self recognition was also observed in other mutants where perinuclear germ granules are disrupted. Together, our results argue that perinuclear germ granules function critically to promote the fidelity of piRNA-based transcriptome surveillance in *C. elegans* and preserve self versus non-self distinction.

W36-6:

Emerging enterococcus pore-forming toxins with MHC/HLA-I as receptors

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Enterococci are a part of human microbiota and a leading cause of multidrug resistantinfections. Here we identify a family of *Enterococcus* pore-forming toxins (Epx), in *E. faecalis*, *E. faecium*, and *E. hirae* strains isolated across the globe. Structural studies reveal that Epxs forma branch of β-barrel pore-forming toxins with a unique β-barrel protrusion (designated the top domain) sitting atop the cap domain. Through a genome-wide CRISPR-Cas9 screen, we identify human leukocyte antigen – I complex (HLA-I) as a receptor for two members (Epx2 and Epx3), which preferentially recognize human HLA-I and homologous MHC-I of equine, bovine, and porcine, but not murine origin. Interferon exposure, which stimulates MHC-I expression, sensitizes human cells and intestinal organoids to Epx2 and Epx3 toxicity. Co-culture with Epx2-harboring *E. faecium* damages human peripheral blood mononuclear cells and intestinal organoids, and this toxicity is neutralized by an Epx2 antibody, demonstrating the toxin-mediated virulence of Epx-carrying *Enterococcus*.

Workshop #37:

W37-1:

mRNA Nanomedicine for Precision Cancer Immunotherapy

Dr. Jinjun Shi

Loss or mutation of tumor suppressor genes is a dominant force in cancer onset and progression and in the development of therapeutic resistance. Recent studies have alsosuggested that loss of tumor suppressors (e.g., PTEN and p53) may correlate with an immunosuppressive tumor microenvironment (TME) and poor response of different cancers to immune checkpoint blockade therapy. Synthetic mRNAs have shown enormous potential in biomedical applications, as exemplified by the recent FDA approval of mRNA vaccines for COVID-19. We recently demonstrated the feasibility of using synthetic mRNA nanoparticles to reconstitute tumor suppressors in human and murine cancer cells. The reactivation of PTEN/p53 could not only inhibit tumor growth, but also promote CD8+ T cell infiltration to tumor tissues and reverse the immunosuppressive TME. The combination of PTEN/p53 mRNA nanoparticles with anti-PD-1 antibody led to a highly potent and safe anti-tumor effect in immunocompetent mouse models of melanoma, prostate cancer, and hepatocellular carcinoma. Our studies suggest that synthetic mRNA nanomedicine may provide a novel, tumor suppressor-specific precision immunotherapy strategy for more effective cancer treatment along with immune checkpoint blockade.

W37-2:

Precision nanomedicine treating vascular diseases

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Vascular disease is the leading cause of morbidity and mortality in the United States and globally. Vascular complication such as atherosclerosis preferentially develops at arterial curvatures and bifurcations where disturbed blood flow activates endothelium. Nevertheless, current atherosclerosis therapies mainly target systematic risk factors but not the vasculature *per se*. Our objective is to devise innovative precision nanomedicine approaches targeting disease-causing endothelial pathways. Our recent studies demonstrated that disturbed blood flow significantly increases the endothelial expression of TXNDC5 (thioredoxin domain containing 5) and microRNA-92a (miR-92a), two atherogenic molecules driving atherogenesis. Novel targeted nanomedicine platforms were engineered to deliver miR-92a inhibitors or TXNDC5-targeting CRISPR/Cas9, which significantly reduced atherosclerosis and stenosis in vivo. Our results provide a proof of concept of innovative targeted nanomedicine approaches toaddress a major unmet medical need in vascular therapies.

W37-3:

Post-GWAS Functional Analysis to Define Genetic and Acquired Pathogenesis of Pulmonary Arterial Hypertension as A Complex Disease

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Disclosure: None.

RATIONALE: Pulmonary arterial hypertension (PAH) is an enigmatic and morbid disease where insights are emerging regarding genetic susceptibility. Genome-wide Association Studies (GWAS) have identified SOX17 as the most significant PAH-associated genomic locus. The allele of the tag SNP in this locus is associated with 1.8-fold higher PAH risk. It has been challenging to define the mechanisms underlying the contribution of the PAH-associated functional SNPs (fSNPs) to pathogenesis of the disease.

METHODS: We developed a post-GWAS functional genomics strategy to define the causative fSNPs and identify the associated biological mechanisms. This analysis includes Reel-seq (Regulatory element-sequencing), an EMSA-based high-throughput technique to identify fSNPsin a synthetic DNA library containing PAH-associated SNPs; SDCP-MS (SNP-specific DNA competition pulldown-mass spectrometry) to identify proteins that specifically bind to fSNPs; and AIDP-Wb (allele-imbalanced DNA pulldown-Western blot) to show allele-imbalanced binding ofthese proteins to fSNPs. The regulation of risk gene expression by these fSNP-binding proteinsand their pathogenicity were determined in human pulmonary arterial endothelial cells (PAECs)and confirmed in PAH animal models and patients.

RESULTS: By using high-throughput Reel-seq and subsequent validation with EMSA, intergenic SNP rs4738801 in SOX17 locus was identified as a fSNP from a library containing 254 PAH- associated haplotype SNPs. This fSNP resides in a remote upstream enhancer region of SOX17, an endothelial effector increasingly associated with PAH pathogenesis. Using SDCP-MS and AIDP-Wb, we found that the transcription factor FUBP1 binds to rs4738801 risk allele C with lower affinity than non-risk allele G, resulting in a decrease in SOX17 expression. FUBP1 and target gene SOX17 controlled PAH-associated pathophenotypes in PAECs, includingproliferation, apoptosis, and angiogenesis. Downregulated by the major acquired PAH trigger hypoxia, FUBP1 and SOX17 were decreased in lungs and pulmonary ECs isolated from PAH patients and mouse models. A 3.77-fold enrichment of fSNP rs4738801 risk allele C was found in patients with PAH induced by hypoxia, but not in PAH associated with connective tissue disease or congenital heart disease.

CONCLUSIONS: FUBP1 controls SOX17 expression via allele-specific binding to PAH- associated fSNP rs4738801. The reduced binding of FUBP1 to risk allele C defines the genomic architecture contributing to the SOX17-dependent genetic susceptibility of PAH. The downregulation of FUBP1-SOX17 by hypoxia results in endothelial dysfunction, contributing to the acquired pathogenesis of PAH. These findings identify a novel role of FUBP1 in the functional regulation of SOX17 locus and elucidating a pathogenic mechanism that combines the acquired PAH-triggering factors and altered genetic susceptibility.

W37-4:

Endothelial Cell-Based Precision Therapy for Cardiovascular Disease

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Endothelial cells (EC) play critical roles in maintaining cardiovascular homeostasis. The clinical relevance of EC dysfunction in cardiovascular disease comes from prospective outcome studies, which revealed an independent significant association of EC dysfunction with a negative outcome in many cardiovascular diseases. However, the underlying molecular mechanism remains largely unknown, making it hard to target ECdysfunction in the treatment of cardiovascular disease. Thus, it is critical important to understand the EC regulators that regulate EC function and lineage specification.

Recent studies found that unique epigenetic signatures preferentially regulate cell identity genes. We thus systematically investigated the epigenetic landscape of EC lineage and identified MECOM to be the leading candidate as an EC lineage regulator. Single-cell RNA-Seq analysis revealed

that MECOM-positive cells are exclusively enriched in the cell cluster of bona fide EC derived from induced pluripotent stem cells. Further experiments verified that MECOM was required for EC differentiation and functions. Through integrative analysis of Hi-C, DNase-Seq, ChIP-Seq, and RNA-Seq data, we find MECOM binds enhancers that form chromatin loops to regulate EC identity genes. Specifically, we identified and verified the VEGF signaling pathway to bea key target of MECOM. Our work provides new insights into epigenetic regulation of cell identity and uncovered MECOM as an EC lineage regulator. MECOM could further serve as a therapy target for EC dysfunction related cardiovascular disease.

W37-5:

Improving allele-specific genome editing with mismatched gRNA

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CRISPR/Cas9-mediated allele-specific genome editing holds immense potential to correct disease-associated heterozygous mutations and to elucidate molecular functions of these mutations. However, the selectivity of allele-specific targeting by CRISPR/Cas9 remains a great challenge due to the sequence similarity between the mutant and wildtype alleles, which differ byonly 1-bp for most point mutations. Hence, current targeting strategies are limited to the mutationsthat occur in the PAM or seed regions. Based on an “epistasis-like” combinatorial effect of dual mismatches of gRNAs that we revealed, here we report an improved strategy for allele-specific editing with computer-aided design of mismatched gRNAs. Through a high-throughput allele- editing screen with perfectly matched, truncated, and mismatched gRNAs on 18 cancer hotspot mutations using a dual-target synthetic system, we find a vast majority of hotpot mutations locatedeither in the seed or non-seed regions are selectively targetable by mismatched gRNAs. The improvement by mismatched gRNAs is further confirmed by experimental validations on 6 selected hotspot mutations in reporter cells. In a proof-of-concept application, NRAS-WT and - G12D alleles are selectively edited by mismatched gRNAs in leukemia cells harboring heterozygous NRAS-G12D mutation. In addition, delivery of the mismatched gRNA by ribonucleoprotein complex shows promising editing efficiency in NRAS reporter cells. Thus, our results proved applicability of mismatched gRNAs for robust allele-specific genome editing.

