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P1: Research and Development of Chemical Control Drugs for Plant Bacteria and Mechanisms of Antibacterial Action

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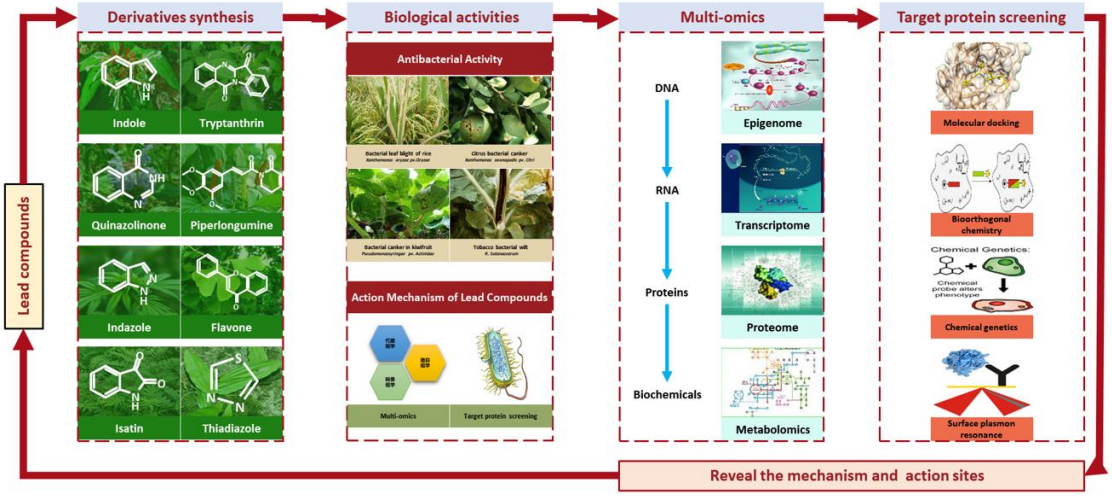
Plant bacterial diseases such as *Xanthomonas oryzae pv. oryzae* (*Xoo*) and *Xanthomonas axonopodis subsp. Citri* (*Xac*) pose significant threats to crops. We used organic synthesis method to construct over 1000 small molecule compounds containing nitrogen heterocyclic compounds derivfed from Chinese herbs, and successfully identified key compounds including indole, indazole, quinazoline, tryptamine and piperlongine. More than 60 lead molecules were screened for their effective inhibition of plant pathogens. Moreover, we have established a comprehensive research platform for the isolation and identification of plant pathogens, as well as *in vitro* and *in vivo* screening and evaluation of inhibitory lead compounds. Our approach integrates chemical genetics, multi-omics, molecular biology, computational biology, epigenetics, and other cutting-edge technologies to investigate the mechanisms underlying antibacterial action.



P2: Mutants Produced by the Evolution of Marine

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Microorganisms have caused serious economic losses to our ocean. Traditional techniques do not meet the growing demand for single-base resolution and PCR-free rapid diagnosis and high-throughput analysis of marine microorganisms. The project intends to solve two key scientific questions: (1) single-base resolution mechanism of marine microbial subtypes, and (2) High sensitivity of single-base resolution detection. Based on theoretical innovation (the first author proposed the concept of microbial defense sensor in Chemical Review), the project research team carried out multidisciplinary research on new microbial technologies for the CRISPR-Cas13/Cas14 system as a biosensor system.

Main contents: (1) CRISPR-Cas13/Cas14 sensing response, (2) Highly specific single-base resolution mechanism; (3) Synergistic enhancement mechanism of

high-sensitivity signals. The project is expected to propose a new mechanism for marine microbial diagnosis based on CRISPR/Cas system, which will provide a theoretical basis for marine microbial monitoring and protection technology.

P3: Glia-Derived Lipid Metabolites Drive Pathological Angiogenesis in Proliferative Retinopathy

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Proliferative retinopathy (PR) is a prominent microvascular complication of premature infants and diabetic adults, and is a leading cause of blindness. Long-chain polyunsaturated fatty acids (LCPUFA) either protect (ω-3) or exacerbate (ω-6) PR, but the precise mechanisms remain ill-defined. Cytochrome P450 oxidase (CYP) 2J metabolizes LCPUFA into bioactive lipid mediators with pro-angiogenic effects. Here, we show that CYP2J is mainly expressed in retinal glial cells and its lipid metabolites are indirectly involved in neovascular disease. Oxygen-induced retinopathy (OIR) model was used to mimic PR. Comprehensive single-cell RNA-sequencing (scRNA-seq) was performed in human and mouse retinas with PR to delineate the expression profile of CYP2J and its alterations in PR. The pro-angiogenic activity of CYP2J was blocked by its potent and selective inhibitor flunarizine, in OIR mice fed with diets containing either ω-3 or ω-6 LCPUFA. Cellular localization of CYP2J and the lipid-activated receptor, peroxisome proliferator-activated receptor (PPAR)-delta was assessed by immunohistochemistry in OIR retinas. scRNA-seq was used to identify proangiogenic effects of CYP2J-derived metabolites. ScRNA-seq analysis revealed a predominant expression of CYP2J in astrocytes and Müller glia in both human and mouse retinas. CYP2J6 and CYP2J9 were the main CYP2J subtypes expressed in OIR retinas. Importantly, inhibition of CYP2J activity with flunarizine resulted in a significant reduction ($p < 0.001$) of pathological neovascularization in mice fed with ω-6 and ω-3 LCPUFA diets. CYP2J protein was localized in astrocytes by immunofluorescence. Finally, CYP2J-derived lipid metabolites were shown to activate PPAR-delta localized to retinal pathological neovessels, but the PPAR-delta inhibitor, GSK3787, significantly reduced ($p < 0.001$) pathological angiogenesis. This study reveals the expression patterns and retinal localization of CYP2J subtypes, as well as their significant role in pathological retinal neovascularization. Pharmacologic modulation of CYP2J or PPAR-delta may be promising alternatives to mitigate pathologic neovascularization in PR and other vascular diseases.

Key Words: proliferative retinopathy, long-chain polyunsaturated fatty acid, cytochrome P450 oxidase 2J, peroxisome proliferator-activated receptor, pathological angiogenesis

P4: Cathepsin K Expressing Progenitor Cells Activate Hedgehog Signaling to Drive Bony Fusion

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ABSTRACT.
Objective Pathological bone formation characterized by ossification within soft tissues is a complication of ankylosing spondylitis (AS), and involves abnormal osteogenesis by stem/progenitor cells. However, the cells of origin and mechanisms involved in the pathogenesis of spine fusion remain elusive.
Methods Ossification was analyzed by micro-CT. Chondrocytes and ossification in the AF were detected by H&E and Safranin O/fast green (SOFG) and immunofluorescence. Primary annulus fibrosis (AF) derived progenitor cells(AFDPCs) were sorted by FACS and induced to different differentiation. The relative expression of Hedgehog (Hh) signaling and differentiation relative genes were detected by qRT-PCR.
Results Micro-CT of the 20-, and 40-week-old Cathepsin K(Ctsk)-Cre; Suppressor of fused(Sufu) $^{flox/flox}$ mice showed spontaneous and progressive AF ossification and spine fusion compared with Ctsk-Cre mice($P < 0.01$). Histologically, SOFG staining and immunofluorescence staining of type II collagen and osteopontin showed chondrocytes and ossification in the AF from the Ctsk-Cre;Sufu$^{flox/flox}$ mice. Lineage tracing studies using Ai9 reporter mice to label all Ctsk lineage cells with tdTomato fluorescence. Ctsk-Cre was expressed in AF cells.The Ctsk-Cre–expressing cells expressed the AF marker Scleraxis(Scx) after crossing with Scx-GFP mice.Ctsk^{+}Scx^{+} AFDPCs identified by FACS are enriched for stem cell markers. Ctsk^{+} cells from the AF of the Ctsk-Cre;Sufu$^{flox/flox}$;Ai9 mice and Ctsk-Cre;Ai9 mice were induced to different differentiation. qRT-PCR showed higher expression of osteogenic genes(Bsp($P < 0.001$), Runx2($P < 0.01$)), chondrogenic genes (Sox9($P < 0.001$), Col2a1($P < 0.01$)) and Hh target genes (Gli1($P < 0.05$), Gli2($P < 0.01$), Patch1($P < 0.05$))in the Ctsk^{+} cells from the AF of the Ctsk-Cre;Sufu$^{flox/flox}$; Ai9 mice.Furthermore, knocking down Gli1 or Gli2 genes which directly activate transcription of Hh target genes in the Ctsk-Cre;Sufu$^{flox/flox}$ mice by crossing with Gli1$^{lacZ/lacZ}$ knockin mice or Gli2 $^{flox/flox}$ mice, suppressed the development of spine fusion compared with the Ctsk-Cre mice.
Conclusion Ctsk-Cre labels a subpopulation of AFDPCs. Sufu deficiency caused enhanced chondrogenic and osteogenic differentiation of Ctsk-Cre expressing AF-derived cells via upregulation of Hh signaling. Intervention of Hh target genes suppressed the development of spine fusion, which may be a new therapeutic way for spine fusion in AS.

Key Words: Stem cell, Annulus fibrosis, Spine fusion

P5: Host Cyclophilin a Facilitates SARS-CoV-2 Infection by Binding and Stabilizing Spike on Virions

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ABSTRACT
SARS-CoV-2 continues to evolve and remains highly transmissible, which is primarily mediated by spike (S) protein. S is cleaved at S1/S2 boundary during biosynthesis, and cleaved S1 and S2 are associated noncovalently to maintain S trimeric state. Although the structure and function of S trimers have been well-investigated, the regulation and maintenance of the S1/S2 association remain unknown. In this study, we found that a host protein can facilitate S1 engagement to S oligomers after S cleavage, via increasing S1/S2 interaction. By a whole-genome screen, human cyclophilin A (PPIA or CyPA) was identified to interact with both S1 and S2, and cyclic peptide inhibitors of PPIA can disrupt the infection of SARS-CoV-2 via blocking PPIA-S interaction. Moreover, PPIA expression in target cells contributes to viral infectivity and membrane fusion activity of SARS-CoV-2.
Targeting PPIA can disrupt the infection of multiple SARS-CoV-2 variants of concern (VOCs). Mechanically, we highlight that it is PPIA binding, but not its isomerase activity, that facilitates S1 engagement to S trimers on the surface of virions and probably maintains prefusion S trimer state ready for receptor binding.
Together, our results clearly demonstrate a previously unknown mechanism that maintains S trimeric structure on SARS-CoV-2 virions to facilitate viral infection, and emphasize PPIA as a therapeutic target for COVID-19.

Key Words: SARS-CoV-2; Spike; PPIA; S-Trimer; viral infection

P6: Miniaturized Click Chemistry and Crystallography Enabled Rapid Discovery of Unique Diazabicyclooctane-based SARS-CoV-2 Mpro Inhibitors

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ABSTRACT.
The ongoing global spread of SARS-CoV-2 and its variants underscores the urgent need for the development of effective broad-spectrum anti-drug resistance agents. In this study, focused compound libraries were synthesized in 96-well plates utilizing click chemistry to rapidly discover potent inhibitors targeting SARS-CoV-2 main protease (M^{pro}). Subsequent direct biological screening identified novel 1,2,3-triazole derivatives as potent M^{pro} inhibitors with high anti-SARS-CoV-2 activity. Notably, **C5N17B** demonstrated sub-micromolar M^{pro} inhibitory potency (IC_{50} = 0.12 μM) and excellent antiviral activity in Calu-3 cells determined through an immunofluorescence-based antiviral assay (EC_{50} = 0.078 μM, CC_{50} > 100 μM). **C5N17B** showed superior potency to nirmatrelvir (EC_{50} = 1.95 μM) and similar

efficacy to ensitrelvir (EC_{50} = 0.11 μM). Importantly, this compound displayed high antiviral activities against several SARS-CoV-2 variants (Gamma, Delta and Omicron, EC_{50} = 0.13 - 0.26 μM) and HCoV-OC43, indicating its broad-spectrum pan-coronaviral activity. C5N17B retained its antiviral activity against nirmatrelvir-resistant strains with T21I/E166V and L50F/E166V mutations in M^{pro} (EC_{50} = 0.26 and 0.15 μM, respectively). Futhermore, **C5N17B** displayed favorable pharmacokinetic properties. Crystallography studies revealed a unique, non-covalent multi-site binding mode. In conclusion, these findings substantiate the potential of **C5N17B** as a promising drug candidate for clinical therapy.

Key Words: Click Chemistry; Crystallography; SARS-CoV-2; Mpro; Drug design

P7: Spatiotemporally Controlled Function of a Chromatin Remodeler Distinguishes Pancreatic Malignancy from Tissue Regeneration

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ABSTRACT.

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease, and the average survival rate of stage IV PDAC patients 5 years after diagnosis is less than 1%. The cell of origin for PDAC is adult acinar cells. Acute pancreatitis induces a transient progenitor-like cell fate switch called acinar-to-ductal metaplasia (ADM), which allows pancreatic tissue regeneration or chronic pancreatitis in the presence or mutant Kras, which then can progress into PDAC upon loss of tumor suppressor genes. Tumor cell intrinsic and environmental cues both have recently been demonstrated to induce the series of neoplastic reprogram and the accompanied epigenetic remodeling. In addition, the Kras signaling is activated in both wild type or mutant Kras setting, and its activity has been paradoxically shown to be required for both normal tissue regeneration (with wile type Kras) and tumorigenesis (mutant Kras). Thus, an intervention that specifically reverses the mutant Kras activity without affecting normal Kras signaling would be a therapeutically preferred. Although many studies have tried to identify epigenetic determinants of early chromatin alterations during pancreatitis, factors such as BRD4 and BRG1 ablation not only stops cancer formation, but also causes tissue atrophy . Thus, the effects of general chromatin factors such as Brd4 inhibitors are less than satisfying. An optimal intervention of cancer formation would be such measure that only prevent malignant transformation without affecting the ability of normal adult tissue to regenerate.

In this study, we set out to map the series chromatin switches from the earlies time point during acute pancreatitis to tumorigenesis, aiming to reveal a mechanism that can differentiate malignancy and tissue regeneration.

Key Words: Chromatin remodeler, pancreatic cancer, transcription factor

P8: Hypoxia-activated Selectivity-improved Anti-PKM2 Antibody Combined with Prodrug TH-302 for Potentiated Targeting Therapy in Hepatocellular Carcinoma

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Background: Hypoxia induces hepatocellular carcinoma (HCC) malignancies; yet it also offers treatment opportunities, exemplified by developing hypoxia-activated prodrugs (HAPs). Although HAP TH-302 combined with therapeutic antibody (Ab) has synergistic effects, the clinical benefits are limited by the on-target off-tumor toxicity of Ab. Here, we sought to develop a hypoxia-activated anti-M2 splice isoform of pyruvate kinase (PKM2) Ab combined with TH-302 for potentiated targeting therapy.

Methods: Codon-optimized and hypoxia-activation strategies were used to develop H103 Ab-azo-PEG5k (HAP103) Ab. Hypoxia-activated HAP103 Ab was characterized, and hypoxia-dependent antitumor and immune activities were evaluated. Selective imaging and targeting therapy with HAP103 Ab were assessed in HCC-xenografted mouse models. Targeting selectivity, systemic toxicity, and synergistic therapeutic efficacy of HAP103 Ab with TH-302 were evaluated.

Results: Human full-length H103 Ab was produced in a large-scale bioreactor. Azobenzene (azo)-linked PEG5k conjugation endowed HAP103 Ab with hypoxia-activated targeting features. Conditional HAP103 Ab effectively inhibited HCC cell growth, enhanced apoptosis, and induced antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) functions. Analysis of HCC-xenografted mouse models showed that HAP103 Ab selectively targeted hypoxic HCC tissues and induced potent tumor-inhibitory activity either alone or in combination with TH-302. Besides the synergistic effects, HAP103 Ab had negligible side effects when compared to parent H103 Ab.

Conclusion: The hypoxia-activated anti-PKM2 Ab safely confers a strong inhibitory effect on HCC with improved selectivity. This provides a promising strategy to overcome the on-target off-tumor toxicity of Ab therapeutics; and highlights an advanced approach to precisely kill HCC in combination with HAP TH-302.

Key Words: Hypoxia-activated antibody, On-target off-tumor toxicity, TH-302, PKM2, Hepatocellular carcinoma

P9: Multiple Roles of the Natural Product MAM in Promoting Cell Death

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ABSTRACT
Natural products are important sources of anticancer drug research and discovery. A panel of natural compounds such as paclitaxel, camptothecin, vinblastine, and vincristine, etc, have been widely used for cancer therapy for decades. 2-Methoxy-6-acetyl-7-methyljuglone (MAM) is a naphthoquinone isolated from Polygonum cuspidatum Sieb.et Zucc. (虎杖), a traditional Chinese herb with the function of fever relieving and detoxification. Previous studies revealed its antibacterial, neuroprotective, and antiviral activities. We focus on its anticancer effect and found that it is a potent cytotoxic compound. MAM significantly kills a panel of cancer line cells in vitro and inhibited tumor growth in xenograft animal models. Interestingly, it may trigger different types of programmed cell death (PCD) in different cell lines including apoptosis, necroptosis, and ferroptosis. Reactive oxygen species (ROS), JNK1/2, and calcium play important roles in mediating MAM-induced PCD. Receptor interacting protein kinase 1 (RIP1) and NAD(P)H quinone oxidoreductase 1 (NQO1) might be MAM's molecular targets. Collectively, MAM shows a potent anticancer effect by inducing several forms of cell death.