W37-6:

AtheroSpectrum reveals novel macrophage foam cell gene signatures associated with atherosclerotic cardiovascular disease risk

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Background: Atherosclerosis remains one of the main causes of death in US and worldwide, demanding precision ASCVD risk assessment. Monocytes and macrophages play central roles in atherosclerosis, but previous work has yet to provide a detailed view of macrophage populations involved in increased ASCVD risk. **Methods:** A novel macrophage foaming analytics tool, AtheroSpectrum, was developed using quantitative indices depicting actions of macrophages. Next, a machine-learning algorithm was developed to analyze gene patterns in the peripheral monocyte transcriptome of MESA participants (MESA-set1, n=911). A listof 30 genes was generated and integrated with traditional risk factors to create an ASCVD risk prediction model (CR-30), which was subsequently validated in the remaining MESA-set2 (n=228) and two independent human atherosclerotic tissue transcriptome datasets (GTEx and GSE43292). **Results:** Using single-cell transcriptomic profiles (GSE97310, GSE116240, GSE97941, FR-FCM-Z23S), AtheroSpectrum detected two distinct programs in plaque macrophages: homeostatic-foaming and inflammatory pathogenic-foaming, the latter was positively associated with severity of atherosclerosis in multiple studies. A pool of 2209 pathogenic foaming genes was extracted and screened to select a subset of 30 genes correlated with CVE in MESA-set1. A CVD risk score model (CR-30) was then developed byincorporating this gene-set with traditional variables sensitive to CVE in MESA-set1 after cross-validation generalizability analysis. The performance of CR-30 was then tested in MESA-set2 (p=2.60×10-4 , AUC=0.742), and two independent datasets (GTEx, p=7.32×10-17, AUC=0.664; GSE43292, p=7.04×10- 2 , AUC=0.633). Model sensitivity tests confirmed the contribution of the 30-gene panel to the prediction model (likelihood ratio test, df=31, p=0.03).**Conclusion:** Our novel computational program (AtheroSpectrum) identified a specific gene expression profile associated with inflammatory macrophage foam cells. A subset of 30 genes expressed in circulatingmonocytes jointly contributed to prediction of symptomatic atherosclerotic vascular disease. Incorporating a pathogenic foaming gene-set with known risk factors can significantly strengthen the power to predict ASCVD risk.

Workshop #38:

W38-1:

Single-cell profiling of the developing *Drosophila* heart

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Drosophila has been instrumental in identifying novel genes and pathways required for heart development. However, what cell-types the developing embryonic heart contains and what genes are specifically expressed in each embryonic heart cell type remain unclear. We performed single-cell RNA-seq using thousands of *Hand*-GFP embryos collected at five consecutive developmental stages, ranging from bilateral rows of cardiac progenitors to a fused and beating heart tube. We identified six distinct cardiac cell types (cardioblasts and five types of pericardial cells) with unique markers and revealed the localization of most pericardial cell types. The fly cardioblasts showed combined transcriptional features of mammalian first and second heart fields. The genes associated with human cardiomyocyte maturation are mostly conserved in *Drosophila* and displayed similar dynamic transcriptional changes. For two of these newly identified genes, *rhea* and *LamC*, which are highly conserved to human *TLN2*/*TLN1* and *LMNA*, we demonstrate that they are essential for *Drosophila* cardioblast maturation. Our data provide evidence for interspecies correlation of heart development at the transcriptome level and enable characterization of *Drosophila* heart development at the single-cell resolution.

W38-2:

Molecular Regulation of Cardiac Function, Regeneration and Disease

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It is now recognized that more than 98% 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 gene expression and function in the heart, as well as how that is related to cardiac diseases. Using combination of gain- and loss- of function approaches in animal models and molecular dissection, our studies demonstrate the function of miRNAs, lncRNAs and RBPs in the cardiovascular system. Our ultimate goal is to develop these molecules into novel therapeutic targets for cardiac disease. (1) Demonstrate genetic evidence to support the function of miRNAs in the heart. (2)Understand basic molecular mechanism of how miRNAs and lncRNAs work. (3) Learn how miRNAs and RBPs could become novel therapies for disease

W38-3:

Coordinated transcriptome and cell state dynamics of non-myocytes in heart regeneration

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Cardiac regeneration occurs primarily through proliferation of existing cardiomyocytes, yet the regenerative response also involves complex interactions between distinct cardiac cell types including not only cardiomyocytes, but also non-cardiomyocytes (nonCMs). However, the subpopulations, distinguishing molecular features, cellular functions, and intercellular interactions of nonCMs in heart regeneration remain largely unexplored. Using the LIGER algorithm, we assembled an atlas of cell states from 61,977 individual nonCM scRNA-seq profiles isolated at multiple time points during heart regeneration in both wildtype and mutant fish. This analysis revealed extensive nonCM cell diversity, including multiple macrophage, fibroblast and endothelial subpopulations with unique spatiotemporal distributions and cooperative interactions during the process of cardiac regeneration. Genetic and pharmacological perturbation of macrophage functional dynamics compromised interactions among nonCM subpopulations, reduced cardiomyocyte proliferation, and caused defective cardiac regeneration. Furthermore, we developed a computational algorithm called Topologizer to map the topological relationships and dynamics of nonCMs during heart regeneration. We uncovered dynamic transitions between macrophage functional states and identified factors involved in mRNA processing and transcriptional regulation associated with the transition. Together, our single-cell transcriptomic analysis of nonCMs during cardiac regeneration provides a blueprint for interrogating the molecular and cellular basis of cardiac regeneration.

W38-4:

Inhibition of adrenergic b1-AR/Gas signaling promotes cardiomyocyte proliferation through activation of RhoA-YAP axis

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The regeneration potential of the mammalian heart is incredibly limited, as cardiomyocyte proliferation ceases shortly after birth. β-adrenergic receptor (β-AR) blockade has been shown to improve heart functions in response to injury; however, the underlying mechanisms remain poorly understood. Here we inhibited β-AR signaling in the heart using a cardiomyocyte specific β1-adrenergic receptor (β1-AR) blocker (metoprolol) to examine its role in heart maturation and regeneration at the neonatal stage. We found that metoprolol robustly enhanced cardiomyocyte proliferation and promoted cardiac regeneration post myocardial infarction, resulting in reduced scar formation and improved cardiac function. Moreover, the increased cardiomyocyte proliferation was also induced by the genetic deletion of *Gnas*, the gene encoding G protein alpha subunit (Gαs), a downstream effector of β-AR. Genome wide transcriptome analysis revealed that the cardiomyocytes of □-blocker treated and *Gnas* cKO hearts maintained an immature proliferating status even at the young-adult age, and that the loss of Gas function enhanced the activity of the Hippo-effector YAP, which is associated with immature cardiomyocyte proliferation. We also found that the increased YAP activity is modulated by RhoA signaling. Our pharmacological and genetic studies reveal a previously unrecognized β1-AR-Gαs-YAP signaling axis for regulating cardiac regeneration. These results suggest that inhibiting β-AR-Gαs signaling promotes the regenerative capacity and extends the cardiac regenerative

window in mice by activating YAP-mediated transcriptional programs. Thus, targeting β–AR-Gαs signaling may serve as a novel therapeutic target for the treatment of ischemic heart.

W38-5:
Precision Medicine for Cardiomyopathies via Zebrafish Genetics
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Cardiomyopathy is a group of cardiac muscle diseases that often lead to heart failure. Despite a monogenic disease, more than one hundred genes have been linked to cardiomyopathies. Thus, it is plausible that each genetic type of inherited cardiomyopathies might have its unique pathological events, which converge to common pathological signaling pathways at later stages. To decipher the complex genetic basis of cardiomyopathies and to implement precision medicine, we decided to leverage adult zebrafish as an efficient vertebrate model. We have been generating adult zebrafish models for cardiomyopathies of different genetic etiology, developing phenotyping tools to discern their pathogenesis processes, and identifying both unique and common therapeutic avenues. Using a zebrafish model of anthracycline-induced cardiotoxicity, 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 identified from our screen, uncovered spatiotemporally specific mechanisms of their modifying effects, which lead to the development of two mechanism-based therapeutic strategies for anthracycline-induced cardiotoxicity. Moreover, we demonstrated that a MMEJ-based genome editing technology can be used to establish genotype-phenotype relationship in F0 adult zebrafish, enabling rapid discovery of genetic modifiers for each genetic type of inherited cardiomyopathy. In summary, our work established adult zebrafish as a powerful *in vivo* model that would accelerate the implementation of precision medicine for cardiomyopathy.

Workshop #39:

W39-1:

Identification of New Therapeutic Targets in Kidney Cancer

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Von Hippel Lindau (*VHL*) is the most important tumor suppressor in kidney cancer. *VHL* loss leads to stabilization of HIF2α, which contributes largely to the development of clear cell renal cell carcinoma (ccRCC). Recent reports showed that the specific HIF2α inhibitor PT2399 inhibits primary tumor growth and invasion of a subset of kidney cancer. However, a significant portion of kidney cancer remains resistant to HIF2α inhibitor treatment, highlighting the importance of identifying additional therapeutic vulnerabilities of VHL-deficient kidney cancer. In this talk, I will discuss some of our latest findings on new VHL regulatory pathways, including ZHX2, SFMBT1 and TBK1. I will discuss on they may contribute to ccRCC tumorigenesis and their therapeutic implications.