Key Words: MAM; PCD; Necroptosis; Ferroptosis; ROS

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P10: Discovery of Natural Product Derivative Triptolidiol as a Direct NLRP3 Inhibitor by Decreasing K63-specific Ubiquitination

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ABSTRACT
NLRP3 inflammasome activation is implicated in various inflammatory and autoimmune conditions. However, developing effective NLRP3 inhibitors is challenging due to the potential side effects and toxicity associated with current therapies. Triptolide, a natural product known for its anti-inflammatory properties, exhibits a narrow therapeutic window, prompting the exploration of its derivatives. Our study involved the screening of triptolide derivatives for inhibition of the NLRP3 inflammasome in human THP-1 cells and mouse bone marrow-derived macrophages (BMDMs). The interaction between NLRP3 and inflammasome components was examined in AD293 cells and BMDMs. The efficacy of triptolidiol, a potent NLRP3 inhibitor, was assessed in lipopolysaccharide (LPS)-induced models of acute lung injury and septic shock. Triptolidiol emerged as a highly potent and selective NLRP3 inhibitor, effectively inactivating the NLRP3 inflammasome in primed macrophages, without affecting the AIM2 or NLRC4 inflammasomes. It specifically inhibits procaspase 1 cleavage downstream of NLRP3. Structural analyses revealed that the C8-β-OH group of triptolidiol is crucial for its interaction with NLRP3 residue C280,

which blocks the interaction with NEK7, thereby preventing inflammasome oligomerization and assembly. Notably, triptolidiol decreases the K63-specific ubiquitination of NLRP3, leading to an inactive conformation of the inflammasome. In vivo, triptolidiol significantly reduced the severity of LPS-induced acute lung injury and septic shock. Triptolidiol represents a novel, structurally distinct NLRP3 inhibitor that modulates inflammasome activation through a unique mechanism involving the reduction of K63-linked ubiquitination. Its distinct properties and significant therapeutic potential underscore its value as a candidate for treating inflammatory diseases.

Key Words: Triptolidiol, NLRP3 inflammasome, anti-inflammation.

Acknowledgement: This work was partially supported by Macau Science and Technology Development Fund 0092/2021/A2, 0131/2022/A3, and SKL-QRCM (UM)-2023-2025.

P11: Design, Assembly and Test Large Synthetic DNA in Yeast and Mouse

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Synthetic biology confers new functions to hosts by introducing exogenous genetic elements, yet rebuilding complex traits that are based on large-scale genetic information remains challenging. Here,

1) We developed a CRISPR/Cas9-mediated haploidization method that bypasses the natural process of meiosis. Based on the programmed haploidization in yeast, we further developed an easy-to use method designated HAnDy (Haploidization-based DNA Assembly and Delivery in yeast) that enables efficient assembly and delivery of large DNA, with no need for any fussy in vitro manipulations.

2) A de novo designed 1.024 Mb synthetic accessory chromosome (synAC) encoding 542 exogenous genes was parallelly assembled and then directly transferred to six phylogenetically diverse yeasts using HAnDy. The synAC significantly promotes hosts' adaptations and increases the scope of the metabolic network, which allows the emergence of valuable compounds.

3) A de novo designed 180 Kb synthetic *Igh* locus (*SynIG*) embedded with an artificial Cre/loxPsym rearrangement system was synthesized and delivered to generate a mouse model. In B cells of the *SynIG*$^{+}$ mouse, all 60 V genes, 31 D genes, and 13 J genes were detected by the VDJ-seq. More important, implementing of the inducible artificial rearrangement before V(D)J recombination resulted in significant alterations in the V(D)J generation pattern, thereby enhancing the diversity of V(D)J recombination.

Our work should facilitate the design and manipulation of large DNA for expanding and deciphering complex biological functions.

Key Words: Synthetic Genomics, large DNA assembly, Synthetic Biology

P12: The Moonlighting Functions of Metabolic Enzyme

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ABSTRACT
We elucidated instrumental mechanisms of the Warburg effect, discovered the protein kinase and phosphatase activity of metabolic enzymes, and revealed the non-metabolic functions of metabolic enzymes in tumorigenesis. (1) Our work elucidated important mechanisms underlying the RTK-promoted Warburg effect, which are regulated by nuclear function of pyruvate kinase M2 (PKM2) and mitochondrial function of phosphoglycerate kinase 1 (PGK1). RTK activation induces translocation of the glycolytic enzyme PKM2 into the nucleus, where it binds to and activates tyrosine-phosphorylated β-catenin, thereby upregulating expression of glycolytic genes and enhancing the glucose uptake and lactate production. In addition, we revealed that activation of RTKs, expression of K-Ras G12V and B-Raf V600E, and hypoxia induce the mitochondrial translocation of the glycolytic enzyme PGK1, which phosphorylates and activates pyruvate dehydrogenase kinase 1 (PDHK1) to inhibit mitochondrial pyruvate metabolism, thereby promoting the Warburg effect. (2) We discovered that metabolic enzymes (PKM2, PGK1, PCK1, HK2, and fructokinase KHK-A) can function as protein kinases to regulates cell cycle progression, mitochondrial function, autophagy, and de novo nucleic acid synthesis. (3) We discovered for the first time that metabolic enzymes can function as protein phosphatase. Fructose-1, 6-bisphosphatase 1 (FBP1) dephosphorylates histone H3 at T11 and suppresses gene transcription. (4) Our work revealed that metabolic enzymes, including fumarase, acetyl-CoA synthetase 2 (ACSS2), and α-ketoglutarate dehydrogenase (KGDH) can possess nonmetabolic functions in regulation of instrumental cellular activities including DNA repair and gene expression. The discoverers of tumor-promoting functions of metabolic enzymes provide novel approaches for diagnosis and treatment of human cancer.

Key Words: Warburg effect; metabolic enzymes; nonmetabolic function; protein kinase; phosphatase; cancer.

P13: Synergistic Effects in Immunology

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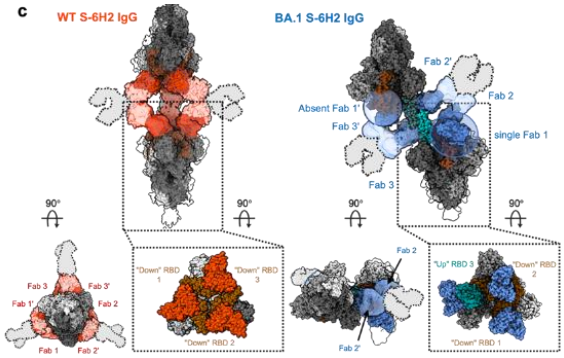
Multivalent interactions are crucial in biological recognition and signaling processes, such as immune recognition, cell adhesion, virus-host interactions, and protein-protein interactions. This study focuses on the synergistic enhancement effect and mechanism of weak interactions in two scenarios: folding and assembly of nucleic acid molecules and antigen-antibody interactions. DNA and RNA assembly structures contain many

unstable and complementary building blocks. Studies have shown that these unstable building blocks tend to form a single closed loop dimer due to the synergistic enhancement effect between two complementary building blocks in a dimer. In antigen-antibody interactions, the synergistic effect of multivalent interactions also depends on the optimal spatial matching between multiple ligands and receptors. The structure of antigen-antibody complexes was analyzed using cryo-electron microscopy, which showed that bivalent binding of IgG to three RBDs on the Spike protein promotes enhanced binding affinity and specificity, resulting in the formation of higher-order multivalent complexes. Multivalent interactions are especially critical in whole virus systems. These research results reveal the synergistic enhancement effect and mechanism of multivalent interactions of unstable building blocks in the biological system, which has important implications for understanding the nature of life systems.

Key Words: antibody, cryoEM, multivalency



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P14: Targeting NUP214 Eradicates Leukemia Stem Cells by Inducing Ferroptosis

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ABSTRACT
Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by the accumulation of abnormal myeloblasts. A small population of

leukemia stem cells give rise to mature leukemia blasts and are the major cause of chemotherapy resistance and relapse in AML, but effective therapies against LSCs are lacking. Using shRNA library screening and RNA-seq, we discovered essential genes in murine LSCs and found that a number of essential genes were more highly expressed in LSCs than in hematopoietic stem cells (HSCs). Among these genes, we determined that nucleoporin 214 (*Nup214*) was a dose-dependently essential gene (DDEG) and a target for selective eradication of LSCs. Homozygous deletion of *Nup214* eradicated LSCs in AML mice and impaired HSC maintenance. *Nup214* haploinsufficiency induced LSC ferroptosis while sparing HSCs. Mechanistically, Nup214 translocated into the nucleus and interacted with Sub1 to inhibit the transcription of *Alox15* and *Hmox1*, thus protecting LSCs against lipid peroxidation. To leverage this dependency therapeutically, we screened and identified 0449-b as a highly potent small-molecule inhibitor of NUP214. 0449-b functioned as a molecular glue that linked NUP214 and a NEDD8 E3 ligase, Rbx2, to induce neddylation and degradation of NUP214 protein. Inhibition of NUP214 showed efficacy in several AML cell lines and primary AML cells from patients, while having a negligible effect on normal hematopoietic stem and progenitor cells. These biological and chemical findings provide a valuable strategy to eradicate LSCs by targeting DDEGs.

P15: Unilateral Disturbed Function of Striatal DRD1 MSN Underlies Autism-like Behaviors in *Sh3rf2* Null Mice

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

P16: LINE-1 Transcription Activates Long-range Gene Expression

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ABSTRACT
Long interspersed element-1 (LINE-1 or L1) constitutes 17% of the human genome and shows variable expression across cell types. However, the control of L1 expression and its function in gene regulation are incompletely understood. Here we show that L1 transcription activates long-range gene expression. Using genome-wide CRISPR/Cas9 screening against a reporter driven by the L1 5' untranslated region (5' UTR) in human cells, we identify functionally diverse genes affecting L1 expression. Unexpectedly, altering L1 expression by knockout of regulatory genes impacts distant gene expression. L1s can physically contact their distal target genes, with these interactions becoming stronger upon L1 activation and weaker when L1 is silenced. Remarkably, L1s contact and activate genes essential for zygotic genome activation (ZGA), and L1 knockdown impairs ZGA, leading to developmental arrest in mouse embryos. These results uncover the regulation and function of L1 in long-range gene activation and reveal its importance in mammalian ZGA.

Key Words: LINE-1, Enhancer, Looping, Transcription, Zygotic Genome Activation, Retrotransposon

P17: Lymphotoxin-β Promotes Breast Cancer Bone Metastasis Colonization and Osteolytic Outgrowth

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

P18: The CDK1-RNF40-PLK1 Cascade Regulates Cell Cycle-dependent Centrosome Maturation and Chromosome Segregation

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

P19: Broad-spectrum Antiviral Peptide Studies against Multiple Respiratory Viruses

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The high rates of mutation in RNA viruses pose a significant challenge to the effectiveness of antiviral drugs. Throughout the last century, an extensive range of variants of respiratory viruses such as influenza, SARS-CoV-2, parainfluenza virus and rhinovirus have continued to cause outbreaks of pandemics, epidemics, or endemics in human populations. Specific antiviral drugs have palied important roles in controlling the viral infectious diseases, while antivirals with new mechanism are urgently needed to combact with new emerging viruese and drug-resistance mutants. In our studies, we are systemicly studing broad-spectrum antiviral peptides derived from human, mice and frog to investigate the broad-specturm antriviral activities against respiratory viruses including influenza virus, SARS-CoV-1, SARS-CoV-2,

MERS-CoV, rhinovirus and parainfluenza virus in vitro and in vivo. The results provide that engineered-branch peptides could show potent and broad-spctrum antiriviral activities against multip respiratory virses with a high barrier to drug resistance.

P20: Dynamic Regulation of Fact on MacroH2A-nucleosome at Transcription

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

Key Words: FACT, nucleosome, macroH2A, transcription, macrophage

P21: The Regulatory Mechanism of Bona Fide Neoantigen-specific CD8$^+$ T Cells in Antitumor Immunity

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ABSTRACT.
CD8$^+$ T cells respond simultaneously to various tumor antigens via a diverse T-cell receptor (TCR) repertoire, which determines both the specificity and strength of the

antitumor immune response. However, little is known about how the intrinsic regulation of dominant neoantigen (DNa)-specific $CD8^+$ T cells contributes to the efficacy of immune checkpoint blockade therapy. To address this, we conducted a comprehensive analysis of tumor-infiltrating DNa-expressing T cells using combined single-cell TCR/RNA sequencing. By characterizing the DNa-specific T cells, we determined that the key transcription factor downstream of the TCR signaling pathway, which is associated with enhanced metabolic function in conditions of immune checkpoint blockade. Mechanistically, the transcription factor promotes glucose metabolism and mitochondrial function by directly increasing *c-Myc* transcription in tumor microenvironment. This study identifies a pivotal factor that contributes to the metabolic fitness of bona fide DNa-specific $CD8^+$ T cells and provides crucial insights into T-cell-based cancer immunotherapy.

Key Words: $CD8^+$ T cells, T-cell receptor (TCR), dominant neoantigen (DNa), metabolism, antitumor immunity

P22: Autophagy Targeting Nanobody Chimera (ATNC) Degrades Disease-related Proteins

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ABSTRACT.
Targeted protein degradation (TPD) is emerging as a new modality in drug discovery. Compared to traditional small molecule inhibitor drugs which usually rely on occupancy-driven mechanism, TPD is able to directedly modulate cellular protein levels, and holds great potential in targeting undruggable proteins. Our group recently reported autophagy targeting nanobody chimera (ATNC) which is able to degrade unligandable proteins, a class of challenging targets for small molecule degraders. ATNC is a nanobody chimera which consists of two key modules, a nanobody module for recruiting a protein of interest, and an autophagy-directing module, like a LC3B protein or LC3 interacting region (LIR) peptide sequence. We show that ATNC can be versatilely implemented in different ways including (i) expressed ATNC intrabodies for ease of use, (ii) chemically induced proximity (CIP)-operated logic-gated degradation, and (iii) cyclic cell-penetrating peptide-tethered cell-permeable phagobodies. We show that the phagobody recognizing HE4 can selectively degrade the undruggable therapeutically relevant HE4 protein, resulting in effective suppression of ovarian cancer cell proliferation and migration. Overall, ATNC represents a general, modular, and versatile targeted degradation platform that degrades unligandable proteins and offers therapeutic potential.

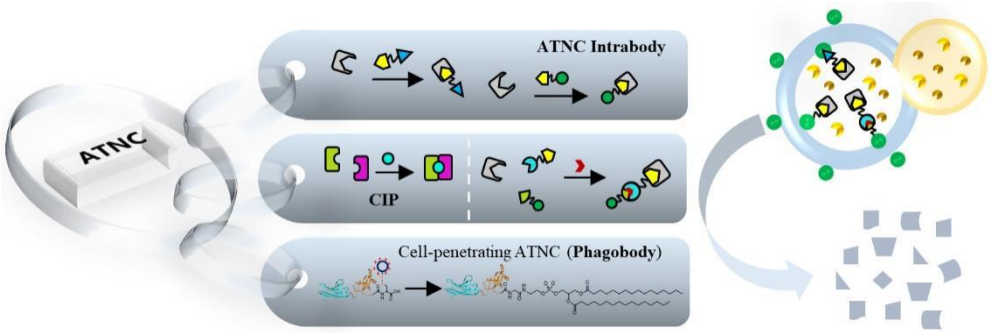

Figure 1. ATNC degradation platform can be implemented in three different ways.

Keywords: Targeted Protein Degradation, Autophagy, ATNC, Chemically Induced Proximity, TPX2, HE4

References:
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P23: Gentle Dyes for Imaging Mitochondrial Structure and Insulin Secretion
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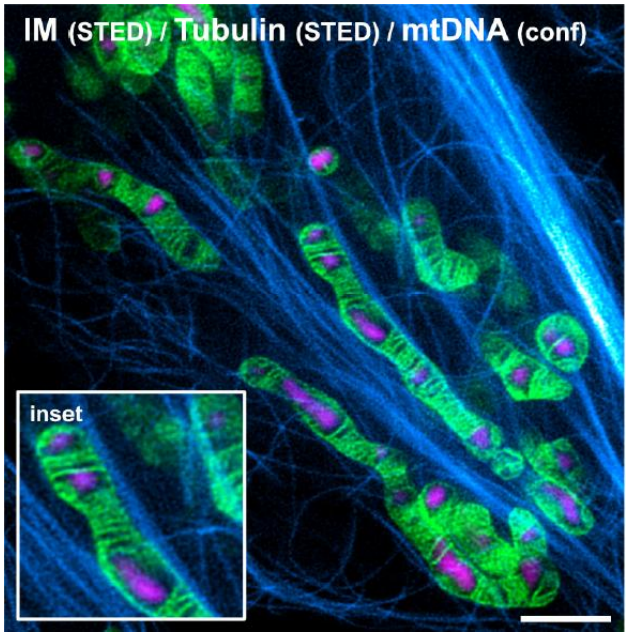
ABSTRACT.
Phototoxicity has become a prevailing issue in the super-resolution era when boosted illumination is applied, compromising the physiological relevance of the recorded data. We advocate leveraging chemical approaches to tackle phototoxicity. By exploiting chemical motifs such as triplet state quenchers and biocompatible auxiliaries, we systematically upgrade the commonly used fluorescent markers toward alleviated phototoxicity. These gentle dyes can be directed to various cellular targets spanning mitochondria, DNA, cytoskeleton, insulin granule, and specific proteins, enabling time-lapse super-resolution imaging with minimal photodamage. For example, PK Mito Orange probe is a mitochondrial inner membrane stain that enables 30 frames of STED recording and multi-color imaging of mitochondrial components. PK Zinc dyes enable multiplexed imaging of insulin secretion in isolated islets. These biocompatible probes, with high specificity and gentle behavior under excitation light, promise to offer reliable spatial-temporal information in the era of 4D multiplexed nanoscopy.