W39-2:

Tumor suppression by BAP1: STING and Interferon pathway

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BRCA1-associated protein 1 (BAP1) is a tumor suppressor gene mutated in 10-15% of clear cell renal cell carcinoma (ccRCC). Although BAP1 loss is tightly associated with poor clinical outcome, it is not clear of the critical tumor suppressor function(s) of BAP1, and no specific therapy exists for BAP1-deficient patients. We found previously that both Hypoxia Inducible Factor 2α (HIF2α) and BAP1, activate Interferon Stimulated Gene Factor 3 (ISGF3). We also found that ISGF3 is a transcription factor activated by type I interferon signaling and is tumor suppressive in ccRCC xenograft models. Here we found that in ccRCC cells, loss of the VHL, the founding mutation for ccRCC development, activates interferon beta (IFN-β) expression in a HIF2α-dependent manner. IFN-β is found to activate ISGF3 and suppresses tumor growth. We further discovered that in a deubiquitinase-dependent manner, BAP1 enhances the expression of IFN-β and Stimulator of Interferon Genes (STING). Both genetic ISGF3 activation and STING agonist treatment suppress the growth of BAP1-deficient tumors in xenografts. Altogether, BAP1 loss lowers type I interferon signaling which enhances tumor growth. Reactivation of this pathway may serve as a novel therapeutic strategy in BAP1-deficient ccRCC.

W39-3:

Structural Insights on the MLL Complex for its Canonical and Non-canonical Functions in Cancer

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The MLL family histone methyltransferases deposit histone H3 lysine 4 (H3K4me) and play important roles in eukaryotic gene expression. Biochemical and structural studies of the MLL complexes have led to mechanism-based development of potential cancer therapeutics. Recently, we have have solved the single particle cryo-EM structures of the MLL1 core complex on the nucleosome core particles (NCP). We revealed a surprisingly dynamic nature of the MLL1 complex on the NCP. We show that DPY30 and the intrinsically disordered regions (IDRs) of ASH2L work together to restrict the rotational dynamics of the MLL1 complex and dramatically increase processivity and activity of the MLL1 complex. This is a general mechanism that apply to all members of the MLL/SET1 family enzymes. We further show that DPY30 is causal for *de novo* establishment of H3K4me3 in cells and plays a role in establishing the heterogenous epigenetic landscape in cells. We will also discuss unique functions for the MLL family enzymes in cancer and provide a new paradigm of how discrete MLL activities regulate fidelity of genetic epigenetic inheritance and how this is exploited in cancer with MLL1 overexpression.

W39-4:

Targeting the chromatin reader ENL as a strategy against acute myeloid leukemia

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Cancer cells are characterized by aberrant epigenetic landscapes and often exploit chromatin machinery to activate oncogenic gene expression programs. Recognition of modified histones by 'reader' proteins constitutes a key mechanism underlying these processes; therefore, targeting such pathways holds clinical promise. Acute myeloid leukemia (AML) is a poor-prognosis disease for which new therapeutic approaches are desperately needed. We have previously identified the histone acylation 'reader' ENL as being a critical dependency in AML. Here, we develop a potent and orally bioavailable small-molecule inhibitor of ENL, TDI-11055, which displaces ENL from chromatin by competitively blocking the interaction of ENL YEATS domain with acylated histones. TDI-11055 selectively inhibits the proliferation of leukemia cells harboring MLL1 (KMT2A) translocations or NPM1 mutations. A CRISPR/Cas9 mutagenesis screen uncovers an ENL mutant that confers resistance to TDI-11055, thus providing compelling genetic proof for the compound's on-target activity. Displacement of ENL from chromatin results in decreased recruitment of select ENL-associated complexes and impaired Pol II elongation which, in turn, leads to the suppression of critical oncogenic gene expression programs. Finally, pharmacological inhibition of ENL *in vivo* leads to reduced AML growth and prolonged survival in both cell line xenotransplantation and patient-derived

xenograft (PDX) models. Collectively, these biological and chemical insights into an emerging epigenetic therapeutic target in AML will support rapid clinical translation of these findings.

W39-5:

Epigenetic Regulation of Cancer Metastasis and Immune Evasion

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Epigenetic dysregulatoin often leads to cancer and other human diseases. Tumors exploit epigenetic mechanisms to evade immune surveillance and metastasize to distant organs, which is the major cause of cancer-related deaths. We recently identified multiple epigenetic regulators of immune evasion and/or metastasis, including histone demethylase KDM5B, histone methyltransferase SETDB1, and bromodomain protein CECR2. KDM5B recruits the H3K9 methyltransferase SETDB1 to repress endogenous retroelements such as *MMVL30*. Derepression of these retroelements activates cytosolic RNA-sensing and DNA-sensing pathways and the subsequent type-I interferon response, leading to tumor rejection and induction of immune memory. CECR2 is recruited by NFκB protein RELA to promote chromatin accessibility and activate the expression of their target genes. These genes include multiple metastasis-promoting genes, such as *TNC*, *MMP2*, and *VEGFA*, and cytokine genes *CSF1* and *CXCL1*, which are critical for immunosuppression at metastatic sites. CECR2 is required for cancer metastasis in multiple mouse models, with more profound effect in the immunocompetent setting. These results show that these epgienetic regulators are novel therapeutic targets for cancer treatment.

W39-6:

Altered Super Enhancer – Promoter Interactions Mediated by YY1 Underlie Age-induced Transcriptome

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Significant changes in transcriptome during aging have been observed in various tissues and cell types, even at the single-cell level. However, the molecular mechanisms that underlie these changes remain poorly understood. Using an ex vivo mesenchymal stem cells (MSC) replicative aging model and Hi-C technology,we discovered that, rather than large-scale changes in chromosome conformation, alterations to super enhancer – promoter looping interactions had the best positive correlation with age-associated transcriptome. Furthermore, we found evidence that these age-induced transcriptomic changes are mediatedby YY1, a key regulator of promoter-enhancer looping. This work, for the first time, unveiled a molecularmechanism elucidating the changes in transcription during aging.

Workshop #40:

W40-1:

Genomics and AI-enabled Cancer Immunotherapeutics

X. Shirley Liu
GV20 Therapeutics

Despite the exciting clinical benefits of immune checkpoint inhibitors, only a minority of cancer patients respond to treatment. Addressing resistance to immune checkpoint inhibitors is an urgent unmet need and requires new approaches for target identification and drug discovery. GV20 Therapeutics adopts an interdisciplinary approach integrating functional genomics, big data AI, and cancer immunology for IO target identification and drug discovery. We use proprietary CRISPR screens to identify IO targets of interest and bioinformatics on big data for target validation. We previously published a computational algorithm TRUST to extract the tumor-infiltrating antibody sequences from tumor RNA-seq profiles. Using an AI approach trained on hundreds of millions of tumor-infiltrating antibodies and billions of circulating antibodies, we could de novo design antibodies against targets without any known antibody sequences against the targets. This approach not only designs antibodies enriched in functional binders and good developability profiles, but also provides insights on target identification and validation. Using this approach, we identified a novel IO target, its antibodies, and its mechanism. Through multiple syngeneic tumor models, we also demonstrated its single-agent efficacy as well as its synergy with anti-PD1 in controlling tumor growth. We are submitting an Investigational New Drug (IND) Application to the U.S. Food and Drug Administration (FDA) to test this new drug in the clinic. Our efforts represent just the beginning of combining genomics and AI to unlock the hidden gems from patient tumors to develop cancer immunotherapeutics.

W40-2:

AI-driven Big Data Analysis for Drug Development

Han Liang
The University of Texas MD Anderson Cancer Center

Recently available large-scale cancer genomics data have provided unprecedented opportunities for developing novel cancer drugs. Many people believe that “AI + Big Data” represents a major force facilitating the paradigm shift from “fast follower” to “first-in-class”. I will discuss how AI-driven big data analysis helps make the decisions about key questions in the drug development pipeline from target evaluation, biomarker identification, disease indication, MOA, to drug combinations. This effort will substantially increase the efficiency and accuracy of decision making in drug development.

W40-3:

Ultra-fast Deep Screening of >1,000 Cancer Cell Lines

Shuxing Zhang
MD Anderson Cancer Center

High-throughput screening (HTS) has been the cornerstone for drug discovery, but there are significant challenges due to the long time needed for assay development and optimization, high cost of acquiring reagents, and requirement of specialized expertise. For instance, when considering over 1,500 cancer cell lines available today, it is practically impossible to screen all of them experimentally in one HTS campaign for therapeutic discovery. Therefore, there is an urgent unmet need to develop artificial intelligence (AI) technologies, which have demonstrated significant successes recently. In the present study we to implemented an AI-based platform for ultra-high throughput screening of most, if not all, cancer cell lines for drug responses, by curating big data sets of chemical compounds with associated bioactivity data and cancer cell line genomic profiles, developing novel data representation schemes, designing novel deep neural network training engines, and building robust AI models to conduct deep cancer cell line screening and employ them to intelligently design anticancer agents. We expect that the impact of such disruptive technology is enormous as it tremendously helps efficient identification and rational design of potent therapeutics for either common or rare cancers, with significantly high efficiency and low cost.