Key Words: Mitochondria, Inner Membrane, Insulin Secretion, Super-Resolution Imaging, Phototoxicity

P24: Developing New Cancer Cellular Immunotherapies for Solid Tumors
Li Peng

Adoptive cell therapy is highly personalized and represents a promising cancer therapy. ACT therapy uses both T cells and NK cells. Engineered T and NK cells that express chimeric antigen receptors (CARs) with PD-1 ablation have been successfully used to treat hematopoietic malignancies but exhibit limited clinical benefits for solid tumor patients. Given the cellular and molecular heterogeneity in solid tumors, new sources of immune cells are demanded to use multiple machineries to recognize and eliminate tumor cells. In addition, the tumor microenvironment (TME), which contains immune suppressive molecules, including PD-L1 and TGFb, also represses the antitumor effects of T cells directly and indirectly. I am leading a team to study tumor immunology in three aspects: 1. Design new CAR vectors for treating solid tumors such as pancreatic cancer and lung cancer; 2. Derive NK-like cells from T cells as new sources of ACTs; 3. Develop new CAR-T cells to disrupt the TME. In the presentation, I will show recent progress in these three initiatives. Briefly, we have designed a TLR2-CAR vector and are conducting clinical trials for treating B cell malignancies in China and New Zealand. We have also developed anti-mesothelin or anti-GPC3 CAR-T cells with improved killing and tumor penetration capacities and CAR molecules that rewire immune suppressive signals in the TME. Preliminary clinical trial results of these CAR-T cells will be discussed. In addition, preclinical and clinical data on NK-like cells derived from T cells will presented.

P25: Thyroid Control of Glucose Homeostasis Via a Liver-gut Axis
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ABSTRACT
Thyroid hormone regulates systemic glucose metabolism through incompletely understood mechanisms. Here, we show that improved glucose metabolism in hypothyroid mice after T3 treatment is accompanied with increased glucagon-like peptide-1 (GLP-1) production and insulin secretion. By using mice lacking hepatic thyroid hormone receptor β (TRβ), we demonstrate that TRβ-mediated hepatic TH signaling is required for both the regulation of GLP-1 production and the glucose lowering effects of T3. Mechanistically, T3 suppresses Cyp8b1 expression, resulting in increased the levels of Farnesoid X receptor (FXR)-antagonistic bile acids, thereby potentiating GLP-1 production and insulin secretion by repressing intestinal FXR signaling. T3 correlates with both plasma GLP-1 and fecal FXR-antagonistic bile acid levels in people with normal thyroid function. Thus, our study reveals a role for hepatic thyroid hormone signaling in glucose homeostasis through the regulation of GLP-1 production via bile acid-mediated FXR antagonism.

Key Words: glucose homeostasis, thyroid hormone, GLP-1, thyroid hormone receptor, bile acids

P26: Spatially Organized Tumor-stroma Boundary Determines the Efficacy of Immunotherapy in Colorectal Cancer Patients

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ABSTRACT
Colorectal cancer (CRC) patients with mismatch repair (MMR)-deficient (dMMR) but not MMR-proficient (pMMR) tend to benefit from immune checkpoint blockade (ICB) therapy. To uncover the rules governing these varied therapeutic responses, we integrated spatial enhanced resolution omics-sequencing (Stereo-seq), single-cell RNA sequencing, and multiplexed imaging analysis to create high-definition spatial maps of tumors from treatment-naïve and ICB-treated CRC patients. Our results identified the spatial organization and immune status of the tumor-stroma boundary as a distinctive feature of dMMR and pMMR CRCs, which determines ICB response. The physical interactions and abundance of *LAMP3*$^+$ DCs and *CXCL13*$^+$ T cells shape the ICB-responsive tumor-stroma boundary, whereas *CXCL14*$^+$ cancer-associated fibroblasts remodel extracellular matrix to form a structural barrier in non-responders. Our work therefore uncovered the black box of how the molecular and cellular spatial structures of tumors determine ICB response, raising the possibility of reprogramming tumor-stroma boundary for sensitizing immunotherapies in the majority of CRCs.

Key Words: Colorectal cancer, immunotherapy, stereo-seq, spatial organization, tumor-stromal boundary

P27: Prevalent Chemical Carcinogen-related Mutational Signature in Adenocarcinoma of Bladder

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ABSTRACT.
Adenocarcinoma of the bladder is a rare urinary bladder carcinoma with limited therapy options due to lack of molecular characterization. Here, we aimed to reveal the mutational and transcriptomic landscapes of adenocarcinoma of the bladder and assess any relationship with prognosis. Between February 2015 and June 2021, a total of 23 patients with adenocarcinoma of the bladder were enrolled. These included 16 patients with primary bladder adenocarcinomas and seven patients with urachal adenocarcinoma. Whole exome sequencing, whole genome sequencing, bulk RNA sequencing (RNA-seq), and single-cell RNA-seq were conducted for the specimens. Prevalent T>A substitutions were observed among somatic mutations, and major trinucleotide contexts included 5'-CTC-3' and 5'-CTG-3'. This pattern was mainly contributed by COSMIC signature 22 related to chemical carcinogen exposure (probably aristolochic acid), which has not been reported in bladder adenocarcinoma. Moreover, genes with copy number changes were also enriched in the KEGG term 'chemical carcinogenesis'. Interestingly, a small fraction of samples with an APOBEC-derived mutational signature exhibited a higher risk of disease progression compared with samples with only a chemical carcinogen-related signature, confirming the molecular and prognostic heterogeneity of bladder adenocarcinoma. This study presents mutational and transcriptomic landscapes of bladder adenocarcinoma, and for the first time indicates that a chemical carcinogen-related mutational signature may be related to a better prognosis compared with an APOBEC signature in adenocarcinoma of the bladder.

Key Words: adenocarcinoma of bladder; mutational signature; chemical carcinogen; multi-omics landscape; single-cell sequencing

P28: Selective Vulnerability of Coronary Arteries to Hypercholesterolemia and Angiotensin II-induced Hypertension

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ABSTRACT

Aims: Hypercholesterolemia and hypertension are major risk factors for atherosclerosis. This study determined their combined effects on coronary atherogenesis.
Methods and Results: A mouse model to accelerate coronary atherogenesis was induced by hypercholesterolemia via blocking two genome-wide association loci-apolipoprotein E and scavenger receptor B1 ($ApoE^{SA/SA}$) in combination with angiotensin II (AngII)-induced hypertension. The resulting atherosclerotic lesions exhibited endothelial erosion, myeloid cell infiltration, and plaque rupture, leading to spontaneous myocardial infarction, heart failure, and a male propensity for sudden death. AngII-treated $ApoE^{SA/SA}$ mice developed severe atherosclerosis in coronary arteries but not in femoral arteries, while norepinephrine-treated $ApoE^{SA/SA}$ mice did not develop coronary atherosclerosis, despite similar degree of hypertension. Proteomic analyses revealed notable differences between coronary and femoral arteries, which encompassed vasocontractility, extracellular matrix metabolism, angiotensinogen metabolism and inflammation. Endothelium-dependent dilatation of coronary arteries was highly susceptible to hypercholesterolemia and AngII-induced hypertension in contrast to femoral arteries. Losartan treatment restored coronary endothelium-dependent dilatation and eliminated coronary atherosclerosis. Additionally, coronary artery dilatation was more dependent on prostaglandins than femoral artery dilatation. Hypercholesterolemia and hypertension suppressed coronary prostaglandin biosynthesis and dilatation of coronary arteries. Conversely, an elevated coronary production of prostaglandins after methotrexate administration was associated with improved endothelial dysfunction and better cardiovascular survival. In patients with hypercholesterolemia and hypertension, coronary arteries exhibited impaired endothelium-dependent dilatation, compared to internal mammary arteries. However, their endothelium-independent dilatation remained intact.
Conclusion: The combination of hypercholesterolemia and AngII-induced hypertension significantly increased coronary atherogenesis, which led to spontaneous myocardial infarction. This makes coronary arteries particularly vulnerable to AngII exposure and prostaglandin inhibition.

Key Words: Coronary artery disease, Atherosclerosis, Hypercholesterolemia, Hypertension, Angiotensin, Prostaglandin

P29: Effective Production of Highly Functional CAR-macrophages from Human Pluripotent Stem Cells for Cancer Immunotherapy

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Generation of chimeric antigen receptor macrophages (CAR-M) from human pluripotent stem cells (hPSCs) offers new prospects for cancer immunotherapy, but is currently challenged by low differentiation efficiency and limited function. Here, we develop a highly efficient monolayer-based system that can produce around 6,000 macrophages from a single hPSC within 3 weeks. Based on screens of modified structures, we identify macrophage-compatible CAR structures with optimized ability to generate hPSC-CAR-M with stable CAR expression and potent tumoricidal activity

in vitro. To overcome the loss of tumoricidal activity of hPSC-CAR-M that acquire an anti-inflammatory phenotype after infusion *in vivo*, we use interferon-γ and monophosphoryl lipid A to activate an innate immune response *in vivo* to repolarize the infused hPSC-CAR-M to tumoricidal macrophages. Moreover, through combined activation of T cells by hPSC-CAR-M to initiate an adaptive immune response, we demonstrate that activating a collaborative innate-adaptive immune response can further enhance the anti-tumor effect of hPSC-CAR-M in both liquid and solid tumor xenograft models. Collectively, our study provides feasible methodologies that significantly improve the production and function of hPSC-CAR-M to support their translation into clinical applications.

P30: Discovery of a Novel Biomarker Predicting Liver Metastasis in Non-functional Pancreatic Neuroendocrine Tumors

Objective: Nonfunctional pancreatic neuroendocrine tumors (NF-PanNETs) exhibit varied clinical behaviors ranging from indolent to highly malignant. Current understanding of the molecular pathology of NF-PanNETs is insufficient for their clinical management, with a recognized challenge being the need to identify patients with aggressive tumors who should receive intensive therapeutic interventions. This study aims to characterize the gene expression and chromatin regulatory landscape of NF-PanNET tumors, to explore pro-cancer regulatory mechanisms, and to identify both potential therapeutic targets and prognostic biomarker(s) for NF-PanNETs.
Design: We generated single cell RNA-seq data from 10 clinically diverse patients, single cell ATAC-seq from 4 patients and then used this to construct a large-scale atlas providing an in-depth view of the NF-PanNET microenvironment. We then harnessed this atlas and employed various tumor models for follow-up analyses underlying NF-PanNET malignancy and to identify potential vulnerable therapeutic targets. Finally, we validated the prognostic relevance of simple IHC staining against a newly identified biomarker in a two-center cohort of 167 NF-PanNET patients.
Results: We mapped 6 major cell clusters (16 subclusters) within NF-PanNET samples and identified a pro-cancer subcluster. We discovered a specific transcription factor directly regulates the proliferative transcriptional program in these subcluster cells and promotes NF-PanNET growth. Chemically targeting this transcription factor confers benefit against pancreatic neuroendocrine tumors. We also show that the positivity of this subcluster marker by IHC staining is associated with several adverse prognostic clinical features (larger tumor size, higher WHO grade and more frequent synchronous liver metastasis) and with poorer patient outcomes (shorter PFS and worse OS).
Conclusion: Beyond generating an atlas of the NF-PanNET tumor microenvironment based on multiple advanced analyses of clinically resected NF-PanNET tumors, our study identified a pro-cancer subcluster and discovered a proliferative transcriptional program that underlies the malignancy of NF-PanNET. Clinical inhibition the specific transcription factor offers a potential therapeutic avenue for NF-PanNET treatment. Additionally, the subcluster marker positivity by simple IHC staining serves as a valuable prognostic marker for NF-PanNETs.

P31: Mismatch Repair Protein MutSα Promotes Nascent Strand Degradation at Stalled Replication Forks
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Mismatch repair (MMR) is a replication-coupled DNA repair mechanism and plays multiple roles at the replication fork. The well-established MMR functions include correcting misincorporated nucleotides that have escaped the proofreading activity of DNA polymerases, and recognizing non-mismatched DNA adducts and triggering DNA damage response. In an attempt to determine if MMR regulates replication progression in cells expressing an ultramutable DNA polymerase ε carrying a proline-to-arginine substitution at amino acid 286 (Polε-P286R), we identified an unusual MMR function in response to hydroxyurea-induced replication stress. Polε-P286R cells treated with hydroxyurea exhibit increased MRE11-catalyzed nascent strand degradation. This degradation by MRE11 depends on the mismatch recognition protein MutS α and its binding to stalled replication fork. Increased MutS α binding at replication forks is also associated with decreased loading of replication fork protection factors FANCD2 and BRCA1, suggesting blockage of these fork protection factors from loading to replication forks by MutSα. We find that the MutS α-dependent MRE11-catalyzed fork degradation induces DNA breaks and various chromosome abnormalities. Therefore, unlike the well-known MMR functions of ensuring replication fidelity, the newly identified MMR activity of promoting genome instability may also play a role in cancer avoidance by eliminating rogue cells.

P32: Deciphering the Spatial Organization of Fibrotic Microenvironment in Silica Particles-induced Pulmonary Fibrosis

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ABSTRACT
Silicosis represents a form of interstitial lung disease induced by the inhalation of silica particles in production environments. A key pathological characteristic of silica-induced pulmonary fibrosis is its localized tissue heterogeneity, which presents significant challenges in analyzing transcriptomic data due to the loss of important spatial context. To address this, we integrate spatial gene expression data with single-cell analyses and achieve a detailed mapping of cell types within and surrounding fibrotic regions, revealing significant shifts in cell populations in normal and diseased states. Additionally, we explore cell interactions within fibrotic zones using ligand-receptor mapping, deepening our understanding of cellular dynamics in these areas. We identify a subset of fibroblasts, termed inmt fibroblasts, , that play a suppressive role in the fibrotic microenvironment. Validating our findings through a comprehensive suite of bioinformatics, histological, and cell culture studies highlights the role of monocyte-derived macrophages in shifting Inmt fibroblast populations into profibrotic Grem1 fibroblast, potentially disrupting lung homeostasis in response to

external challenges. Hence, the spatially detailed deconvolution offered by our research markedly advances the comprehension of cell dynamics and environmental interactions pivotal in the development of pulmonary fibrosis.

Key Words: crystalline silica, pulmonary fibrosis, fibroblast-macrophages homeostasis, scRNA-seq

P33: Exploring the Mechanisms and Physiological Functions of Vesicle-Mediated Organelles Export

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Mitochondrial export into the extracellular space is emerging as a key process in a variety of physiological activities. While some studies have begun to illuminate the processes for discarding damaged mitochondria, the mechanisms of mitochondrial export, as well as the physiological implications of such release, remain largely enigmatic. In particular, the existence of a mitochondria-specific export mechanism remains underexplored. Our lab has recently discovered 'mitopherogenesis', a previously unknown process that specifically secretes mitochondria from cells via a unique extracellular vesicle, termed 'mitopher'. In this presentation, I will share our latest findings on the general and conserved role of mitophers. Furthermore, the question of whether other organelles can also be exported through the generation of extracellular vesicles remains open, and I will also share our insights on this topic. Our research may identify vesicle-mediated organelle export as a fundamental mechanism for controlling organelle quality and quantity, potentially playing crucial roles in cell development and the maintenance of homeostasis.

P34: Mec1 and Rad53 Kinases Coordinate a Redox Cycle to Gate G1/S Transition

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The essential role of *MEC1*^{ATR} and *RAD53*^{CHK1} kinases is widely known to be bypassed by elevation of dNTP levels. Here, we identify that an extra copy of human GCLC or yeast ortholog *GSH1* gene, encoding the rate-limiting enzyme of glutathione (GSH) biogenesis, can potently suppress the lethality of *mec1Δ* or *rad53Δ* yeast cells. *GSH1* is repressed by Mig1 in G1. During G1/S transition, Rad53 phosphorylates Mig1, which turns Mig1 to be an activator to recruit the Mediator through a direct association with Med15 and thereby induces *GSH1* transcription. All these events create a reductive GSH-rich environment required for proper DNA replication and S phase progression. Mechanistically, phospho-mimicking of Mig1 or directly fusing Mig1 with Med15 can also bypass the essentiality of *MEC1* and *RAD53*. These findings suggest that Mec1ATR and Rad53^{CHK1} play a highly conserved indispensable role in coordinating the reductive state with DNA replication in eukaryotes.

P35: VGLL3 Reduces Chemosensitivity by Promoting DNA Damage Response

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Transcription cofactor Vestigial-like 3 (VGLL3) is a member of the VGLL family that contains a conserved TONDU motif, a region essential for binding with TEA domain-containing transcription factors (TEADs). As a candidate female-biased immune regulator, the elevated VGLL3 expression is associated with inflammatory diseases, especially female-biased autoimmune diseases. Recently, emerging evidence implicate a role of VGLL3 in tumor development. VGLL3 was reported to associate with cancer progression by promoting tumor cell proliferation or motility, with its expression positively correlated with poor prognosis in multiple types of human cancers, including breast, ovarian, colon and gastric cancers. Paradoxically, a tumor suppressor role of VGLL3 was also reported in estrogen receptor (ER) positive breast cancers, in which VGLL3 is essential in mediating Hippo/Yes-associated protein (YAP) signaling to repress ESR1 expression. Moreover, VGLL3 was reported to confer therapeutic resistance to TEAD–YAP blockade through functioning as a transcription co-activator to induce the expression of YAP-suppressed genes. Nevertheless, how VGLL3 functions in tumor development remains largely obscure. Here, we report that VGLL3 can be recruited to DNA double-strand breaks (DSBs) in a PARylation- and chromodomain helicase DNA-binding protein 4 (CHD4)-dependent manner. Depletion of VGLL3 impairs the recruitment of multiple DDR factors, including RNF8, RNF168, RAD51 and RPA, to DSBs. As a functional consequence, VGLL3 deficiency impairs HR repair and sensitizes cells to multiple DSB-inducing agents. Consistently, targeting VGLL3 inhibits tumor growth and sensitizes xenografted tumors to etoposide treatment. Together, our data reveal that VGLL3 can function as a DDR regulator that is essential for optimal DDR signaling, which is independent of its roles in transcriptional regulation.