W40-4:

Exploration of dark chemical, biological and patient spaces for precision drug discovery

Lei Xie
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Modern drug discovery process is dominated by target- and phenotype-based compound screening in a model system. Both of them achieve a limited success. Several complex diseases such as Alzheimer’s disease do not have any effective and safe treatments yet. The challenge is partially due to the ignorance of dark chemical, biological and patient spaces where knowledge and data are limited or completely unavailable. For example, more than 90% druggable proteins have not been explored, and we know little about the drug off-target effects. Fundamentally, drug responses in a disease model are significantly different from those in human bodies, but it is unethical and infeasible to directly perform the compound screening in humans. To accelerate drug discovery and precision medicine for complex diseases, new compound screening strategies that overcome the inherent limitations of existing drug discovery process are urgently needed. Deep learning has a great potential to accelerate drug discovery and development. Because it is impossible to screen compounds in humans directly, computational prediction is essential to translate drug potencies in a disease model to clinical responses. Capability of end-to-end training by deep learning makes it a promising approach to model hierarchical organization and multi-level information transmission in a biological system as well as integrate heterogeneous, biased, noisy, and sparse omics data generated from diverse resources. However, existing machine learning methods faces a fundamental challenge when predicting an unseen chemical or patient sample from limited data with incoherent labels, i.e., an out-of-distribution (OOD) problem. We have developed a systems pharmacology strategy for high-throughput, mechanism-driven, and patient-specific compound screening. Our approach is established on several new deep learning methods that aim to overcome the OOD challenge. It enables us to explore dark matters in drug discovery. We further integrate machine learning with biophysics and systems biology to maximize the value of machine learning and minimize its limitations. Our computational pipeline models drug actions on a multi-scale from genome-wide drug-target interactions to molecular profiles in a patient. It allows us to identify promising personalized drug leads for Alzheimer’s disease and pancreatic cancers.

W40-5:

Targeted intracellular delivery of Cas13 and Cas9 nucleases using bacterial toxin-based platforms

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Targeted delivery of therapeutic proteins toward specific cells and across cell membranes remains major challenges. Here we develop protein-based delivery systems utilizing detoxified single-chain bacterial toxins such as diphtheria toxin (DT) and botulinum neurotoxin (BoNT)-like toxin, BoNT/X, as carriers. The system can deliver large protein cargoes including Cas13a, CasRx, Cas9, and Cre recombinase into cells in a receptor-dependent manner, although delivery of ribonucleoprotein containing guide RNAs is not successful. Delivery of Cas13a and CasRx, together with guide RNA expression, reduces mRNAs encoding GFP, SARS-CoV-2 fragments, and endogenous proteins PPIB, KRAS, and CXCR4 in multiple cell lines. Delivery of Cre recombinase modifies the reporter loci in cells. Delivery of Cas9, together with guide RNA expression, generates mutations at the targeted genomic sites in cell lines and iPSC-derived human neurons. These findings establish modular delivery systems based on single-chain bacterial toxins for delivery of membrane-impermeable therapeutics into the cytosol of targeted cells.

Poster Presentation Abstracts:

P-1:

Construction and characterization of two SARS-CoV-2 minigenome replicon systems

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The ongoing COVID-19 pandemic severely impacts global public health and economies. To facilitate research on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virology and antiviral discovery, a noninfectious viral replicon system operating under biosafety level 2 containment is warranted. We report herein the construction and characterization of two SARS-CoV-2 minigenome replicon systems. First, we constructed the IVT-CoV2-Rep complementary DNA template to generate a replicon messenger RNA (mRNA) with nanoluciferase (NLuc) reporter via in vitro transcription (IVT). The replicon mRNA transfection assay demonstrated a rapid and transient replication of IVT-CoV2-Rep in a variety of cell lines, which could be completely abolished by known SARS-CoV-2 replication inhibitors. Our data also suggest that the transient phenotype of IVT-CoV2-Rep is not due to host innate antiviral responses. In addition, we have developed a DNA-launched replicon BAC-CoV2-Rep, which supports the in-cell transcription of a replicon mRNA as initial replication template. The BAC-CoV2-Rep transient transfection system exhibited a much stronger and longer replicon signal compared to the IVT-CoV2-Rep version. We also found that a portion of the NLuc reporter signal was derived from the spliced BAC-CoV2-Rep mRNA and was resistant to antiviral treatment, especially during the early phase after transfection. In summary, the established SARS-CoV-2 transient replicon systems are suitable for basic and antiviral research, and hold promise for stable replicon cell line development with further optimization.

P-2:

Bioadhesive tape for gastrointestinal anastomotic leaks sealing

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Risk-adjusted mortality and morbidity define a quality standard for surgical care. Among postoperative complications, anastomotic leaks inflict more pain and sufferingthan any other surgical. The standard care to close wounds still mostly rely on traditional methods such as sutures. Sutures placed by hand uniformly invoke an inflammatory response because dragging the thread through the bowel wall injures tissue and causes reductions in ischemia, tissue necrosis, or narrowing of the lumen. During the last two decades, various attempts with fibrin glue, collagen patches, and degradable stents have been carried out to prevent anastomotic leaks. However, there is no convincing evidence thus far to demonstrate the effectiveness of these methods indecreasing occurrence of anastomotic leaks. The outcomes are closely related to the limitations of the commercial patches, which lack the high-strength bonding to tissue surfaces required in the fluid-rich intraperitoneal cavity. For example, Fibrin glue shows low adhesion force upon tissue contact and suffers from the risk of virus or prion contamination. Cyanoacrylate derivatives, which exhibit cytotoxicity due to degraded byproducts. Thus, it is highly desirable to develop biocompatible and mechanically stable adhesives for use in intraperitoneal spaces. In this talk, we will introduce our body fluid-resistant, biocompatible adhesive patches inspired by mussel adhesion. Weaddressed two long-existing challenges, aimed at the prevention of anastomotic leaks and promoting the wound healing.

P-3:

Antibacterial and fluorescence staining properties of an innovative GTR membrane containing 45S5BGs and AIE molecules *in vitro*

Yu-Wen Wei[1,†], Sayed Mir Sayed[2,†], Weiwen Zhu[1], Ke-Fei Xu[2], Fu-gen Wu[2], Jing Xu[1], HepengNie[1], Yuli Wang[1], Xiaolin Lu[2,*] and **Qian Ma[1,*]**

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Periodontitis is an inflammatory disease affecting periodontal tissues, and patients with severe periodontal defects require guided tissue regeneration (GTR) surgeries. However the oral cavity is an environment rich in bacteria. Therefore, infection is one of the most common complications after GTR surgery, which often leads to the failure. Our study aimed to develop a new GTR membrane, which had feasible antibacterial property without drug resistance. For this purpose, 45S5BG, a classic type of bioactive glasses, was chosen for its antibacterial activity, healing ability to soft tissues, remineralization characteristics, and adhesion and proliferation properties for osteoblasts. We also synthesized a naphthalimide-based bioprobe named tetraphenylethylene-naphthalimide (TPE-NIM) with aggregation-induced emission (AIE) characteristic and then modified it to prepare TPE-NIM$^+$ which could track and identify G^+ bacteria with fast stainability, high selectivity, and wash-free property. we innovatively introduced TPE-NIM$^+$ into the composite membrane as a selective fluorescent nano bioprobe.As the membrane degraded *in vivo*, TPE-NIM$^+$could be released from the membrane into the body fluid such as gingival crevicular fluid, pus, or blood,which was collected and examined to determine whether the patients' periodontitis was primarily caused by G^+ or G^- bacteria. Identification of bacterial species might help clinicians better evaluate the postoperative status and provide further treatment to the periodontal affected patients.The results showed our PLGA/BG/TPE-NIM+ composite membrane had efficient sterilization performance and surface mineralization ability. Innovatively adding molecules with AIE characteristics, the molecules aggregate into nanoaggregates to emit fluorescence, so that the membrane can be used to detect pathogens in a simple, fast, and wash-free protocol. Owing to its good biocompatibility, this membrane will have good effect on the bone tissue formation. Overall, this membrane has a wide range of clinical application prospects. Its strong antibacterial properties can prevent post-GTR infections and help clinicians perform rapid, minimally invasive, and convenient clinical diagnoses for infections.

P-4:

3D Printable Hydrogel Bioelectronic Interfaces for Various Organs
Tao Zhou, Xuanhe Zhao

Abstract: Bioelectronic interfaces require intimate and prolonged functional communication with highly fragile and sensitive tissues or organs, and therefore, high biocompatibility and low foreign body responses. Clearly noticeable in the progressive advances in bioelectronic interfaces in recent decades, bioelectronic interfaces have been continuously innovated in both designs and materials to achieve tissue-matching properties to minimize foreign body responses while providing desired electrophysiological functionalities such as recordings and stimulations. Owing to the unique tissue-matching properties, hydrogels have been one of the most promising materials to provide biocompatible long-term interfacing with biological tissues. Here, we report 3D-printed hydrogel bioelectronic interface consists of highly stretchable and soft hydrogels. The unique set of 3D-printable hydrogel inks allows facile and flexible one-step additive manufacturing of bioelectronic interfaces with diverse designs. To validate tissue-matching properties and biocompatibility of the 3D-printed hydrogel bioelectronic interfaces, we perform systematic mechanical and electrical characterizations and in vivo evaluations based on rat models. The resulted hydrogel bioelectronic interfaces will not only provide a promising platform for bioelectronic devices and treatments but also inspire the future development of functional hydrogel devices and machines.

P-5:
Uncovering the cellular mechanism of Schizophrenia associated gene *SETD1A* loss-of-function in ahuman neural model
Xiao Su[1], Yan Hong[2], Hanwen Zhang[3], Le Wang[1], Siwei Zhang[3], HongJun Song[2], Jubao Duan[3,4], Guo-liMing[2], Zhiping P. Pang[1]
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Rare loss-of-function (LoF) mutations in *SETD1A* are strongly associated with schizophrenia (SZ), a debilitating mental disorder affecting 1% of the population, and other severe neurodevelopmental disorders. *SETD1A* encodes a component of the histone methyltransferase complex producing mono-, di, and trimethylated histone H3 at Lysine 4 (H3K4). H3K4 trimethylation (H3K4me3) and H3K4me1 are epigenomic marks of active gene transcriptional promoters and enhancers, respectively. Interestingly, histone methylation has also been suggested as one of the most enriched gene pathways in common variant-based genome-wide associations studies (GWAS) of major psychiatric disorders. However, the detailed molecular mechanism by which it causes neuronal dysfunction is still unclear. Recent advances in stem cell biology have allowed the efficient conversion of human stem cells into defined neuron subtypes allows to address this question. Using CRISPR/Cas9 gene editing, we have generated isogenic hiPSC lines carrying heterozygous LoF mutations on different genetic backgrounds of *SETD1A*. Preliminary results showed that mutant lines were defective in neuronal development with premature neuronal differentiation at early developmental stages. Furthermore, morphological, electrophysiological and transcriptomic analyses of hiPSC neurons carrying *SETD1A* LoF mutation showed defective synaptic neurotransmission. Ongoing experiments are evaluating *SETD1A* LoF with functional, morphological, biochemical and genomic parameters to understand the cellular mechanisms that how *SETD1A* LoF contributes to the pathogenesis of SZ. The study enables us to perform a well-controlled assessment of theimpact of *SETD1A* LoF mutations on the molecular and cellular mechanisms underlying deficits in early neurodevelopment and synaptic properties.

P-6: empty

P-7:
Oxalate-Curcumin-Based Fluorescent ROS Probe: Visualizing ROS in Vascular Endothelial Cells and Atherosclerosis
Fangzhou Cheng[1], Yuxiang Zhou[1], Nadia Oleinik[1], Chongzhao Ran Ph.D.[2], and Jingyan Han Ph.D.[1]
[1]Vascular Biology Section, Evans Department of Medicine, Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA, USA; [2]Molecular Imaging Laboratory, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Reactive oxygen species (ROS), as redox signaling molecules are key players in endothelial dysfunction and atherosclerosis. However, their pathophysiological role remain enigmatic, partly due to the lack of effective methods to unambiguously detect the level and location of intracellular ROS. CRANAD-61, a near-infrared fluorescent imaging probe (λ_{Em}=740nm/λ_{Ex}=670nm)has recently been shown to be a novel ROS sensor via its oxalate moiety, which is readily oxidized by ROS, yielding CRANAD-5 with shorten fluorescent wavelength (λ_{Em}=560nm/λ_{Ex}=465nm). By harnessing this unique feature, we employed it to detect ROS in endothelial cells (EC) and aortic lesions of ApoE deficient ($ApoE^{-/-}$) mice. Using human aortic ECs, we demonstrated that CRANAD-61 was able to detect increasing levels of intracellular H_2O_2, mitochondrial ROS, and signaling ROS in response to treatments with increased doses of H_2O_2(0, 10, 50, 100 μM, 30 min),antimycin-A (0, 10, 50, 100 μM, 30 min), and tissue growth factor-β (TGF-β, 0, 1, 5, 10 ng/ml, 5 min) respectively, as manifested by augmented green fluorescent signals of CRANAD-5. CRANAD-61 showed a greater sensitivity and specificity than Dihydroethidium (DHE), which is a widely used ROS probe. *In vivo* administrating CRANAD-61 (2.0mg/Kg) to WD-fed $ApoE^{-/-}$ mice could detect cellular ROS in aortic lesions, and the oxidized probe CRANAD-5 was mainly accumulated in Mac-2-positive macrophages. Similar results were observed in the cross-sections of aortic lesions incubated with CRANAD-61 *in vitro*. We conclude that CRANAD-61 can effectively detect intracellular ROS in vascular endothelial cells and atherosclerotic lesions, and thus may become an efficient fluorescent imaging probe for detecting ROS in cardiovascular system.

P-8:
Chronic Moderate Alcohol Consumption In $ApoE^{-/-}$ Mice Damages Vascular Endothelial Function Via Redox Dysregulation of Small RhoGTPase Rac1
Xiaoqiu Yang[1], Kerstin Seidel Ph.D.[1], Xueping Wan Ph.D.[1], and Jingyan Han, Ph.D.[1]
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Although it is well established that chronic alcohol consumption impairs endothelial function and thus contributes to cardiovascular disease, findings on the vascular effects of moderate alcohol drinking are inconsistent. To mimic chronic moderate and heavy drinking patterns in humans with hypercholesterolemia, ApoE deficient ($ApoE^{-/-}$) mice on the Lieber-DeCarli liquid diet received ethanol (2g or 5g/kg/day as moderate or heavy drinking pattern, respectively), isocaloric maltose (5 g/kg/day as control) for 12 weeks. *In vivo* flow-mediated vasodilation (FMD) of femoral arterywere assessed, and found both moderate and heavy alcohol consumption significantly impaired endothelium-dependent FMD; whereas endothelium-independent FMD was promoted after 10- wks feeding as reflected by increased FMD value in the presence of L-NAME, an eNOS inhibitor.Aortic endothelial permeability as measured by Evan's blue dye infiltration, and atherosclerotic lesions at aortic root were all promoted by moderate and heavy drinking. In aorta and endothelium- rich lung tissues, overall protein S-glutathione levels were elevated in the moderate and heavy drinking groups, coinciding with eNOS inactivation as reflected by a reduction in its dimer to monomer ratio; the RhoGTPase activity of Rac1 was impaired, and its glutathionylation level waselevated. In human aortic endothelial cells, ethanol exposure dose-dependently promoted protein S-glutathionylation, while lowering the protein levels of glutaredoxin-1, an explicit de- glutathionylation enzyme. Interestingly, moderate ethanol treatment (25mM) per se showed negligible impact on eNOS and Rac1 activities; however, in endothelial cells under metabolic stress, or knockdown of Grx1 by CRISPR system, the moderate ethanol treatment caused endothelial dysfunction as observed in animal studies. We conclude that, in the presence of other cardiovascular risk factors like hypercholesterolemia, moderate alcohol consumption can also cause vascular endothelial dysfunction; the underlying mechanism is attributable at least in part to the aggravated S-glutathionylation-centered redox dysregulation of Rac1 and eNOS.

P-9:

Glutaredoxin1 preserves endoplasmic reticulum stress response in aortic endothelium in aging

Xueping Wan Ph.D.[1], Kerstin Seidel Ph.D.[1], Yuxiang Zhou[1], and Jingyan Han, Ph.D.[1]

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Vascular aging is associated with endoplasmic reticulum (**ER**) stress and oxidative stress, which are closely linked mechanisms that predispose the elderly to cardiovascular diseases. Aging- related ER malfunction features impaired ER stress response also known as unfolded/misfolded protein response (**UPR**). Our previous studies showed glutaredoxin1 (**Grx1**), a potent thiol antioxidant that specifically remove oxidant-induced S-glutathionylation (**PrS-SG**) on protein thiols, plays a protective role in development of age-related metabolic syndrome, and aortic endothelial barrier dysfunction by hypercholesterolemia. PrS-SG of ER proteins is associated with ER stress. As such we hypothesize Grx1 preserves ER homeostasis in endothelial cells (**ECs**) during aging. In aortae from middle-aged Grx1-halpoinsufficient ($Grx1^{+/-}$) and $Grx1^{-/-}$ mice (10- mo. old), the levels of Grx1 and BiP that is a sentinel marker of UPR activation, are decreased. Additionally, compared with age-matched wild-type (**WT**) group, major ER stress mediator CCAAT-enhancer-binding protein homologous protein (**CHOP**) and apoptosis executor cleaved caspase 3 are increased. These aortic changes in Grx1 deficient mice are coincident with an accumulation of PrS-SG, and are comparable to those in 22-mo-old WT mice. In human aortic ECs, Grx1 protein degradation is sensitive to ER stress caused by different stressors (e.g., glycosylation inhibitor tunicamycin, SERCA inhibitor thapsigargin, proteasome inhibitor MG132, S-glutathionylation inducer GSSG)—but not to thiol-reducing agent DT— which implies thiol oxidation is involved in the Grx1 proteolytic process. Using mouse aortic ECs isolated Grx1 transgenic mice, we found upregulation of Grx1 by itself promotes BiP and CHOP expression, and confer resistance, especially to CHOP and apoptosis induced by thiol oxidative stress. In parallel with ER function regulated by Grx1, In EC-enriched lung tissues—but not livers— from 10-mo.-old $Grx1^{-/-}$ mice, phosphorylation of Akt and eNOS upon acute insulin stimulation is massively inhibited. We conclude that Grx1 through preserving UPR improves vascular endothelial dysfunction during aging.

P10:

Atherosclerotic Lesions

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Aortic endothelial cell dysfunction is an early trigger of atherosclerosis, the major cause of cardiovascular disease. Nanomedicines targeting vascular endothelium and lesions hold great promise as therapeutic solutions to vascular disorders. The objective of the present study is to investigate macrovascular targeting efficacy of a redox-responsive degradable polyurethane- polyurea nanocapsule (Puua-NC). Red fluorescent lipophilic cationic indocarbocyanine dye, withDiI as cargo, was encapsulated into Puua-NCs of variable sizes (20, 175, 255 nm in diameter) at aconcentration of 8.60, 6.54, and 6.91ng/mg NCs, respectively. *In vitro* cellular uptake studies with human aortic endothelial cells (HAECs) indicated these different sized Puua-NCs were efficiently taken up by cells, and the cellular uptake of NCs was positively correlated with concentration and incubation time. Exposure of HAECs to dithiothreitol (DTT, a thiol reducing reagent) or diamide(a thiol oxidizing reagent) altered intracellular thiol redox status and broke disulfide bonds on the Puua-NC shell, triggering cell disassembly and release of the encapsulated fluorescent cargo DiI,as evidenced by the loss of bright punctuate red fluorescence and appearance of the weak diffusedsignals of DiI. Using ApoE deficient mice fed a Western-type diet, a mouse model of atherosclerosis, we showed that intravenously administrated Puua-NCs were efficiently taken up by aortic endothelium and atherosclerotic lesions *in vivo*. Importantly, internalized Puua-NCs in vascular cells and lesions were rapidly disassembled to release DiI in response to DTT treatment.We conclude that Puua-NCs demonstrate remarkable affinity to aortic endothelium and atherosclerotic lesions, with disassembly well controlled by redox status. Puua-NCs may be used as a novel redox-responsive degradable nanodevice to deliver drugs and gene materials to the cardiovascular system.