P36: The Metabolic Remodeling of Endothelial Cells and Its Regulatory Role in Angiogenesis of Hepatocellular Carcinoma

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Hepatocellular Carcinoma (HCC) is a highly prevalent malignancy worldwide, characterized by active angiogenesis, high metastasis and mortality. The liver is the metabolic center of the human body. We aim to investigate the regulatory roles of liver-enriched metabolites, including fructose and acetate, on the metabolic remodeling of endothelial cells and HCC angiogenesis. We reveals that the metabolism pathways of both fructose and acetate are upregulated in the endothelial cells of HCC. And fructose and acetate promote HCC angiogenesis and tumor metastasis. Furthermore, fructose metabolism activates AMPK, enhances mitochondrial respiratory capacity, and generates more energy to promote the migration and proliferation of endothelial cells. On the other hand, acetate increases histone acetylation in endothelial cells, thereby upregulating the expression of pro-angiogenic genes and enhancing endothelial cell migration. These findings not only elucidate the relationship between metabolites in the microenvironment as well

as metabolic remodeling of endothelial cells and HCC angiogenesis, but also suggest that limiting dietary intake of fructose and acetate, or interfering with their metabolic processes may be a potential strategy for anti-cancer treatment.

P37: Chemical Modulators Targeting the Oncogenic m^{6}A Demthylase FTO

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The N^6-methyladenosine (m^{6}A), the most prevalent mRNA modification, has emerged in recent years as a new layer of post-transcriptional regulatory mechanism controlling gene expression in eukaryotes. The m^{6}A methylation influences nearly every step of RNA metabolism, and thus broadly affects gene expression at multiple levels. Increasing evidence has revealed connectivity between reversible RNA m^{6}A methylation with tumorigenesis, metastasis and immune evasion via the dynamic alteration of m^{6}A-marked mRNA transcripts. FTO, the first m^{6}A demethylase, revitalized the interest in m^{6}A and created new momentum for the biology of RNA epigenetics, which has been associated to promote leukemogenesis and other types of cancers. Our investigations into FTO inhibitors have yielded substantial advances through a structure-based rational design approach, resulting in the development of highly selective and potent chemical inhibitors. Our inhibitors have proved to eficiently altere FTO-mediated aberrant epitranscriptome and significantly inhibite acute myeloid leukemia (AML) progression in vivo. We also have developed FTO-targeting proteolysis targeting chimera (PROTAC) degraders and demonstrated that FTO degradation exerted antileukemic effects. Collectively, these chemical modulators that selectively targeting the oncogenic FTO demethylase will promote in-depth studies on the regulation of gene expression and potentially accelerate anticancer target discovery

P38: The Extracellular Matrix Regulates Pulmonary Macrophage Polarization and Responsiveness in Type 2 Inflammation of Acute Respiratory Distress Syndrome

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ABSTRACT

The extracellular matrix (ECM), as a dynamic structure, is now recognized as a highly dynamic partner of inflammation. We focused on the dynamic alterations of ECM in the development of acute respiratory distress syndrome (ARDS), and explored the complex interconnectivity between ECM and macrophages. This research provides a better understanding of the underlying mechanisms responsible for the diagnosis and treatment of ARDS. Cecal ligation and puncture (CLP) was performed in mice to induce sepsis as a model to study ARDS. Quantitative mass spectrometry (MS)-based proteomics was performed to explore the dynamic alterations of lung ECM in ARDS.

Specific macrophage populations in single-cell RNA sequencing (scRNA-Seq) in ARDS were analyzed. Based on the ECM-cell transplantation model, we deciphered the downstream molecular mechanisms of the interaction between ECM and specific macrophage populations in ARDS. We highlighted aberrant ECM remodeling, which was characterized by a time-dependent decrease in collagen and structural disruption in CLP mice at 8h, 16h, and 24h. An intriguing phenomenon observed in CLP mice pertains to ECM remodeling, which, despite reducing the chemical attraction and adhesion of macrophages, paradoxically attracts monocytes and fosters their differentiation into macrophages. Primary macrophages isolated from CLP mice exhibited a pro-inflammatory phenotype. After being seeded onto ECM derived from CLP mice, the macrophages transformed towards an anti-inflammatory phenotype. Specifically, the binding of Annexin A1 (Anxa1) in ECM to integrin αvβ3 on macrophages seems to mediate this process. Collectively, our findings demonstrate that ECM regulates inflammatory responses by mediating reprogramming of pulmonary macrophage in ARDS.

Key Words: Extracellular matrix; Macrophage; Acute respiratory distress syndrome.

P39: Circular RNA SCMH1 Suppresses Kynurenine 3-monooxygenase Expression to Inhibit Mitophagy and Functional Recovery Following Stroke

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Metabolic dysfunction is considered a key event after ischemic stroke. However, the underlying mechanisms of metabolic disorders in cerebral ischemia are unknown. In this study, the kynurenine pathway, which is the major pathway of tryptophan metabolism, was identified after ischemic stroke through coordinated metabolomic and RNA-seq analysis. Circular RNA SCMH1 (circSCMH1) is a circular RNA that has been reported to play a role in brain repair after stroke, and was herein confirmed to be involved in kynurenine pathway regulation. Specifically, we found that circSCMH1 inhibited the kynurenine pathway and promoted mitochondrial fusion after cerebral ischemia. Mechanistically, circSCMH1 bound to STAT5B and inhibited its translocation to the nucleus, resulting in decreased expression of kynurenine 3-monooxygenase (KMO), a key enzyme in the kynurenine pathway. Our findings suggest that circSCMH1 inhibits post-stroke mitophagy via STAT5B mediated regulation of KMO, providing insight into the mechanism by which circSCMH1 promotes stroke recovery.

P40: Histamine H2 Receptor Deletion in VTA Dopaminergic Neurons Induces Mania-like Behavior in Mice

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Hyperfunction of dopamine system has been implicated in the pathogenesis of manic episodes in bipolar disorders. How dopaminergic neuronal function is regulated in the pathogenesis of mania still remains elusive. Histaminergic neurons project dense efferents into the midbrain dopaminergic nuclei. Here, we present mice lacking dopaminergic H2R in ventral tegmental area (VTA), but not in substantia nigra pars compacta, exhibit mania-like phenotypes characterized by increased locomotor activity, reduced anxiety- and depression-like behavior. The mania-like behavior can be reversed by mood stabilizers lithium and valproate. H2R deletion in dopaminergic neurons significantly enhances neuronal activity, concurrent with a decrease in the GABAAR membrane presence and inhibitory transmission. Conversely, either overexpression of H2R in VTA dopaminergic neurons or treatment of H2R agonist amthamine within VTA counteracted amphetamine-induced hyperactivity. Together, our results demonstrate the engagement of H2R in reducing VTA dopaminergic activity, shedding light on the role of H2R as a precise target for mania therapy.

P41: Development of Broad-spectrum Antiviral Drugs

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ABSTRACT

In the past twenty years, viral diseases have had a significant impact on human society. By constructing a multidimensional, multi-target, structurally diverse antiviral compounds library, some drug candidates were obtained. At the onset of COVID-19, based on the structure of SARS-CoV M^{pro}, **FB2001** was designed and synthesized, which exhibited broad-spectrum, potent antiviral activity. **FB2001** does not need co-administration with ritonavir and showed potent activity against various

clinically mutant strains. Injectable and nebulized forms of **FB2001** are undergoing international multicenter phase II/III clinical trials, with related work published in Science (Science, **2020**, *368*: 1331-1335) as a cover paper. Additionally, some inhibitors also were designed to target various stages of the coronavirus life cycle. Dual-target inhibitors **14a** and **14b** targeting host cathepsin L and calpain-1 showed pan-coronavirus inhibitory activity and excellent anti-inflammatory activity in vitro and in vivo. An oral drug candidate **DC406068** was designed to simultaneously inhibit coronaviral M^{pro} and host proteases (cathepsin L and calpain-1), which also exhibited broad-spectrum antiviral activity against coronaviruses and the Ebola virus, and excellent anti-inflammatory activity in vitro and in vivo. Based on the structures of 3C/3CLpro, **DC402209** and **DC402267** were obtained which exhibited potent activity against enteroviruses, noroviruses, and coronaviruses. N protein inhibitors **A43** and **DC056204** displayed potent anticoronaviral effects.

P42: Chromatin-based Regulation of DNA Replication

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ABSTRACT
DNA replication is a tightly regulated process that ensures the precise duplication of the genome during the cell cycle. DNA replication origins are first recognized and licensed at G1 phase, then DNA replication begin to initiate at the selectively activated replication origins during S phase. Aberrant replication licensing and activation can lead to replication stress and affect genomic instability, further promote tumorigenesis. In eukaryotes, DNA wraps around histone octamers to form nucleosomes. Nucleosomes are then hierarchically folded into higher order chromatin. DNA replication also occurs on chromatin templates. However, the chromatin-based regulatory mechanisms on DNA replication process remain largely uncharacterized. Therefore, my lab focuses on deciphering the chromatin-based regulation mechanisms on the stepwise selection and activation of DNA replication origins. In this meeting, I will present our progress on the regulation mechanisms of origin selection.

P43: Construction of pTYNE-VE, a CRISPR-Cas9 Gene Knockout Validation Vector for Liver Cancer VE-statin Gene

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ABSTRACT
Objective: To verify the gene knockout efficiency of the VE-statin gene CRISPR-Cas9 editing plasmid constructed earlier, this experiment aimed to construct

a VE-statin gene knockout validation plasmid pTYNE-VE and subsequently validate the gene editing effect.
Methods: The pTYNE plasmid was amplified, and then digested with XhoI and HindIII enzymes to generate a linearized plasmid. The primer containing the target gene knockout site was annealed and ligated to the pTYNE vector. The ligation product was transformed into competent DH5α bacteria. Colony PCR was performed to identify positive clones. The successfully constructed pTYNE-VE plasmid was co-transfected with the VE-statin gene CRISPR-Cas9 editing plasmid into H293 cells, and by observing the fluorescence results of cells after transfection to identify the gene knockout effect.
Results: The pTYNE-VE validation plasmid was successfully constructed, and the sequencing results showed that the DNA sequence of the recombinant plasmid was completely consistent with the target fragment.
Discussion: The successful construction of the pTYNE-VE validation plasmid indicates that it can be used to validate the gene knockout of the VE-statin gene editing plasmid and precisely modify the VE-statin gene in liver cancer cells, which will play a crucial role in promoting the research of VEGF-statin gene function and liver cancer treatment.

Key Words: CRISPR-Cas9; VE-statin; validation vector;

P44: Application of a Novel Antisense Oligonucleotide Technology to Treat Neuromuscular Diseases
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ABSTRACT
RNA targeted ASO technologies have potential to treat various diseases. However, the ASO efficacies in the present technologies are generally mediocre, limiting their therapeutic application. Therefore, new technologies with higher ASO efficiencies are in urgent need. Here, we developed a novel ASO-mediated exon skipping technology, which has 10-fold efficiency compared to conventional ASO technologies based on studies in cultured cells and animal models. Several ASOs designed using this technology hold great promise for treatment of Duchenne muscular dystrophy and spinocerebellar ataxia.

P45: CAFs secreting CXCL12 Mediated Carboplatin Resistance in LUAD through NF-κB Signaling Pathway
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ABSTRACT
Background: Cancer-associated fibroblasts (CAFs) are the dominant component of the tumor microenvironment (TME) that can be beneficial to the generation and progression of cancer cells leading to chemotherapeutic failure via several mechanisms. Reports have found that CAFs can induce malignant tumor chemoresistance by secreting CXCL12.Nevertheless, the roles of CAFs secreting CXCL12 on Lung adenocarcinoma (LUAD) chemoresistance has not been fully illustrated which need more empirical evidence to study. To investigate whether CAFs secreting CXCL12 induced LUAD chemoresistance.
Methods We isolated corresponding fibroblasts, named pCAFs, mCAFs and normal fibroblasts (NFs), from tumor and adjacent tissues of patients with poorly differentiated and moderately differentiated adenocarcinoma. Immunofluorescence, RT-qPCR and Western blot were used to determine CXCL12 level in NFs, mCAFs, pCAFs. We used RNA-seq to detect the differentially expressed genes and pathways in pCAFs, mCAFs, and NFs. Human LUAD cell lines A549 and H1299 were treated with conditioned media from different CAFs and NFs detected the killing effects by CCK8 experiments, flow cytometry and plate cloning experiments.
Results We found that CAFs secreting CXCL12, whereas NFs secreted less of CXCL12. A549 and H1299 treated with CAFs CM can inhibit the killing of carboplatin, and the degree of resistance is positively correlated with the CAFs derived from different differentiation degree of LUAD. We found that CAFs may secrete CXCL12 by activating the NF-κB pathway through RNA-seq results.
Conclusions Our study found that CAFs derived from LUAD promote carboplatin resistance in LUAD by secreting CXCL12 through the NF-κB pathway.

Key Words: Lung adenocarcinoma, Cancer-associated fibroblasts, CXCL12, NF-κB

P46: CD248^{+}CAFs Secreting CXCL12 Induced M2-polarized Macrophages by Activating STAT3

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ABSTRACT
Objective: In our pervious study, we found that CD248-expressing cancer-associated fibroblasts (CAFs) secreting CXCL12 induced macrophages polarized to M2 type, and then, in turn to promote non-small cell lung cancer progression. However, the mechanism was still unknown. In this study, we will investigate the mechanism of $CD248^{+}$CAFs secreting CXCL12 induced M2-polarized macrophages.
Methods: NFs and CAFs were isolated from normal adjacent tissues or tumour tissues of NSCLC patients, and then used conditional medium of NFs or CAFs treated human monocyte cell line THP-1 to detect the polarization of macrophages. Blocking CXCL12 to detect the polarization of macrophages. Used conditional medium of NFs or CAFs treated human monocyte cell line THP-1 to detect the expression of p65, p-p65, STAT3 and p-STAT3.Results We found that CD248 is expressed mainly in NSCLC-derived CAFs and $CD248^{+}$CAFs secreting CXCL12 induced macrophages polarized to M2 type. Blocking CXCL12 drastically decreased M2 macrophage chemotaxis. The expression of p-STAT3 was increased and the expression of p-p65 was decreased in conditional medium from $CD248^{+}$CAFs treated THP-1 groups.
Conclution: $CD248^{+}$CAFs secreting CXCL12 induced M2-polarized macrophages by activating STAT3.

Key Words: CAFs, macrophage, CD248, CXCL12, STAT3.

P47: CD248 Promoting Immune Escape of NSCLC by Up-regulating PD-L1 Expression on CAFs through FAK-Src-JNK-c-Jun Pathway

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ABSTRACT

Background: Immune escape is one of the vital mechanisms of malignant tumor. Cancer associated fibroblasts (CAFs) prompt it through inhibiting the function of $CD8^+$T cells. CD248 as a new surface marker of CAFs to promote the immune escape of non-small cell lung cancer (NSCLC) remains unknown. We investigated the role of CD248 up-regulates the expression of PD-L1 on CAFs through FAK-Src-JNK-c-Jun pathway to promote NSCLC immune escape.

Methods: TIMER 2.0 database, immunofluorescence, RT-qPCR and Western blot were used to determine CD248 and PD-L1 relationships in NSCLC. We performed co-culturing experiments using CAFs or its the conditional medium with NSCLC cell lines. Then, we employed transwell assay, invasion test and cell scratch test to evaluate the migratory and invasive capabilities of the NSCLC cells in vitro. The killing effect of $CD8^+$T cells on tumor cells was detected by animal imaging apparatus. RNA sequencing (RNA-seq) and Kyoto Encyclopedia of Gene and Genomes (KEGG) were employed to investigate differentially expressed gene and pathway of PD-L1. Additionally, subcutaneous tumor bearing models were created in vivo using fibroblast-specific CD248 gene deletion mice.

Results: Higher levels of CD248 in CAFs promote the expression of PD-L1, which facilitates the invasion and migration of NSCLC cells. CD248+CAFs inhibit the killing effect of $CD8^+$T cells. The activation of the Fak-Src-JNK pathway by CD248+CAFs induces c-Jun expression, which facilitate the expression of PD-L1. Deletion of the CD248 gene in mice resulted in a significantly reduced ability to express PD-L1 to inhibit immune escape.

Conclusion: Our study suggests that CD248-expressing CAFs activate FAK-Src-JNK-c-Jun pathway, resulting in c-Jun expression induction, promoting the expression of PD-L1, which inhibits the proliferation and activation of T cells. This study provides a new theoretical basis for CAFs to promote NSCLC immune escape, and will also provide a new molecular mechanism for CD248 as a potential therapeutic target.