P-11:

Transcriptional and functional motifs defining renal function revealed by single nuclei RNA sequencing

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Recent advances in single cell sequencing provide a unique opportunity to gain novel insights into the diversity, lineage and functions of cell types constituting a tissue/organ. Here, we performed a single nuclei study of the adult *Drosophila* renal system, consisting of Malpighian tubules and nephrocytes, which shares similarities to the mammalian kidney. We identified 11 distinct clusters representing renalstem cells, stellate cells, regionally specific principal cells, garland nephrocyte cells and pericardial nephrocytes. Characterization of the transcription factors specific to each cluster identified *fruitless (fru)*as playing a novel role in stem cell regeneration and *Hepatocyte nuclear factor 4* (*Hnf4*) in regulating glycogen and triglyceride metabolism. In addition, we identified a number of genes, including *Rho guanine nucleotide exchange factor at 64C* (*RhoGEF64c*), *Frequenin 2* (*Frq2*), *Prip* and *CG1093,* that are involved in regulating the unusual star shape of stellate cells. Importantly, the single cell dataset allows visualization of the expression at the organ level of genes involved in ion transport and junctionalpermeability, providing a systems-level view of the organization and physiological roles of the tubules. Finally, a cross-species analysis allowed us to match the fly kidney cell types to mouse kidney cell typesand planarian protonephridia - knowledge that will help the generation of kidney disease models.Altogether, our study provides a comprehensive resource to study the fly kidney.

P-12:

Threshold of neutralizing antibody titers in vaccinated people with SARS-CoV-2 Deltabreakthrough infections

Ping Ren[1], Jing Zou[2], Xuping Xie[2], Mingru Liu[1], Pei-Yong Shi[2]
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Neutralizing antibodies elicited by vaccines protect us from SARS-CoV-2 infection. However, due to waning immunity and evasion of neutralization, breakthrough infections occur in fully vaccinated people, especially during the surges of previous Delta and current Omicron variants.To determine the minimal antibody level required for preventing COVID, we measured the SARS-CoV-2 neutralizing antibody titer against SARS-CoV-2 USA-WA1/2020 in the sera/plasmas from fully vaccinated people who had vaccine breakthrough infection from July to October 2021, the period of Delta variant infection surged. Among 75 qualified patient samples,48 (64%) had no detectable neutralizing antibody against USA-WA1/2020. Other breakthrough samples had neutralizing antibody titers of less than 1:70 against USA-WA1/2020. The neutralizing antibody titers against the Delta variant are under investigation. Our preliminary data suggest that a minimum of 1:70 neutralizing antibody level is potentially required for protection against breakthrough infections of the Delta variant. These clinical laboratory results have implications for vaccine strategy and public health policy.

P-13:

The ubiquitin sensor and adaptor protein p62 mediates EBV LMP1 signal transduction

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EBV LMP1 serves as a paradigm that engages complicated ubiquitination-mediated mechanisms to activate multiple transcription factors. p62 is a ubiquitin sensor and a signal-transducing adaptor that has multiple functions in diverse contexts. However, the interaction between p62 and oncogenic viruses is poorly understood. We have recently reported a crucial role for p62 in oncovirus-mediated oxidative stress by acting as a selective autophagy receptor. In this following study, we further discover that p62 is upregulated in EBV Type 3 compared to Type 1 latency, with a significant contribution from NFκB and AP1 activitiesdownstream of LMP1 signaling. More importantly, p62 participates in LMP1 signal transduction in a positive feedback loop through its interaction with TRAF6, promoting TRAF6 ubiquitination and activation. As expected, shRNA-mediated knockdown (KD) of p62 reduces LMP1-TRAF6 interaction and TRAF6 ubiquitination, as well as p65 nuclear translocation. Strikingly, LMP1-stimulated NFκB, AP1, andAkt activities are all markedly reduced in p62-/- MEFs or in EBV-negative Burkitt's lymphoma (BL) cell lines with CRISPR-mediated knockout (KO) of the p62-encoding gene. In consequence, shRNA-mediatedp62 KD impairs the ability of LMP1 to regulate its target gene expression, promotes etoposide-induced apoptosis, and reduces the proliferation of lymphoblastic cell lines (LCLs). These important findings have revealed a previously unrecognized novel role for p62 in EBV latency and oncogenesis, which advance ourunderstanding of the mechanism underneath virus-mediated oncogenesis. (mBio, 2021. 12:e0109721)

P-14: See W18-6

P-15 :See W32-6

P-16: See W12-5

P-17:

Structure and regulation of the respiratory syncytial virus polymerase

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Respiratory syncytial virus (RSV) is a nonsegmented negative-sense (NNS) RNA virus and shares a similar RNA synthesis strategy with other members of NNS RNA viruses, such as measles, rabies, and Ebola viruses. RSV RNA synthesis is catalyzed by a multifunctional RNA-dependent RNA polymerase

(RdRP), composed of a 250 kDa large (L) protein that catalyzes three distinct enzymatic functions (nucleotide polymerization, cap addition, and cap methylation) and an essential coenzyme tetrameric phosphoprotein (P). How RSV L and P coordinate these activities is poorly understood. Here, we present a 3.67 Å cryo-EM structure of the RSV polymerase (L:P) complex. The structure reveals that the RNA dependent RNA polymerase (RdRp) and capping (Cap) domains of L interact with the oligomerization domain (P_{OD}) and C-terminal domain (P_{CTD}) of a tetramer of P. The density of the methyltransferase (MT) domain of L and the N-terminal domain of P (P_{NTD}) is missing. The structure we obtained is likely to be at an elongation-compatible stage. Besides, we demonstrated that the RSV polymerase could carry out both *de novo* and primer-based RNA synthesis. We defined the minimal length of the RNA template for *in vitro de novo* RNA synthesis using the purified RSV polymerase is 8 nucleotides (nt). We showed that the RSV polymerase catalyzed primer-dependent RNA elongation with different lengths of primers on both short (10-nt) and long (25-nt) RNA templates. We compared the sequence specificity of different viral promoters and identified positions 3, 5, and 8 of the promoter sequence as essential to the *in vitro* polymerase activity, consistent with the results previously mapped with the *in vivo* minigenome assay. Overall, these findings agree well with previous studies, provide enriched insights into the interrelationship, the inhibitors, and the evolutionary implications of the RSV polymerase and extend our understanding of the mechanism of RSV RNA synthesis.

P-18: See W14-5

P-19: See W27-6

P-20: See W34-4

P-21: See W7-6

P-22: See W26-6

P-23: See W35-5

P-24: See W29-6

P-25: See W28-6

P-26: See W9-6

P-27: See W37-6

P-28: See W21-5

P-29: See W31-6

P-30: See W10-6

P-31: See W11-5

P-32: See W39-6

P-33: See W1-6

P-34: See W11-6

P-35: empty

P-36: empty

P-37: See W25-6

P-38: See W21-5

P-39: See W8-5

P-40: See W6-6

P-41: empty

P-42: See W32-5

P-43: See W2-6

P-44: See W22-6

P-45: See W30-6

P-46: See W24-6

P-47: See W40-5

P-48: See W37-5

P-49: See W36-6

P-50: See W16-6

P-51: See W8-4

P-52: See W34-5

P-53: See W4-6

P-54: See W13-6

P-55: See W35-6

P-56: See W19-6

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Tao Liu (刘涛) Volunteer
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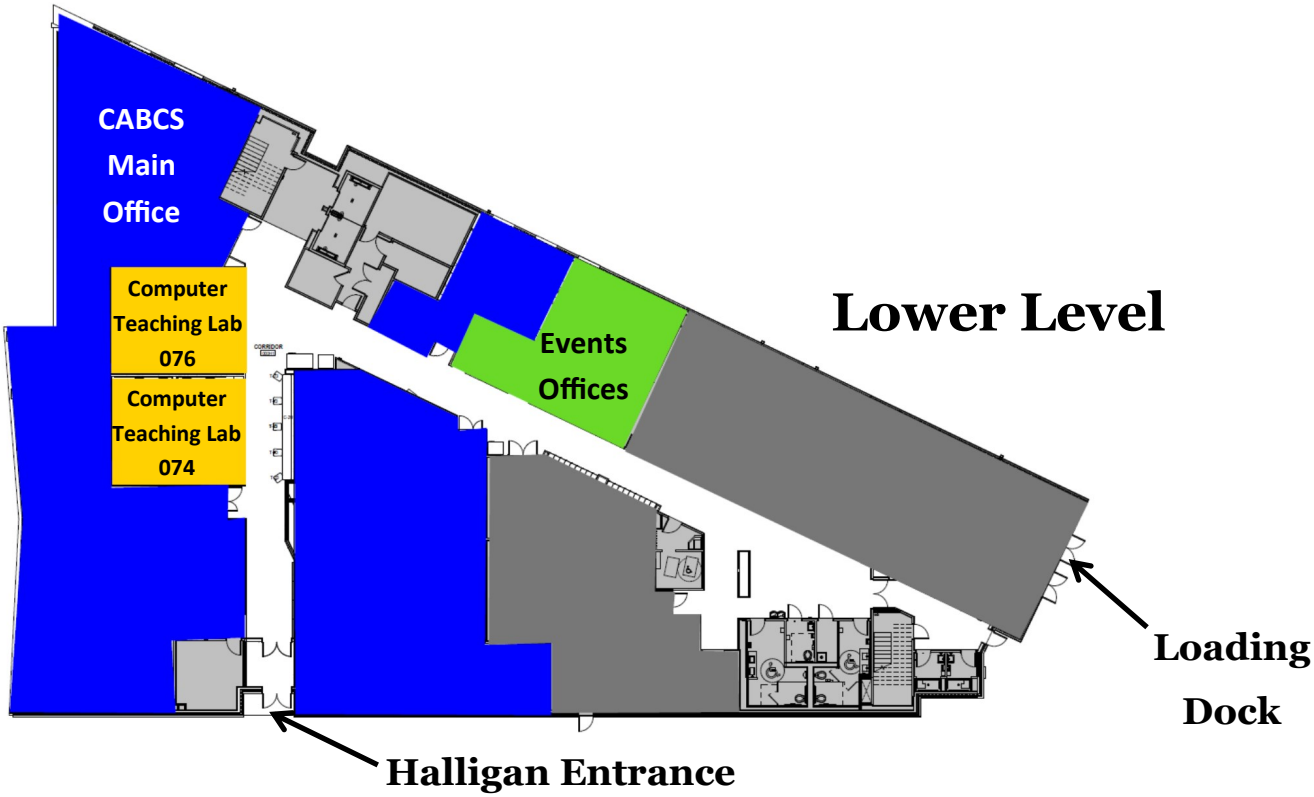