Key Words: non-small cell lung cancer; CAFs; CD248; PD-L1; immune escape

P48: Periostin Derived from CD248-expressing Cancer-associated Fibroblasts Promote EMT in Non-small Cell Lung Cancer through Periostin/ITGB1

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ABSTRACT
Background: Lung cancer is one of the most prevalent malignant tumors, and its morbidity and mortality are among the highest in tumors. Nearly 85% of lung cancer are non-small cell lung cancer (NSCLC). Epithelial-mesenchymal transition (EMT) is the first step in tumor metastasis. The mechanism of CD248 as a potential marker of CAFs to promote EMT of NSCLC remains unclear. We investigated the role of $CD248^{+}$CAFs in promoting EMT of NSCLC through Periostin/Integrinβ1 (ITGB1).
Methods: The relationship between CD248 and Periostin was detected by western blot, RT-qPCR and immunofluorescence. The relationship between the expression level of *ITBG1* gene in NSCLC and EMT-related genes and the prognosis of patients was analyzed by immunohistochemical staining and TCGA database. Western blot was used to detect the expression of EMT-related proteins in NSCLC cell lines A549 and NCI-H460 treated with supernatant from differential expression of CD248 CAFs.
Results: The higher level of CD248 in CAFs can promote the secretion of Periostin, which can significantly reduce the expression of E-cadherin and increase the expression of N-cadherin and TWSIT1 in NSCLC cell lines. After blocking Periotin, the expression of N-cadherin and TWSIT1 was significantly decreased, and the expression of E-cadherin was increased. Periostin receptor ITBG1 was highly expressed in NSCLC, which was closely related to the poor prognosis of patients, promoting EMT in NSCLC.
Conclusions: Our results suggest that Periostin secretion by $CD248^{+}$CAFs may bind ITGB1 on A549 and NCI-H460 by promoting NSCLC cells EMT. This study has the potential to elucidate the underlying mechanisms that $CD248^{+}$ CAFs promote EMT and thus lay the foundation for future therapeutic interventions.

Key Words: NSCLC; CD248; CAFs; Periostin; EMT

P49: CD248-expressing Cancer-associated Fibroblasts Induce Non-small Cell Lung cancer Metastasis via Hippo Pathway-mediated Extracellular Matrix Stiffness

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ABSTRACT
Metastasis is a crucial stage in tumor progression, and cancer-associated fibroblasts (CAFs) support metastasis through their participation in extracellular matrix (ECM) stiffness. CD248 is a possible biomarker for non-small cell lung cancer (NSCLC)-derived CAFs, but its role in mediating ECM stiffness to promote NSCLC metastasis is unknown. We investigated the significance of $CD248^{+}$CAFs in activating the Hippo axis and promoting connective tissue growth factor (CTGF) expression, which affects the stromal collagen I environment and improves ECM stiffness, thereby facilitating NSCLC metastasis. In this study, we found that higher levels of CD248 in CAFs induced the formation of collagen I, which in turn increased extracellular matrix stiffness, thereby enabling NSCLC cell infiltration and migration. Hippo axis activation by $CD248^{+}$CAFs induces CTGF expression, which facilitates the formation of the collagen I milieu in the stromal matrix. In a tumor lung metastasis model utilizing fibroblast-specific CD248 gene knockout mice, CD248 gene knockout mice showed a significantly reduced ability to develop tumor lung metastasis compared to that of WT mice. Our findings demonstrate that $CD248^{+}$CAFs activate the Hippo pathway, thereby inducing CTGF expression, which in turn facilitates the collagen I milieu of the stromal matrix, which promotes NSCLC metastasis.

Key Words: CD248; CAFs; Hippo pathway; NSCLC; ECM; metastasis.

P50: IL-8 from CD248- expressing Cancer- associated Fibroblasts Generates Cisplatin Resistance in Non- small Cell lung Cancer

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ABSTRACT
Chemotherapy-resistant non-small cell lung cancer (NSCLC) presents a substantial barrier to effective care. It is still unclear how cancer- associated fibroblasts (CAFs) contribute to NSCLC resistance to chemotherapy. Here, we found that $CD248^{+}$CAFs released IL-8 in NSCLC, which, in turn, enhanced the cisplatin IC50 in A549 and NCI-H460 while decreasing the apoptotic percentage of A549 and NCI-H460 *in vitro*. The $CD248^{+}$CAFs-based IL-8 secretion induced NSCLC chemoresistance by stimulating nuclear factor kappa B (NF-κB) and elevating ATP-binding cassette transporter B1 (ABCB1). We also revealed that the $CD248^{+}$CAFs-based IL-8 release enhanced cisplatin chemoresistance in NSCLC mouse models in vivo. Relative to wild- type control mice, the CD248 conditional knockout mice exhibited significant reduction of IL-8 secretion, which, in turn, enhanced the therapeutic efficacy of cisplatin in vivo. In summary, our study identified CD248 activates the NF-κB axis, which, consecutively induces the CAFs-based secretion of IL-8, which promotes NSCLC chemoresistance. This report highlights a potential new approach to enhancing the chemotherapeutic potential of NSCLC-treating cisplatin.

Key Words: non-small cell lung cancer, cancer-associated fibroblasts, CD248, IL-8, chemoresistance

P51: CD248-expressing Cancer-associated Fibroblasts Secreting IL-8 Promote Cisplatin Resistance by Sustaining Cancer Stemness in NSCLC

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ABSTRACT
Cancer stem-like cells (CSCs) play pivotal roles in chemoresistance of many cancer types, including NSCLC. Stromal cells from tumor microenvironment (TME) are indispensable for the maintenance of CSCs, CAFs is the component of stromal cells from tumor microenvironment (TME). It is still unclear how CAFs contribute to NSCLC resistance by sustaining cancer stemness. $CD248^{+}$CAFs released IL-8 in NSCLC, which, in turn, enhanced the cisplatin IC50 in A549 and NCI-H460 and promoted colony formation ability of A549 and NCI- H460 in vitro. The $CD248^{+}$CAFs secreting IL-8 induced NSCLC CSCs by stimulating JNK/c-Jun. We also revealed that the $CD248^{+}$CAFs co-cultured with A549/NCI-H460 releasing IL-8 increased the expressing of CD44, OCT4 and inhibited the expressing of CD24 in vitro. Relative to wild-type control mice, the CD248 conditional knockout mice exhibited significant decrease of IL-8 secretion, which, in turn, decreasing CD24-$CD44^{+}OCT4^{+}$cells proportion in vivo. Our study reveals that $CD248^{+}$CAFs secreting IL-8 promote NSCLC cisplatin resistance by sustaining cancer stemness via stimulating JNK/c-Jun axis. The results suggest that targeting the $CD248^{+}$CAFs could be an effective therapeutic strategy against CSC-driven solid tumors.

Key Words: non-small cell lung cancer, cancer-associated fibroblasts, CD248, IL-8, CSCs, chemoresistance

P52: Molecular Mechanisms of IL-6 Secretion by Cancer-Associated Fibroblasts (CAFs) in Promoting Chemoresistance in Lung Squamous Cell Carcinoma

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ABSTRACT
Background: Lung squamous cell carcinoma (LUSC), as one of the main pathological types of non-small cell lung cancer (NSCLC), is primarily treated with surgery and conventional chemotherapy compared to lung adenocarcinoma (LUAD). Chemoresistance in LUSC during clinical treatment is one of the major causes of poor prognosis for patients. Cancer-associated fibroblasts(CAFs) , as an important component of the tumour-microenvironment (TME), promote tumour chemoresistance by secreting IL-6. However, the molecular mechanism through which CAFs secrete IL-6 to promote chemoresistance in LUSC remains unclear.

Aim: To study CAFs can secrete IL-6 to promote chemoresistance in lung squamous cell carcinoma.
Method: We used conditioned media from CAFs or NFs treated human LUSC cell lines (SK-MES-1， NCI-H520) to determine the killing effects of cisplatin and docetaxel through CCK-8 assays, flow cytometry. RNA sequencing and Gene Set Enrichment Analysis (GSEA) were conducted to study the differential gene expression and changes in related signaling pathways between CAFs and NFs. The relationship between IL-6 and CAFs was validated through immunofluorescence (IF), Western blotting, ELISA, and RT-qPCR assays. Furthermore, we investigated the mechanism of action of IL-6 in chemoresistance of LUSC cell lines via CCK-8 assays, flow cytometry.
Result: We found that the conditioned media from CAFs treated SK-MES-1 and NCI-H520 can inhibited the killing effects of cisplatin and docetaxel. Meanwhile, CAFs secreted IL-6 to promote chemoresistance in LUSC cells through activation of the PI3K/AKT/NF-kB pathway. Moreover, The conditioned media from normal fibroblasts (NFs) plus IL-6 can reverse the killing effects of cisplatin and docetaxel in SK-MES-1 and NCI-H520.
Conclusion: Our study found that CAFs secreting IL-6 promote chemoresistance in LUSC cells by activating the PI3K/AKT/NF-kB pathway. Thereby laying the groundwork for future therapeutic interventions.

Key Words: Lung squamous cell carcinoma，Cancer-associated fibroblasts，IL-6, Chemoresistance

P53: Multi-ethnic Genetic Analysis Reveals Structure and Diversity in the Populations of Guizhou in China.

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ABSTRACT.
Purpose Analysis of variations in human genome could provide insights into their population history and uncover the genetic basis of phenotypic diversity and disease etiology. There are 18 resident ethnic groups ling in Guizhou in southwest of China, accounting for 37% of the total population. These ethnic minorities represent a unique resource for genetic studies. However, only few large-scale cohort or whole-genome sequencing (WGS) studies have been conducted in these local populations. Here, we performed the whole-genome sequencing in GZ individuals to understand their population structure and history.
Methods Whole blood samples were collected from three GZ ethnic minorities including 156 Bouyei, 160 Dong, and 152 Miao. DNA was extracted, and libraries were prepared and sequenced on DNBSEQ T1 platform with 100bp paired-end reads. Reads were mapped to bwa software, and variants were called and filtered based on

the standard GATK hard-filter pipeline. 26 samples were excluded due low sequencing depth, or relatedness.
Results After filtering, 437 unrelated individuals passing data quality control were retained for downstream analyses. Their mean ages were 53.7 years and 49.2% were female. The mean sequencing coverage was 33.6 X. A total of 22.4 million variants were identified, of which 23.9 % were novel. Among the 60 pathogenic variants identified, two (rs33986703 at Hemoglobin Beta and rs367956927 at HTR1A) showed a high allele frequency in the GZ population and are associated with diseases specific to the Guizhou population, such as thalassemia and stress response. Furthermore, we confirmed that the largest ancestral component in the GZ genomes is from East Asians, and the ancestral component pattern of GZ shares a high similarity to Miao and Dai samples from the HGDP database. In addition, the results of the natural selection analysis indicate that significant selection of variants in the adaptive immune process was observed.
Conclusions Findings from this study provided fundamental insights into genetic structure, demographic history and natural selection in three GZ ethnic groups. Meanwhile, the genetic evidence of GZ population structure and demographic is consistent with the corresponding geographical distribution of southwest of China. These results highlighted the value of our data as a resource to contribute to human genetics discovery in Chinese populations.

Key Words: Population genetic; whole-genome sequencing; ethnic minorities

P54: Identification of Critical Residues of TMPRSS2 for the Entry and Host Range of Human Coronavirus HKU1

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ABSTRACT.
Human coronavirus (CoV) HKU1 infection typically causes common cold but can lead to pneumonia in children, old people and immunosuppressed individuals. Recently human transmembrane serine protease 2 (hTMPRSS2) was identified as the functional receptor for HKU1, but its region and residues critical for HKU1 S binding remain elusive. In this study, we found that HKU1 only utilized human but not rat, mouse, hamster, or bat TMPRSS2 for virus entry, displaying a narrow host range. Using human-bat TMPRSS2 chimeras, we showed that the serine peptidase (SP) domain of TMPRSS2 is essential for entry of HKU1. Further extensive mutagenesis analyses of the C-terminal regions of SP domains of human and bat TMPRSS2s identified residues 417 and 469 critical for entry of HKU1. Replacement of either

D417 or Y469 with asparagine in hTMPRSS2 abolished its abilities to mediate entry of HKU1 S pseudovirions and cell-cell fusion, whereas substitution of N417 with D or N469 with Y in bat TMPRSS2 (bTMPRSS2) renders it supporting HKU1 entry. Our findings contribute to a deeper understanding of coronavirus-receptor interactions and cross-species transmission.

Key Words: Human coronavirus HKU1、TMPRSS2、Entry、Host range

P55: MicroRNA-19a-3p Inhibits Endothelial Dysfunction in Atherosclerosis by Targeting JCAD

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Endothelial dysfunction occurs early in the progression of atherosclerosis, and much research has been carried out on its role in this disease. MicroRNA-19a-3p (miR-19a-3p), a miR-17-92 family member, has been shown to be associated with atherosclerosis. However, its involvement in endothelial dysfunction in atherosclerosis is unclear. The objective of this investigation was to examine the connection between miR-19a-3p and endothelial dysfunction. Our findings showed that miR-19a-3p expression in endothelial cells decreased after exposure to TNF and/or oscillatory flow, which was consistent with the change in miR-19a-3p found in atherosclerotic plaques. Additionally, endothelial cell dysfunction and inflammation were significantly diminished by miR-19a-3p overexpression but markedly exacerbated by miR-19a-3p inhibition. MiR-19a-3p transfection significantly decreased the expression of junctional protein associated with coronary artery disease (JCAD) by binding to the 3'-UTR of JCAD mRNA. Furthermore, the protective effect of miR-19a-3p against endothelial cell dysfunction and inflammation was achieved by regulating JCAD and was closely linked to the Hippo/YAP signaling pathway. Therefore, our findings provide evidence that miR-19a-3p expression is a crucial molecular switch in the onset of atherosclerosis and that miR-19a-3p overexpression is a possible pharmacological therapeutic strategy for reversing the development of atherosclerosis.

P56: Paeonol Inhibits Cell Growth by InducingFerroptosis in U118 Human Glioma Cells

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Paeonol (PAE) is a phenolic component extracted from peony bark, peony root and Xu Changqing. and has a variety of pharmacological effects. Studies have reported that PAE has notable inhibitory effects on liver cancer, gastric cancer, breast cancer and other cancers. However, there are no relevant reports about its role in human glioma. Ferroptosis is an iron-dependent form of non-apoptotic regulated cell death. Increasing evidence indicate that induction of ferroptosis can inhibit the proliferation, migration, invasion and survival of various cancer cells, which acts as a tumor suppressor in cancer. In this study, we confirmed that PAE can inhibit the cell proliferation, migration, invasion and survival in human nasopharyngeal carcinoma cells. Our finding shows that PAE can induce the ferroptosis axis by decreasing the level of GPX4 and promoting the induction of toxic LPO and ROS. AL-mediated cytotoxicity in human glioma cells is dependent on ferroptosis. Therefore, PAE has good anti-cancer properties and is expected to be a potential drug for the treatment of glioma.

P57: Thyroid Hormone in Skeletal Muscle Stem Cells and Muscle Regeneration

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ABSTRACT. The skeletal muscle (SM) is a well-known target tissue for thyroid hormone (TH), where TH plays a crucial role in regulating SM development, maintenance, and regeneration. Thyroid dysfunction patients commonly exhibit symptoms like muscle atrophy and weakness. Muscle stem cells (MuSCs) are primarily responsible for postnatal SM growth, maintenance, and injury-induced muscle regeneration. They must exit quiescence, re-enter the cell cycle, proliferate, differentiate, and self-renew for muscle repair. Complex regulatory networks of intrinsic and extrinsic factors govern the progression of the myogenic lineage. Defects in these processes can lead to muscle loss in patients with various muscular dystrophies. The intracellular TH concentration is influenced by local levels of TH transporters, deiodinase activity, and TH receptors (TR). During regeneration, the TH level in SC is precisely regulated by deiodinases and TH promotes MuSC differentiation by inducing myogenic factor expression via TR. In addition, TRa mutant mice exhibit impaired muscle regeneration after injury. Interestingly, our recent findings suggest that TR has a significant role in maintaining MuSC homeostasis and the immune microenvironment. Specifically, TR regulates the dynamic changes of macrophages during skeletal muscle regeneration, which is crucial for proper muscle repair and regeneration.TR knockout mice in myeloid cells ($TR^{\Delta Mo}$) have impaired muscle regeneration. Given the extensive metabolic regulatory role of TH, we are investigating the mechanism of TH in remodeling macrophage metabolism, which can regulate the immune microenvironment, promote muscle regeneration, and potentially open new avenues for muscle-related disease treatment.
Key Words: Skeletal Muscle Stem Cells, Muscle Regeneration, Thyroid Hormone Receptor, Macrophage

P58: Thyroid Hormone Action in Adipose Tissues

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

P59: CREB Negatively Regulates Pax6 Expression in LECs through its Direct Binding to the Proximal Promoter of Pax6 Gene

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ABSTRACT.
Purpose: CREB is a transcription factor that mediates the regulation of the cAMP response genes by binding as a dimer to a conserved cAMP-response element (CRE): TGACGTCA. The most prominent function of the cAMP/CREB pathway is its implication in the synaptic plasticity associated with long-term memory. In our recent studies, we demonstrated that CREB regulates oxidative stress-induced apoptosis and aging in lens by inhibiting αB-crystallin expression but promoting P00-P53-Bak/Bax signaling axis (Wang L et al. 2021. Aging Cell). Whether CREB is capable to regulate lens differentiation remains largely unknown. Here, in the present study, we demonstrate that CREB directly regulates Pax6 and other genes to control differentiation of mouse lens. **Methods:** RNAseq analysis was used to compare the transcriptional activity of wild type and mutant CREB-S133A. Gel mobility shifting assays were used to determine binding of CREB to Pax6 gene promoter. ChIP assays were used to confirm the binding of CREB to the Pax6 gene promoter. QRT-PCR and Western blot were used to analyze mRNA and protein expression levels. **Results:** mRNA and immunoblotting analysis revealed the reverse relationship between CREB

and Pax6. EMSA assays demonstrate that CREB directly binds to the promoter region of Pax6. ChIP assay results confirmed that CREB binds to Pax6 promoter in vivo. In mouse lens epithelial cell, overexpression WT-CREB resulted in downregulation of Pax6. Silence of CREB upregulates Pax6. RNAseq analysis also revealed that CREB can regulate a panel of differentiation-related genes. **Conclusion:** Through direct control of Pax6 and other downstream differentiation-related genes, CREB regulates lens differentiation.
Key Words: CREB, Pax6, Lens differentiation, Gene regulation

P60: SFTSV Induced Liver Ferroptosis through M6A-related Ferritinophagy
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ABSTRACT
Severe fever with thrombocytopenia syndrome (SFTS) is a widely prevalent infectious disease caused by severe fever with thrombocytopenia syndrome virus (SFTSV). SFTSV infection carries a high mortality rate and has emerged to be a public health concern. SFTSV infection could induce many classical cell death pathways. Ferroptosis, a novel iron-dependent form of regulated cell death, is shown to participate at various biological processes and considered as a promising new therapeutic target. In the current study, we reported that SFTSV infection downregulated the expression of GPX4 and SLC7A11. The level of ROS and MDA were increased and the level of GSH was decreased after SFTSV infection. Interestingly, we observed the elevation of ATG5 mRNA m6A modification after SFTSV infection. Mutation of the m6A-sites significantly rescued SFTSV infection-induced ferritinophagy, and we further found that NSs protein, the virulent factor of SFTSV played a major role driving the ferritinophagy. Finally, we found that ferroptosis inhibitor Ferrostatin-1 prevented ferroptosis and suppressed SFTSV infection in both in vitro and in vivo models. In summary, our study demonstrated that SFTSV infection could induce ferroptosis in liver, and m6A modified ATG5 mediated ferritinophagy to facilitate this process. Targeting ferroptosis may serve as a potential therapy for the treatment of SFTS.
Key Words: SFTSV, Ferroptosis, Ferritinophagy, NSs, ATG5

P61: RAB37-mediated Autophagy, Mechanisms and Functions
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Intracellular membrane trafficking is essential for eukaryotic cell existence. Autophagy activity is a catabolic process involved in intracellular membrane trafficking that is essential to maintaining cellular homeostasis in various eukaryotes. Autophagy plays important roles in differentiation, development, immunity, and lifespan. Dysfunctions of autophagy regulations have been associated with various

human diseases, including cancer, neurodegenerative diseases, metabolic disorders and microbial infections. Thus, understanding of autophagic mechanisms and roles has important physiologic and therapeutic implications. Here, we show that RAB37, a small GTPase, promotes autophagosome formation through the ATG5-ATG12-ATG16L1 complex in a GTP-dependent manner. Conditional knockout of Rab37 in oocytes impaired autophagy proficiency in the ovary and interfered with follicular homeostasis and ovary development in mice. Flunarizine treatment upregulated autophagy, thus rescuing the impairment of follicular homeostasis and ovarian dysfunction in Rab37 knockout mice by reprogramming of homeostasis. Notably, both the E2F1 and EGR2 transcription factors synergistically activated Rab37 transcription and promoted autophagy. Thus, RAB37-mediated autophagy ensures ovary function by maintaining ovarian homeostasis.
Key Words: Homeostasis; Membrane; Autophagy; Gonad; Development

P62: Silencing of SIRPα Enhances the Antitumor Efficacy of CAR-M in Solid Tumors

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The therapeutic potential of macrophage phagocytosis of tumor cells, facilitated by CD47-specific blocking antibodies, holds promise for cancer treatment, yet raises concerns over its potential toxicities for non-tumor cells. Here, we developed chimeric antigen receptor macrophages (CAR-M) by fusing humanized single-chain variable fragment (scFv) with FcγRIIa and incorporating short hairpin RNA (shRNA) to target SIRPα to disrupt the CD47-SIRPα signaling pathway. The engineered CAR-shSIRPα-M exhibited a M1-like phenotype, demonstrated enhanced phagocytic capability, and exerted significant cytotoxic effects on HER2-positive tumor cells whilst eradicating patient-derived organoids. In vivo experiments revealed that CAR-M with SIRPα silencing substantially inhibited tumor growth and extended survival in tumor-bearing mice. Notably, CAR-shSIRPα-M heightened cytotoxic T cell infiltration within tumors, amplifying the antitumor impact in a humanized immune system mouse model. The study further identified that SIRPα inhibition markedly activated inflammatory pathways and the cGAS-STING signaling in CAR-M, which led to elevated production and secretion of pro-inflammatory cytokines. Moreover, SIRPα inhibition in CAR-M increased the output of reactive oxygen species (ROS) and nitric oxide (NO), contributing to their antitumor efficacy. Collectively, these findings suggest that SIRPα inhibition enhances the antitumor potency of CAR-M against solid tumors, presenting an innovative approach to cancer immunotherapy.

P63: Based on MiR-205-5p to Explore the Inhibitory Effect of Sijunzi Decoction on Migration, Invasion and Epithelial-mesenchymal Transition Process of Gastric Cancer Cells

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Abstract:

Objective: To study the effect of Sijunzi Decoction on gastric cancer cell migration, invasion, and epithelial-mesenchymal transition (EMT) by increasing miR-205-5p levels and identifying its potential target genes.

METHODS: Two gastric cancer cell lines (MGC-803 and AGS) were studied in four groups: normal saline (NS) group, Sijunzi Decoction (SJZT) group, Sijunzi Decoction with inhibitor NC (SJZT+inhibitor NC) group, and Sijunzi Decoction with miR-205-5p inhibitor (SJZT+miR-205-5p inhibitor) group. Expression levels of miR-205-5p, Snail, MMP9, and TIMP1 were measured using qPCR and Western blot. Cell migration and invasion were assessed using Scratch and Transwell assays, respectively. Human gastric cancer MGC-803 cells were studied, divided into a negative control (NC) group and a miR-205-5p mimics group. Differentially expressed genes were identified through transcriptome sequencing and analyzed for enrichment. Downstream target genes were validated with qPCR.

RESULTS: MiR-205-5p expression was significantly increased in the SJZT group compared to the NS group (P<0.01). Inhibiting miR-205-5p reversed the promotion effect of Sijunzi decoction on miR-205-5p expression in gastric cancer cells (P<0.01). SJZT group showed reduced scratch healing rate, cell invasion, and expression of MMP9 and Snail compared to the NS group. Inhibiting miR-205-5p reversed these effects in the SJZT group (P<0.05, P<0.01). Timp-1 mRNA and protein levels showed opposite results compared to other genes mentioned (P<0.05, P<0.01). 401 genes were differentially expressed between the NC and miR-205-5p mimics groups, with 195 up-regulated and 206 down-regulated. The GO analysis revealed enrichment in cell adhesion and migration, while KEGG analysis showed enrichment in cancer-related pathways like PI3K-Akt and Rap1 signaling. The miR-205-5p mimics group showed significantly lower expression levels of FGF7, ENC1, and CSF1 compared to the NC group (P<0.01), which was consistent with the sequencing results.

Conclusion: MiR-205-5p has the capacity to facilitate the inhibitory impact of Sijunzi decoction on the migration, invasion, and epithelial-mesenchymal transition process of gastric cancer cells. The mechanism may be associated with the action of miR-205-5p, resulting in decreased expression of downstream genes including FGF7, ENC1, and CSF1, among others.

Keywords: gastric cancer; Sijunzi decoction; miR-205-5p; migration; invasion;

P64: The Action of Si-Jun-Zi Decoction on N6-methyladenosine Modification in Gastric Cancer Based on LC-MS/MS Study and NetworkPharmacology

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Abstract

PURPOSE: The study aimed to analyze how Sijunzi Decoction treats gastric cancer using network pharmacology and experimental validation.

METHODS: This study investigated the active components by LC-MS/MS. Databases such as ChEMBL,TCMIO, TTD, STRING and GeneCards were used to screen targets of SJZ in the treatment of gastric cancer. An online Venn tool and Cytoscape 3.7.0 were used to analyze the components and targets. The study utilized a xenograft tumor model of gastric cancer and gastric cancer cell lines to explore the impact of SJZ on N6-methyladenosine (m6A) modification. Expression levels of m6A enzymes were assessed using qRT-PCR and Western blotting.

RESULTS: The results revealed 511 active components and 196 related targets in SJZ, with 167 targets associated with gastric cancer treatment. KEGG enrichment analysis showed significant enrichment in pathways related to cancer, metabolism, and immunity. The GO enrichment analysis revealed enriched terms in molecular function (MF), biological process (BP), and cellular component (C), including aging (GO:0007568), plasma membrane raft (GO:0044853), and phosphatase binding (GO:0019902). The protein-protein interaction (PPI) network comprised 274 nodes and 2902 edges, while the Herbal-component-target protein-pathway-disease network consisted of 107 nodes and 345 edges, identifying four components with more than 20 putative targets. Experimental assays demonstrated a significant decrease in METTL3 expression following treatment with SJZ, while METTL14 expression levels were notably higher in the SJZ group compared to the NS group in both gastric cancer cell lines and gastric cancer tissues from a mouse model ($P<0.01$, $P<0.001$ or $P<0.05$).

CONCLUSION: SJZ exhibits multi-component, multi-target, and multi-pathway characteristics in the treatment of gastric cancer, with m6A modification potentially playing a crucial role.

Key words： Si-Jun-Zi Decoction; gastric cancer; N6-methyladenosine； network pharmacology；

P65: A Self-adapting Polygenic Risk Score Model Improves Risk Prediction of Venous Thromboembolism in Chinese Cohorts

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ABSTRACT: Most genome-wide association studies (GWAS) of Venous Thromboembolism (VTE) have used data from individuals of European descent, however, genetic factors for VTE have not been fully identified in Chinese populations, which causes the limited use of existing polygenic risk scores (PRS) to identify subpopulations at high risk of VTE for prevention. We, therefore, aimed to curate all the potential VTE-related single-nucleotide polymorphisms (SNPs) for the construction of a new improved PRS model based on the self-adapting method, and then evaluate its utility and effectiveness in the stratification of VTE risk in Chinese populations. We comprehensively analyzed the mutation spectrum of VTE-associated SNPs in the Chinese cohort, and ranked their individual risk effects independently using risk ratio, logistic regression coefficient, and penalty regression coefficient as evaluation criteria. By integrating various algorithms and evaluating their performance, we trained the optimal prediction model of VTE risk in the Chinese population with the least SNP features, established an adaptive PRS model with progressive SNP overlay, and tested it on an independent Chinese population cohort. Self-adaptive polygenic risk score model based on all 318 SNPs or on the 44 most strongly associated SNPs performed similarly (areas under receiver-operating characteristic curves (AUCs) of 0.739 and 0.709, respectively) on the testing dataset of the Chinese VTE cohort, and that achieve the overall best level of the AUC from a conventional PRS model based on known genetic risk factors (0.620-0.718). In addition, we observed the self-adaptive PRS model was an independent effective risk stratification indicator beyond other clinical characteristics including age and smoking status. Our data revealed that only 44 SNPs-derived PRS model can be effectively used in discriminating subpopulations at high risk of VTE. To become clinically useful, our model could benefit from a practically feasible VTE screening program for precision prevention in Chinese populations.
Key Words: GWAS; VTE; Risk Prediction; Self-adaptive Model; Chinese populations

P66: Oleanolic Acid Inhibits Malignant Progression of Leukemia K562 Cells Through The miR-18a-5p/STK4 Axis

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Abstract
Chronic myeloid leukemia (CML) is a disease caused by the malignant proliferation of hematopoietic stem cells in the bone marrow. Although tyrosine kinase inhibitors have made significant progress in the treatment of chronic myeloid leukemia., the issue of drug resistance remains unresolved. Therefore, finding new therapeutic drugs and targets is crucial. Studies have shown that miRNAs are closely associated with various tumors, and oleanolic acid (OA) has a good inhibitory effect on leukemia cells. Therefore, this article aims to investigate whether oleanolic acid can affect K562 cells

through miR-18a-5p/STK4. Through the analysis of transcriptome sequencing results, we found that a considerable portion of genes in K562 cells were enriched in apoptosis related pathways after treatment with oleanolic acid. EdU and CCK8 experiments found that oleanolic acid reduced the proliferation ability of K562 cells ($P < 0.05$). qRT-PCR, immunofluorescence, and Western blot results showed that after treatment with oleanolic acid, the expression of STK4 was upregulated 1.9 times in K562 cells, and the expression of miR-18a-5p was downregulated compared to the control group ($P<0.05$). Compared with the control group, adding miR-18a-5p mimics to cells can inhibit the expression of STK4; Adding miRNA inhibitor can increase the expression of STK4($P<0.05$). The results of reactive oxygen species (ROS) detection and mitochondrial membrane potential detection showed that oleanolic acid can promote the increase of ROS in K562 cells and reduce mitochondrial membrane potential. After overexpression of STK4, the mitochondrial membrane potential of the cells decreased compared to the control group; after knocking down STK4 and receiving treatment with oleanolic acid, the decrease in mitochondrial membrane potential in the knockdown+oleanolic acid group was inhibited compared to the oleanolic acid group. Flow cytometry analysis showed that oleanolic acid promotes the occurrence of cell apoptosis, with an apoptosis rate of 57%, while the addition of miR-18a-5p mimics reduces the apoptosis rate by 10%; Overexpression of STK4 can promote the transformation of cells towards late stage apoptosis, while the addition of miR-18a-5p mimics can inhibit this phenomenon. Our research results confirm that oleanolic acid can promote apoptosis of K562 cells by maintaining low expression of miR-18a-5p and high expression of STK4.

Key words: OA；K562；miR-18a-5p；*STK4*；apoptosis

P67: Emerging Role of Lipid Droplets in Obscure Puffer Immune Response against Vibrio Harveyi

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ABSTRACT

As dynamic and functionally active organelles, lipid droplets (LDs) mainly function in lipid anabolism, while recent researches showed that mammalian LDs also actively participated in innate immunity, however the specific roles and regulation mechanism remain relatively unexplored, and the existing studies were mainly limited to mammals. In the present study, we firstly found that Vibrio harveyi, a serious pathogen in marine environment, could induce LDs accumulation in the liver of obscure puffer Takifugu obscurus on the histology, morphology and molecular levels, and the induction mainly conducted by promoting the synthesis of neutral lipids. Moreover, the antibacterial activity of LD proteins was significantly enhanced upon V. harveyi stimulation, and showed broad-spectrum characteristic. While the inhibition of LDs formation downregulated the expression of immune related genes and immune signaling elements, highlighting the potential critical roles of LDs during the bacterial infection. The isolated LDs from obscure puffer liver were examined via proteomic analyses, and the data supported the conservative property of LDs from bacteria to

humans, and revealed that numerous innate immune system-related components were enriched on the surface of LDs. These results will deepen the understanding of LDs biology and host immune defense mechanism, shedding light on the new strategies for the development of anti-infective therapies.
Key Words: Lipid droplets (LDs); Vibrio harveyi; Takifugu obscurus; Antibacterial activity; Proteomic analyses.