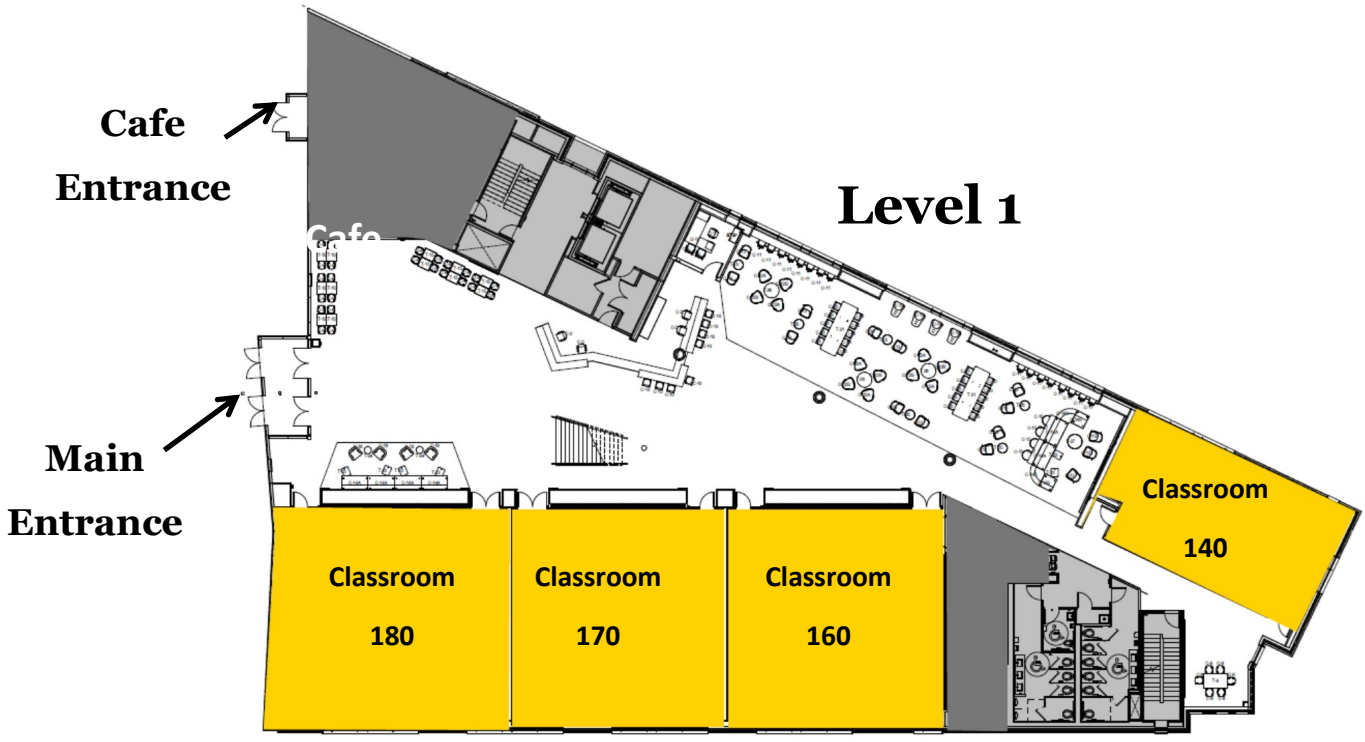

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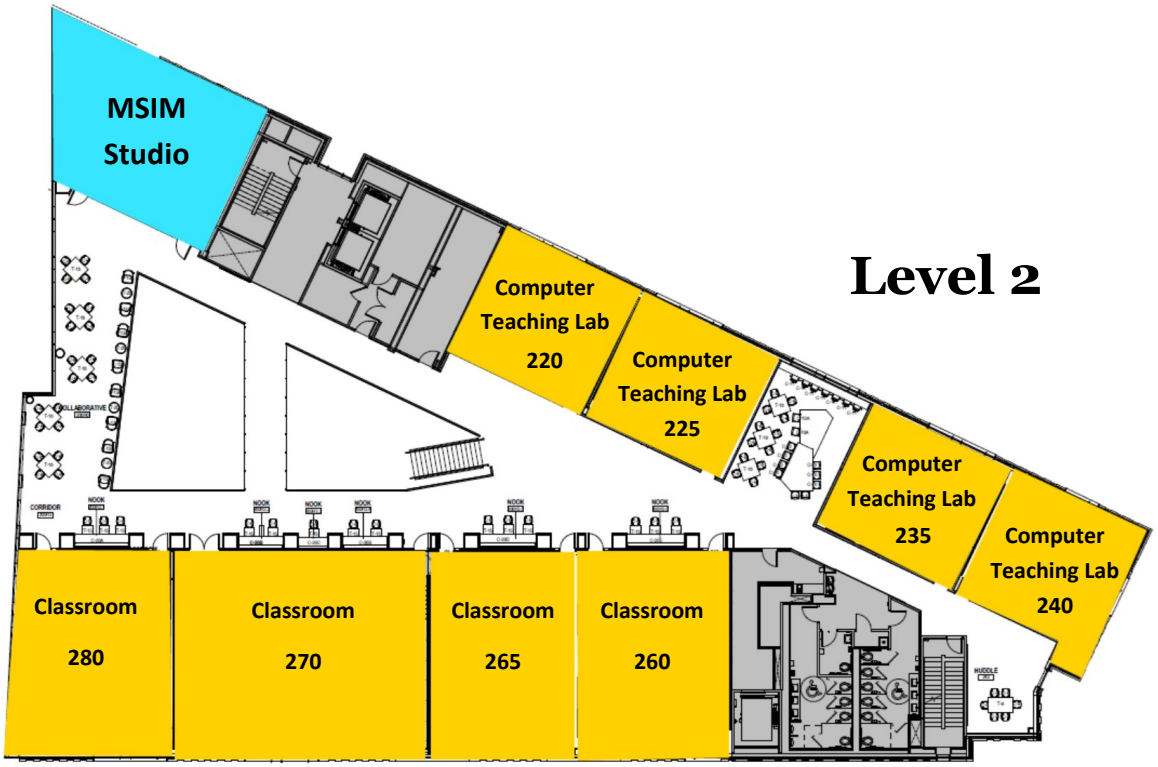

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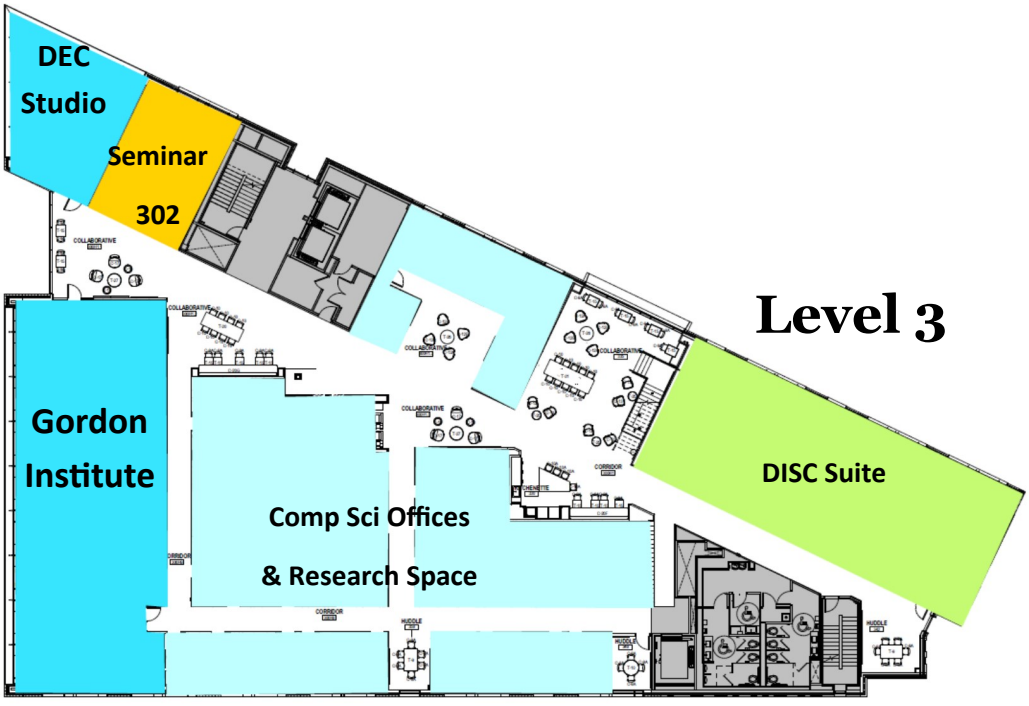