P68: Schisandrin A Enhances Pathogens Resistance by Targeting A Conserved P38 MAPK Pathway

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ABSTRACT.
Schizandrin A (SA), also known as deoxyschizandrin, is one of the most biologically active lignans isolated from the traditional Chinese medicine Fructus schisandrae chinensis. Schisandrin A has proven benefits for anti-cancer, anti-inflammation, hepatoprotection, anti-oxidation, neuroprotection, anti-diabetes. But the influence of Schisandrin A to the innate immune response and its molecular mechanisms remain obscure. In this study, we found that Schisandrin A increased resistance to not only the Gram-negative pathogens Pseudomonas aeruginosa and Salmonella enterica but also the Gram-positive pathogen Listeria monocytogenes. Meanwhile, Schisandrin A protected the animals from the infection by enhancing the tolerance to the pathogens infection rather than by reducing the bacterial burden. Through the screening of the conserved immune pathways in Caenorhabditis elegans, we found that Schisandrin A enhanced innate immunity via p38 MAPK pathway. Furthermore, Schisandrin A increased the expression of antibacterial peptide genes, such as K08D8.5, lys-2, F35E12.5, T24B8.5, and C32H11.12 by activation PMK-1/p38 MAPK. Importantly, Schisandrin A-treated mice also enhanced resistance to P. aeruginosa PA14 infection and significantly increased the levels of active PMK-1. Thus, promoted PMK-1/ p38 MAPK-mediated innate immunity by Schisandrin A is conserved from worms to mammals. Our work provides a conserved mechanism by which Schisandrin A enhances innate immune response and boosts its therapeutic application in the treatment of infectious diseases.
Key Words: Schisandrin A, Innate immunity, p38 MAPK pathway, Caenorhabditis elegans, Mice

P69: Cynaroside Extends Lifespan and Improves the Neurondegeneration Diseases via Insulin/IGF-1 Signaling Pathway in Caenorhabditis Elegans

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ABSTRACT.
The evolutionarily conserved insulin/IGF-1 signaling pathway plays a central role in aging and aging related diseases such as neurodegeneration diseases. Inhibition of insulin/IGF-1 signaling pathway has been proposed as an effective way to extend lifespan and delay neurodegeneration diseases in different organisms. Cynaroside (Cyn), a flavonoid contained in many medical plants and in vegetables, had been shown to exhibit pharmaco-logical properties such as anti-inflammatory, anti-tumor, and anti-oxidant effects. The study demonstrated that lifespan extension and neurodegeneration diseases improving could be achieved by targeting evolutionarily conserved insulin/IGF-1 pathway through using pharmacological interventions. Via using this approach in tractable model Caenorhabditis elegans, we found that 10 μM Cyn significantly promoted the healthy lifespan in wild-type animals. Furthermore, via genetic screen, we showed that Cyn acted on IGF-1-R/DAF-2, which was followed by the activation of transcription factor DAF-16/FOXO to extend the healthy lifespan. Intriguingly, Cyn also improved neurodegeneration diseases such as Alzheimer's and polyglutamine disease by suppressing insulin/IGF-1 signaling pathway. Our work suggests that Cyn may be a promising candidate for the prevention and treatment of aging and neurodegeneration diseases.
Keywords: Cynaroside ; Lifespan ; Insulin/IGF-1 signaling pathway ; Neurodegeneration diseases; Caenorhabditis elegans

P70: Cdc14B/Cyclin B1 Signaling Modulates the Pathogenesis of Sonic Hedgehog Subtype Medulloblastoma

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ABSTRACT
Objective: Medulloblastoma (MB) is a severe malignancy of the central nervous system that predominantly occurs in the cerebellum of children. Overactivation of the sonic hedgehog (Shh) signaling pathway is the primary cause of the development and progression of Shh subtype MB, although the detailed mechanisms underlying this process remain largely elusive.
Methods: Western blot analysis was employed to examine the expression of Cdc14B and CyclinB1 in medulloblastoma (MB) cells and a mouse MB model. The impact of Cdc14B and CyclinB1 on tumor cell proliferation was assessed using cell proliferation assay, colony formation assay, and immunofluorescence. Western blotting was utilized to detect changes in the expression of p-Cdc25C and CDK1 upon overexpression of Cdc14B. Co-immunoprecipitation assay was performed to determine the interaction between CyclinB1 and Ptch1. Immunofluorescence staining confirmed the localization of CyclinB1 in MB cells with or without Shh presence. Immunohistochemical staining was conducted to evaluate the expression levels of

Cdc14B and related proteins in mouse and human MB tissues.
Results: In this study, we have demonstrated that non-canonical Hedgehog signaling mediated by Shh can effectively enhance proliferation in MB cells. This mechanism involves the binding of Shh to Patched 1, resulting in the disruption of its interaction with Cyclin B1. Consequently, nuclear translocation of Cyclin B1 is facilitated, leading to the activation of genes associated with cell division and proliferation. Additionally, our observations indicate that deregulation of Cdc14B in SHH group MB results in the stabilization of the Cyclin B1/CDK1 complex within MB cells through the activation of Cdc25C, a phosphatase known for its role in maintaining Cyclin B1 stability.
Conclusions: Our findings underscore the pivotal role of Cdc14B/Cyclin B1 in orchestrating Hedgehog signaling-driven pathogenesis in SHH group medulloblastoma, thereby offering valuable insights for identifying potential therapeutic targets.
Key Words: Medulloblastoma, sonic hedgehog (Shh), Cdc14B, Cyclin B1, cell proliferation, cell cycle

P71: Hepatic Glycerol Kinase Promotes the Development of Non-alcoholic Hepatic Steatosis by Stimulating SREBP-1c Transcription and Catalyzing Glycerol Metabolism
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Glycerol kinase (GK) participates in triglyceride (TG) synthesis by catalyzing glycerol metabolism. It is unclear whether GK contributes to non-alcoholic fatty liver (NAFL), in which hepatic de novo lipogenesis is increased. In this study, we found that hepatic Gk expression is increased in diet and genetic mouse models of NAFL and is correlated with elevation of SREBP-1c expression. Cholesterol and fatty acids stimulate GK expression in hepatocytes. In mice with HFD-induced NAFL, knockdown of hepatic Gk decreases expression of SREBP-1c and its target lipogenic genes, elevates serum levels of glycerol, lowers serum levels of TG, and alleviates hepatic TG accumulation. Conversely, overexpression of GK in hepatocytes in mice or in culture results in opposite outcomes. Mechanistic studies reveal that GK stimulates SREBP-1c transcription by binding to the SREBP-1c gene promoter and enhances SREBP-1c-induced self-gene transcription by binding to the SREBP-1c protein. Studies using truncated GKs and mutant GKs indicate that GK induces SREBP-1c transcription independently of its enzyme activity. GK contributes to lipid homeostasis under physiological conditions by catalyzing glycerol metabolism rather than regulating SREBP-1c transcription. Together, these results demonstrate that elevation of hepatic GK promotes the development of NAFL by catalyzing glycerol metabolism and stimulating SREBP-1c transcription.

P72: Isoliquiritigenin Attenuates Myocardial Ischemia-reperfusion Injury by Regulating AKT/GLUT4 Pathway Related Myocardial Insulin Resistance
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ABSTRACT
Background. Myocardial ischemia/reperfusion injury (MIRI), a frequent complication in cardiovascular diseases, is characterized by complex and abnormal insulin metabolic and signaling pathways. Isoliquiritigenin (ISL), a prominent flavonoid isolated from the roots of *Glycyrrhiza* species, possesses the ability to improve insulin sensitivity. However, the potential cardio-protective effects and mechanism of ISL in MIRI remain unclear. ***Methods.*** *In vivo,* SD rats underwent left anterior descending coronary artery ligation/reperfusion to induce MIRI. Chest echocardiography was performed to monitor cardiac function post-reperfusion, followed by measurement of myocardial injury and IR markers. *In vitro* model of MIRI was established using H9C2 cardiomyocytes subjected to hypoxia and re-oxygenation. ***Results***. *In vivo* experiments demonstrated that ISL attenuated myocardial infarct size, decreased serum markers of myocardial injury, improved left ventricular systolic function, and enhanced insulin sensitivity. *In vitro* data revealed that ISL ameliorated glucose uptake and cell survival rate. Furthermore, ISL increased AKT phosphorylation and up-regulated membrane-bound GLUT4 (M-GLUT4) protein expression levels. ***Conclusions***. ISL protects against myocardial damage caused by MIRI through the regulation of IR via the AKT/GLUT4 pathway.
Keyword: Isoliquiritigenin, myocardial ischemia/reperfusion injury, insulin resistance, GLUT4, AKT
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P73: Effect of the UFMylation System on Protein Stability and Drug Sensitivity of Gastric Cancer Cells

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Ubiquitin-fold modifier 1 (UFM1) modifies protein substrates (UFMylation) through a cascade of enzymatic reactions similar as protein ubiquitination. Recently it has been discovered that several proteins are modified by UFM1 and UFMylation regulates protein stability, DNA damage response, ER stress, immunotherapy, etc.

However, it is unknown how this modification system regulates protein stability under physiological and pathological conditions and whether it regulates the sensitivity of chemotherapeutic drugs.
In this work, we discovered that the key component in the UFMylation system UFBP1 (UFM1-binding and PCI domain-containing protein 1) is closely associated with the survival of gastric cancer patients treated with cisplatin-based chemotherapy. Proteomic and biochemical studies disclosed that UFBP1 regulates the protein and mRNA levels of aldo-keto reductase 1Cs (AKR1Cs) in gastric cancer cells and UFBP1 modulates the sensitivity of gastric cancer cells to cisplatin. Mechanistic studies further revealed that UFBP1 promotes the ubiquitination of a transcription factor Nrf2 (nuclear factor erythroid-2-related factor 2) and therefore suppresses the expression of AKR1Cs, leading to the enhanced sensitivity of gastric cancer cells to cisplatin. In addition, using affinity purification and quantitative proteomic analysis, we identified many potentially UFMylated proteins. Cell-line based studies revealed that protein UFMylation participated in the regulation of protein stability and functions.
Key Words: UFMylation, ubiquitination, protein degradation, gastric cancer, drug sensitivity

P74: Paraptosomes: Unique Golgi-Derived Structures in Paraptotic Cell Death
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ABSTRACT.
Paraptosis is a novel form of programmed cell death characterized by distinct morphological features such as swelling of the endoplasmic reticulum and mitochondria, and cytoplasmic vacuolation. Unlike apoptosis, paraptosis does not involve the activation of caspases or DNA fragmentation. Recent studies have shown that compounds such as glabridin, morusin, and honokiol can induce paraptotic cell death in tumor cells. This study aims to elucidate the mechanisms of paraptosis induced by these natural compounds.
Using morphological observations, protein immunoblotting, and ELISA, we reveal that paraptosis involves structural and functional changes in the Golgi apparatus, as well as alterations in lysosome and autophagosome-associated proteins, in addition to the previously reported organelle swelling. Interestingly, we identified a previously unreported high-density circular structure during the paraptotic process, which we have named the “paraptosome.” These structures originate from the Golgi apparatus, appearing as high-density formations under light microscopy and colocalizing with the trans-Golgi marker β4GALT1-RFP. Time-lapse confocal microscopy and immunostaining demonstrated that paraptosomes form due to Golgi stress or disintegration, leading to severe disruption of Golgi function. Electron microscopy further revealed their complex and disordered Golgi-like composition. Notably, the formation of paraptosomes is not induced by isolated endoplasmic reticulum or Golgi stress, indicating that paraptosomes are a unique feature of paraptosis.
The discovery of paraptosomes provides a novel marker for defining paraptotic cell death and offers new insights into the characteristic pathological phenomena

associated with multiple organelle dysfunction. This finding broadens the scope of cell biology research by introducing a new structural paradigm linked to paraptosis.
Key Words: Paraptosis, Paraptosomes, Golgi apparatus, Organelle dysfunction

P75: P62/SQSTM1 UFMylation Enhances the Autophagic Flux and Promotes the Degradation of Pathogenic Protein Aggregates

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Ubiquitin-fold modifier 1 (UFM1) covalently modifies protein substrates on their lysine residues through a cascade of multiple enzymatic reactions (termed as UFMylation), but its substrates and biological functions are largely unknown. Genetic screen revealed that enzymes in the UFM1-conjugation system play important roles in autophagy. However, it is unclear which protein is UFMylated and how this modification executes its biological functions in autophagy.
Here, we used double affinity purification and quantitative proteomics to identify more than 700 putative UFMylated proteins in a human cell line. Proteomics and biochemical experiments disclose that p62/SQSTM1 is a new UFMylated protein and identify two major UFMylation sites K420 and K435 on p62. Mutating them to Arg (p622KR) entirely abolishes the effect of p62 on the autophagic activity. Fusion of the C-terminal four residue truncated UFM1 (UFM1 Δ C4) to p622KR (p622KR-UFM1 Δ C4) almost completely restores the p62-mediated autophagic degradation. Furthermore, p62 UFMylation enhances its interaction with LC3, augments autophagic flux, and eliminates pathogenic protein aggregates. Therefore, this work discovers a new PTM, UFMylation, on p62 and elucidates the mechanism by which p62 UFMylation modulates autophagic activity, paving a new avenue for exploring the autophagy-associated protein degradation and associated diseases at the post-translational modification level.
Keywords: p62/SQSTM1, UFM1, UFMylation, Quantitative proteomics, Autophagic degradation

P76: DCAF7 Modulates Ferroptosis and Hepatocellular Carcinoma Tumorigenesis via BMAL1-HIF1α-SLC7A11 Axis

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Ferroptosis is a newly identified cell death form characterized by iron-dependent lipid peroxidation and may be a potential strategy to improving the therapeutic treatment for cancer. Although DDB1- and CUL4-associated factor 7 (DCAF7) is a substrate receptor of a cullin 4-RING E3 ligase, it also functions as a scaffold protein. Bioinformatic analyses suggested that DCAF7 might be a key player in ferroptosis. However, it is unknown whether and how it regulates ferroptosis.
Here, we discovered that DCAF7 was highly expressed in hepatocellular carcinoma (HCC), and silencing DCAF7 significantly induced ferroptosis and inhibited the progression of HCC cells. Furthermore, we revealed that DCAF7 deficiency reduced intracellular GSH level and lipid droplet formation and promoted reactive oxygen

species (ROS) and malondialdehyde (MDA) production to induce ferroptosis by modulating the transcription of SLC7A11. Mechanistically, DCAF7 increased the interaction between deubiquitinating enzyme USP2 and the core clock protein BMAL1, promoted deubiquitination and increased the stability of BMAL1. In accordance with this, DCAF7 deficiency reduced BMAL1 protein level, inhibited HIF1α, its downstream genes SLC7A11, and other HIF1α downstream ferroptosis-related gene FABP3/7 and CP expression, decreased GSH production and lipid storage, and increased ROS and MDA production to enhance ferroptosis. Taken together, our findings identified DCAF7 as a novel suppressor for ferroptosis and elucidated the underlying molecular mechanism. Targeting DCAF7 may be a novel and promising therapeutic strategy for HCC.
Keywords: ferroptosis; DCAF7; BMAL1; IP-MS; HCC.

P77: Advanced Microfluidic Culture System Enhances Precision in Human Whole-Brain Organoid Modeling and Neurological Disease Studies

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Human whole-brain organoids derived from pluripotent stem cells provide invaluable in vitro models for simulating brain development, replicating the intricate structure and functionality of the human brain, and mimicking characteristics of both normal development and neurological diseases. Despite their potential, current brain organoid culture systems lack precise control over morphogenesis and functional maturation. In this study, we present a novel all-in-one microfluidic plate capable of generating high-fidelity and size-uniform brain organoids directly from human pluripotent stem cells within a single device, eliminating the need for transfer.
Our methodology integrates brain partition data acquired from studies on fetal brain tissue, as demonstrated by Sofi et al., and data from the Allen Human Brain Atlas. This comprehensive integration enables a detailed examination of organoid maturation processes and the distribution of brain regions. As a result, we can assess the fidelity of whole-brain organoid models in simulating the intricacies of the human brain under various culture conditions.
Using the whole-brain organoid model, we conducted comprehensive research by combining high-depth proteomics and transcriptomics. Our study delves into the neurotoxic effects of Tau fibrils, characteristic proteins in neurodegenerative diseases, on brain organoids. Our findings reveal that Tau fibrils induce neuron loss in brain organoids, mirroring observations in patients with neurodegenerative diseases. This suggests that exogenous Tau fibrils exert neurotoxic effects, providing important insights into the pathology of such diseases.
Key words: human whole-brain organoids; proteomics; transcriptomics; neurotoxic;

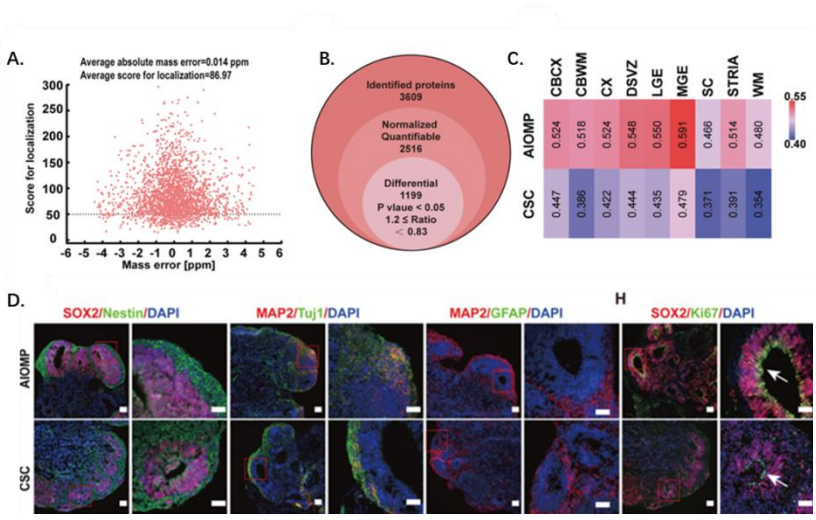

Fig. 1. All-in-one microfluidic plate improves neurogenesis in the brain organoids.

P78: The Inhibitory Effect and Mechanism of rLj-RGD4 Peptide on B16 Melanoma-bearing Mice

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ABSTRACT

rLj-RGD4 is a 6.27 kDa recombinant peptide with 4 RGD (Arg-Gly-Asp) motifs. Here we report the results of effect and mechanism of rLj-RGD4 on the mice xenografted with B16 melanoma in vivo. The results showed that, rLj-RGD4 inhibited B16 tumor growth in a dose-dependent manner. The maximum inhibition rates of tumor volume and weight in the high-dose group of 50.0 μg/kg were 89.26% and 86.51%, respectively. The staining results showed that the tumor tissue became looser and fibrotic, and the apoptotic cells in the tumor tissues were observed. Meanwhile, the results of Western Blot showed that rLj-RGD4 peptide induced apoptosis in tumor tissues through mitochondrial-regulated apoptosis signal pathway, death receptor signaling pathway and MAPK signaling pathway, which inhibited tumor growth in xenografted mice. The results on the action manner of rLj-RGD4 peptide on B16 cells indicated that rLj-RGD4 peptide could be internalized into the cells. The internalization pathway of rLj-RGD4 peptide was studied using protein internalization inhibitors such as filipin III, sodium azide (NaN3), and cytochalasin D. The results showed that the internalization of rLj-RGD4 peptide depended on macropinocytosis and actin rearrangement. In addition, the co-localization of rLj-RGD4 peptide with epidermal growth factor (EGFR) by immunofluorescence indicated that rLj-RGD4 peptide was able to interact with EGFR. Also, Western Blot results of EGFR-related signaling proteins further indicated that rLj-RGD4 peptide can inhibit the expression of EGFR and p-EGFR on the cell surface, and then reduced the expression of FAK, p-FAK, AKT, p-AKT and p-src, which eventually led to the inhibition on tumor growth.