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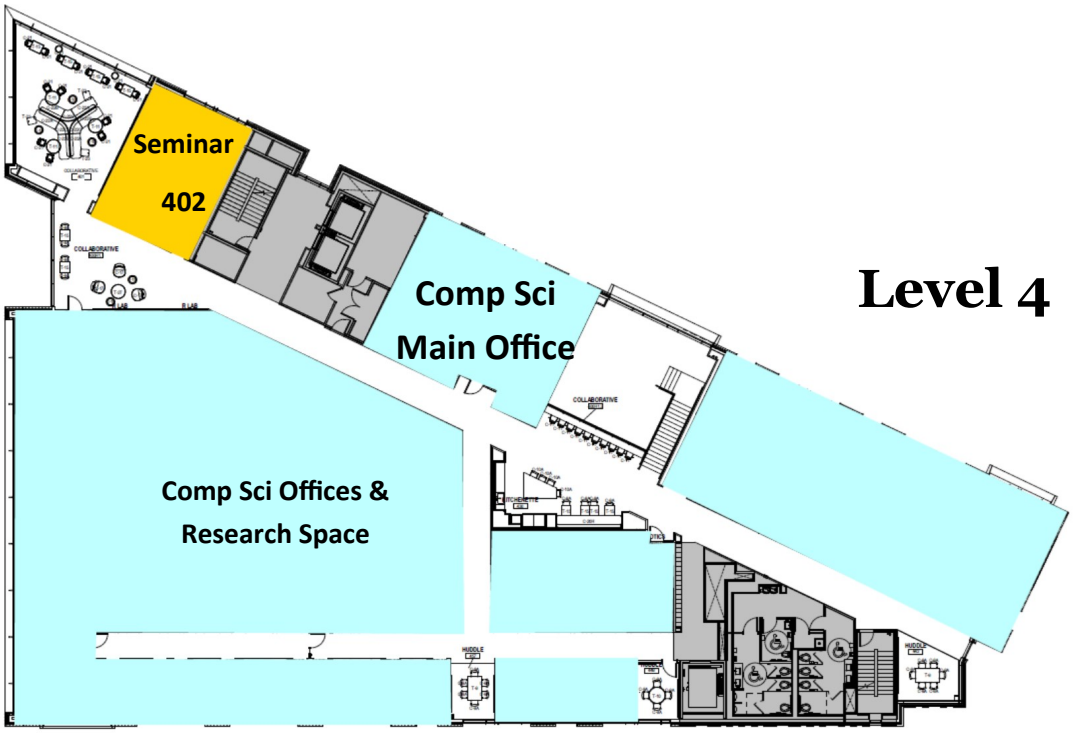

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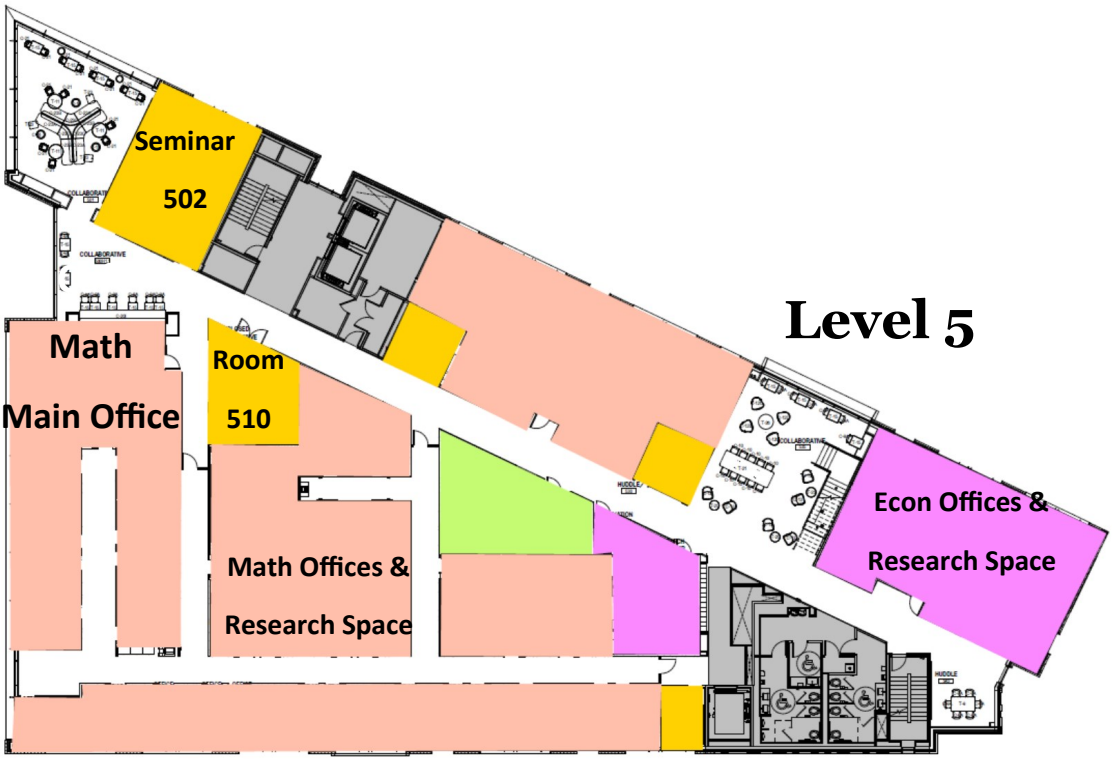

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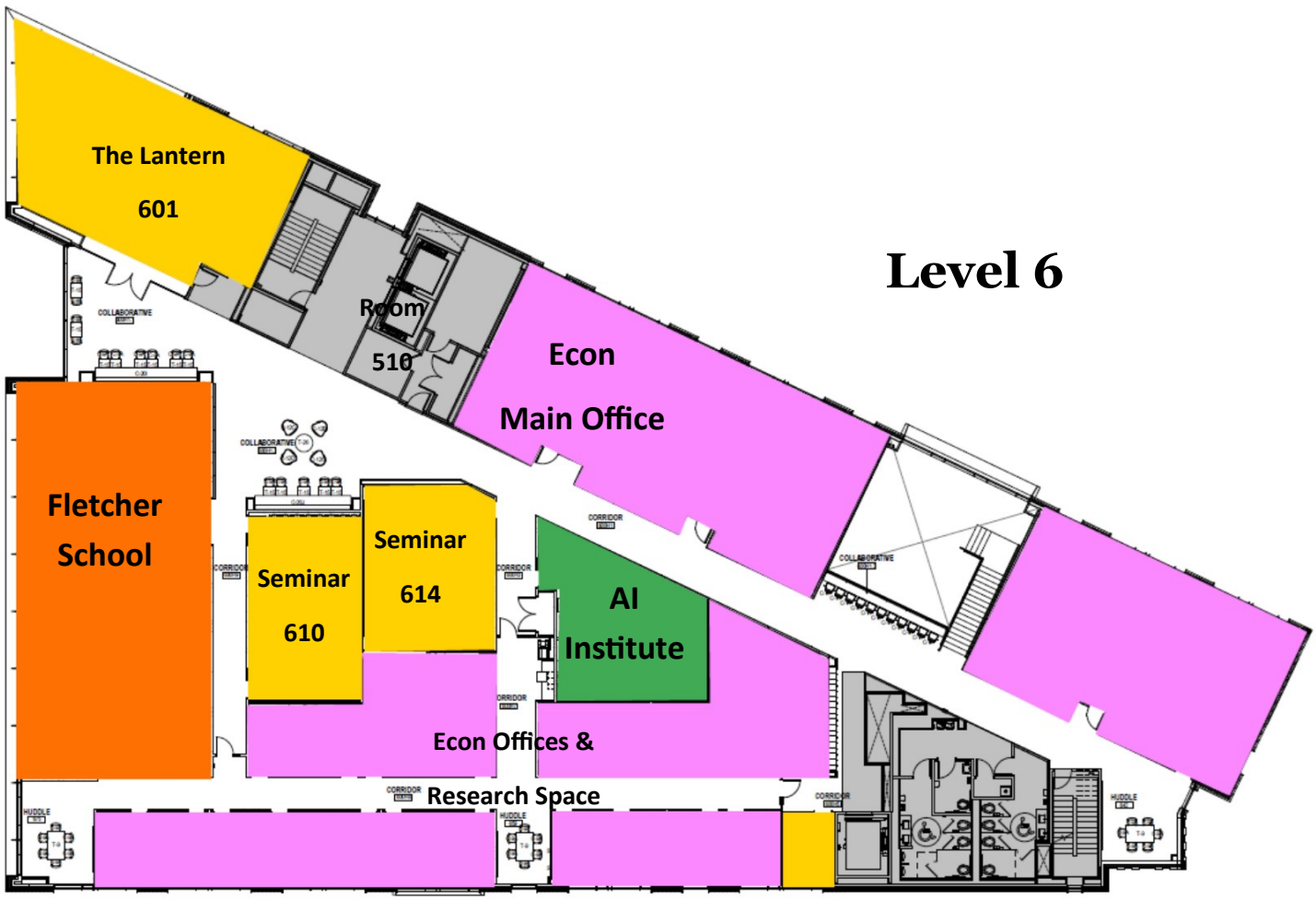

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