In summary, rLj-RGD4 peptide possesses the significantly inhibitory effects on tumors of B16 melanoma-bearing mice in vivo, which can act as a tumor suppressor by internalizing itself and promoting EGFR internalization.
Key Words: rLj-RGD4, Anti-tumor, Cell apoptosis, internalization, EGFR

P79: A Novel Class of Kink-turn RNAs Functions in RNA Splicing
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ABSTRACT
A kink-turn (K-turn) is a three-dimensional RNA structure that exists in all three primary phylogenetic domains. In this study, we developed the RIP-PEN-seq method to identify the full-length sequences of RNAs bound by the K-turn binding protein 15.5K and discovered a previously uncharacterized class of RNAs with backward K-turn motifs (bktRNAs) in humans and mice. All bktRNAs share two consensus sequence motifs at their fixed terminal position and have complex folding properties, expression and evolution patterns. We found that a highly conserved bktRNA1 guides the methyltransferase fibrillarin to install RNA methylation of U12 small nuclear RNA in humans. Depletion of bktRNA1 causes global splicing dysregulation of U12-type introns by impairing the recruitment of ZCRB1 to the minor spliceosome. Most bktRNAs regulate the splicing of local introns by interacting with the 15.5K protein. Taken together, our findings characterize a class of small RNAs and uncover another layer of gene expression regulation that involves crosstalk among bktRNAs, RNA splicing and RNA methylation.
Key Words: Noncoding RNA, RNA splicing, RNA modification, Kink-turn, bktRNA

P80: RNA-binding Motif Protein RBM39 Promotes Gastric Cancer Cell Proliferation through An Oncogenic Splicing Switch
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ABSTRACT
Gastric cancer is a malignant gastrointestinal disease with high morbidity and mortality globally. The occurrence and progression of gastric cancer are associated with many factors, including abnormal alternative splicing of key genes. Recently, RBM39 has been discovered as a tumor biomarker and regulates alternative splicing

in several types of cancer. However, the function and the key alternative splicing event it modulates in gastric cancer are still mysterious.
In this work, bioinformatic analysis of TCGA database and immunoblotting analysis of patient tissue samples reveal that RBM39 is highly expressed in gastric cancer tissues and its high expression significantly reduces the overall patient survival rate. Cell line-based experiments disclose that RBM39 expression promotes while *RBM39* knockdown attenuates the proliferation and colony formation of gastric cancer cells. Mechanistically, using minigene and RT-PCR, we discover that RBM39 inhibits the exon 3 skipping to modulate the splicing of *MRPL33*. The long isoform MRPL33-L, which contains exon 3, but not the short isoform MRPL33-S, which lacks exon 3, significantly promotes the proliferation and colony formation of gastric cancer cells. Moreover, we also observe the increased percent-splice-in (PSI) of *MRPL33* in gastric cancer tissues and cell lines expressing RBM39. Genetic manipulation and pharmacological treatment with an RBM39 degrader indisulam demonstrate that RBM39 modulates the proliferation by regulating the splicing switch in *MRPL33* in gastric cancer cells and a xenograft mouse model. Our findings reveal that RBM39 regulates the oncogenic splicing of *MRPL33* and suggest that RBM39 may be a potential therapeutic target for gastric cancer.
Keywords: RBM39, MRPL33, gastric cancer, proliferation, alternative splicing

P81: Lipid Droplets are Critical Organelles in Inhibiting Type I IFN Response against SFTSV

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ABSTRACT.
Severe fever with thrombocytopenia syndrome phlebovirus (SFTSV), a novel bunyavirus with increasing incidents of infection and spatial distribution, is associated with high case mortality. The nonstructural protein (NSs) of SFTSV sequesters several factors involved in type I antiviral IFN signaling into NSs-induced cytoplasmic structures, resulting in the inhibition of IFN signaling and ISG expression. Lipid droplets (LDs) are known to accumulate during early stage of viral infection. Recent evidence has demonstrated that LDs play pivotal roles in facilitating the magnitude of the early antiviral immune response, specifically through the enhanced modulation of IFN following viral infection and the control of viral replication. Here, we report that LDs significantly contribute to SFTSV replication. Most importantly, we identify that the LDs are critical organelles in the SFTSV-inhibited type I IFN signaling pathway. The suppression of LD formation promotes the nuclear translocation and phosphorylation of STAT1, IRF3, and TBK1, corresponding with the reinduction of type I IFN and ISGs in SFTSV-infected cells. Using Alphafold2 and Heliquest, we preliminarily predicted the amphipathic helices in NSs, which can selectively target to LDs. Therefore, we hypothesize that NSs may mediate the inhibition of the type I IFN signaling pathway through LDs. Our findings provide a novel insight into the mechanisms of viral immune evasion.
Key Words: SFTSV; lipid droplet; innate immune response; NSs

P82: SMAD7 Expression in CAR-T Cells Improves Persistence and Safety for Solid Tumors

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Abstract. **Objective**: As the key protein in the TGF-β-SMADs negative feedback regulatory pathway, SMAD7 is expressed in CAR-T cells to exert a greater antitumor activity. **Methods**: The function of CAR-T cells was verified by several in vitro experiments included the specific lysis, antigen stimulated proliferation and continuous antigen exposure. The antitumor effect of CAR-T cells was evaluated by monitoring the progression of solid tumors in NSG mice. The infiltrating ability and phenotypic changes of CAR-T cells in tumor tissues were analyzed using patient-derived tumor organoids (PDOs). **Results**: SMAD7 CAR-T cells showed stronger killing ability and more durable proliferation ability in vitro. SMAD7 expressing attenuates the expression of TGF-β receptor type I in CAR-T cells, which consequently inhibits the phosphorylation of SMAD2/3. SMAD7 also induced the upregulation of background IκB levels, prevented nuclear translocation of p65, inhibit the activation of NF-κB pathway, thus significantly reduced the inflammatory cytokines produced by CAR-T cells in the process of tumor killing. SMAD7 expressed CAR-T cells persistently inhibited tumor growth and promoted the survival of tumor-challenged mice, also reduced the risk of cytokine storms caused by the release of inflammatory cytokines. In addition, SMAD7 coexpression enhanced the antitumor efficacy of CAR-T cells in PDOs of TGF-β-enriched, and the results showed superior infiltration, killing, proliferation and downregulated the expression of exhaustion markers in SMAD7-expressing CAR-T cells. Accordingly, we found a pronounced higher proportion of memory phenotype. **Conclusions**: SMAD7 expression in CAR-T cells could effectively relieve the inhibitory effect of TGF-β signaling, and significantly reduced the level of cytokine, accordingly improves persistence and safety for solid tumors.

Key words: TGF-β pathway; SMAD7; NF-κB pathway; CAR-T-cell therapy; Cytokine release syndrome (CRS)

P83: The TRIP6/LATS1 Complex Constitutes the Tension Sensor of α-catenin/ Vinculin at Both Bicellular and Tricellular Junctions

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ABSTRACT

Cell-cell mechanotransduction regulates tissue development and homeostasis. α-catenin, the core component of adherens junctions, functions as a tension sensor and transducer by recruiting vinculin and transducing signals that influence cell behaviors. α-catenin/vinculin complex-mediated mechanotransduction regulates multiple pathways, such as Hippo pathway. However, their associations with the α-catenin-based tension sensors at cell junctions are still not fully addressed. Here, we uncovered the TRIP6/LATS1 complex co-localizes with α-catenin/vinculin at both bicellular junctions (BCJs) and tricellular junctions (TCJs). The localization of TRIP6/LATS1 complex to both TCJs and BCJs requires ROCK1 and α-catenin. Treatment by cytochalasin B, Y-27632 and blebbistatin all impaired the BCJ and TCJ junctional localization of TRIP6/LATS1, indicating that the junctional localization of TRIP6/LATS1 is mechanosensitive. The α-catenin/vinculin/TRIP6/LATS1 complex strongly localized to TCJs and exhibited a discontinuous button-like pattern on BCJs. Additionally, we developed and validated an α-catenin/vinculin BiFC-based mechanosensor that co-localizes with TRIP6/LATS1 at BCJs and TCJs. The mechanosensor exhibited a discontinuous distribution and motile signals at BCJs. Overall, our study revealed that TRIP6 and LATS1 are novel compositions of the tension sensor, together with the core complex of α-catenin/vinculin, at both the BCJs and TCJs.

Key Words: Mechanotransduction; Bicellular junction; Tricellular junction; α-catenin/vinculin; TRIP6/LATS1; Tension sensor

P84: Kras-mediated Tight Junction Disassembly Promotes Tumorigenesis of Pancreatic Ductal Adenocarcinoma

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ABSTRACT.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most devastating cancers in the world with a five-year survival rate of less than 10%, due to early metastasis and lack of therapeutic treatment. Numerous studies focusing on pancreatic cancer have described the formation and progression of pancreatic cancer from both genetic and epigenetic perspectives. However, tight junction (TJ) integrity and its regulatory mechanism between tumor cells during the progression from pancreatic precursor lesions, mostly PanIN (pancreatic intraepithelial neoplasia), to PDAC has not been studied thoroughly. Here, we present a novel perspective of PDAC progression targeting the disassembly and reassembly of TJ. Our results demonstrate that the KrasG12D-mediated tight junction disassembly promotes tumorigenesis of PDAC. In further investigation, the critical genes involved in TJ disassembly process may serve as new therapeutic targets for PDAC.

Key Words: PanIN, PDAC, KRAS, tight junction

P85: Specific ECM Degradation Potentiates the Anti-tumor Activity of CAR-T Cell in Solid Tumors

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ABSTRACT
Objective: We generated a CAR-T cells targeting HER2 positive tumors, and added a synNotch receptor that can expression of ECM-degrading enzymes (MMP9, MMP12 and HPSE) regulated by MSLN located in tumor ECM to enhance the infiltration ability of CAR T cells in solid tumor tissues. Methods: Conventional CAR-T (Conv. CAR-T) and synNotch CAR-T cells were successfully built using lentivirus infection. The positive of CAR-T cells was about 50% and used for functional assessment. Transwell matrix degradation and 2.5D tumor spheres were used to simulate tumor extracellular matrix, and NSG mouse subcutaneous tumor bearing and tumor organoid model were used to evaluate the infiltration of CAR-T cells into solid tumors and the tumor therapeutic ability. Results: Compared with Conv. CAR-T cells, synNotch CAR-T cells can degrade dense ECM and efficiently kill HER2-positive tumor cells, as shown by significant destruction of stromal glue in Transwell and 2.5D tumor models and significantly increased apoptosis level of intracellular stromal tumor cells. In the NSG mouse tumor bearing treatment model, the number of CAR T cell infiltration in tumor tissue of mice treated with synNotch CAR T cell was significantly increased, tumor growth was significantly inhibited, and the survival period of mice was significantly prolonged. In addition, the infiltration of synNotch CAR-T cells in HER2-positive tumor organoids and the ability to kill tumor cells were significantly improved, and the proliferation and cell viability of CAR-T cells were significantly enhanced. Conclusions: This new type of CAR T cells can effectively overcome the dense ECM of solid tumors and exert better invasion and killing ability, providing a more effective way for the treatment of HER2-positive cancer.

Key Words：CAR-T cell therapy；synNotch receptor; ECM-degrading enzymes; tumor infiltration; HER2 positive tumor

P86: Regulatory Roles of Tissue-specific Genes in Liver Tumor Heterogeneity

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Liver cancer is the fourth leading cause of cancer-related death worldwide with significant heterogeneity features. Hepatocyte specific genes are functionally important in liver metabolism, whereas it remains limited whether and how they act in the cancer development and progression of hepatocellular carcinoma (HCC, the first most common liver cancer). We have revealed several hepatocyte specific genes with

important roles in inhibiting HCC initiation and progression. The hepatocyte-specific miRNA, miR-192-5p, was highly abundant in hepatocytes, accounting for 20% of miRNA reads in hepatocytes. Via the axis of TP53 mutation/ mir-192 promoter hyper-methylation/ miR-192-5p silencing/deregulation of a group glycolysis regulatory genes, miR-192-5p significantly suppressed the stemness features of HCC cells. Moreover, a member of cytochrome P450 family, CYP39A1, as a hepatocyte-specific gene, significantly inhibited HCC formation in vitro and in vivo. Its suppressor role in HCC did not rely on its known P450 enzyme activity but its C-terminal region, by which CYP39A1 impeded the transcriptional activation activity of c-Myc, leading to a significant inhibition of hepatic carcinogenesis. In intrahepatic cholangiocarcinoma (iCCA), the second most common liver cancer, we focused on genes with specific high level in iCCA tumor tissue compared to normal bile duct and non-tumor liver tissue. Laminin subunit gamma-2 (LAMC2) was identified. Among 26.3% of iCCA tissues, LAMC2 gene was amplified, contributing to its over-expression. Silencing LAMC2 significantly blocked tumor formation in orthotopic iCCA mouse models. Mechanistically, it promoted the EGFR protein translation via LAMC2/ BiP/ unglycosylated EGFR axis in endoplasmic reticulum (ER), contributing to an activated EGFR signaling. Consistently, LAMC2-high iCCA patients had poor prognosis and LAMC2-high iCCA cells were highly sensitive to EGFR tyrosine kinase inhibitors (TKIs) treatment both in vitro and in vivo. All of these findings indicated the important roles of tissue specific genes in liver cancer and its heterogeneity features. Now, we continue to perform the comprehensive studies to explore the whole spectrum of these tissues specific genes in liver cancer and their roles in liver cancer development and progression.

P87: Co-option of Tumor Clonal Evolution and Immunosuppressive Myeloid Cells at Single Cell Resolution

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B lymphocytes are essential mediators of humoral immunity and play multiple roles in human cancer. In the tumor microenvironment (TME), tumor infiltrating B cells display considerable functional heterogeneity, broadly spanning naïve B cells, memory B cells, germinal center B cells, and antibody-secreting cells, primarily on the basis of their immunophenotype. To decode the functions of tumor-infiltrating B cells, we generated a B cell blueprint encompassing single-cell transcriptome, B cell–receptor repertoire, and chromatin accessibility data across 20 different cancer types. B cells harbored extraordinary heterogeneity and comprised 15 subsets, which could be grouped into two independent developmental paths (extrafollicular versus germinal center). Tumor types grouped into the extrafollicular pathway were linked with worse clinical outcomes and resistance to immunotherapy. The dysfunctional extrafollicular program was associated with glutamine-derived metabolites through epigenetic-metabolic cross-talk, which promoted a T cell–driven immunosuppressive program. These data suggest an intratumor B cell balance between extrafollicular and germinal-center responses and suggest that humoral immunity could possibly be harnessed for B cell–targeting immunotherapy.

P88: Genome-wide CRISPR Screening for the Identification of Psoriasis Regulating Genes Using HaCaT Cell Models

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Psoriasis is an inflammatory skin disease that occurs in many forms, such as erythema, induration, pustules, and itchy patches of skin. Keratinocytes play an important role in amplifying psoriasis inflammation, which is characterized by the hyperproliferation and abnormal differentiation of keratinocytes. However, the underlying mechanisms of abnormal keratinocytes behaviors in psoriasis are still incompletely understood. To identify genes involved in the regulation of psoriasis pathogenesis, we applied genome-wide CRISPR screening for cell survival genes using psoriasis HaCaT cell models. We first established the psoriasis HaCaT cell model by stimulation with TNF and IL17A for 24 hours. This mimics the inflammatory responses of psoriasis *in vivo*, including the increased expression of key cytokines such as *CCL20*, the antimicrobial peptide like *S100A8*, as well as the NFκB pathway genes. The genome-wide CRISPR screening identified known pathway gene like NFκB pathway and novel genes, as well as some miRNAs that might regulate cell survival in control group at day 12 compared to day 0. The enriched NFκB signaling pathway indicates our genomic wide-CRISPR screening for cell survival at different conditions was successfully performed. We will further validate the functions of possible candidate genes both in psoriasis HaCaT cells and mouse genetic models by knocking out target genes.
Key words: Psoriasis; CRISPR screening; Cell survival

P89: Loss of LZP Protects against Atherosclerosis by Triglyceride Lowering
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A liver-specific zona pellucida domain-containing protein (LZP), also named OIT3, has been shown to be mainly expressed in human and mouse liver. In hepatocyte, Lzp interacted with apolipoprotein B (ApoB) to attenuate its ER-associated degradation. Both hepatocytic and plasma ApoB levels were decreased in *Lzp* deletion mice. *Lzp* deletion inhibited very low-density lipoprotein (VLDL) secretion, leading to hepatic triacylglycerol (TG) accumulation and lower serum TG levels. We observed that both mRNA and protein levels of LZP were significantly elevated in the mice fed a high-fat diet, implying that LZP can serve as a response factor to lipid burden. Consistently, the mRNA levels of LZP in livers from obese patients were also significantly higher than those of healthy controls. Deletion of *Lzp* reduced plasma TG, macrophage and lipid accumulation in the intima of aorta, reducing the development of atherosclerosis in Western-diet-fed *Apoe*$^{-/-}$mice. Our study uncovers LZP as a player in the VLDL secretion and lipid homeostasis, it also shows that deletion of LZP can lower plasma TG and protects against atherosclerosis.
Key Words: LZP, triacylglycerol, Atherosclerosis

P90: The Abscisic Acid Induced Cargo Loading in Exosomes
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ABSTRACT
Exosomes are nanoscale extracellular vesicles secreted by cells that carry proteins, lipids, nucleic acids, and other substances. They participate in intercellular communication, regulating gene expression and cellular functions. Due to their biocompatibility, high stability, and low immunogenicity, exosomes are widely regarded as potential drug delivery vehicles, transporting bioactive "cargo" between cells. However, there is currently a lack of understanding and efficient methods for the specific loading of contents into exosomes. We have developed a method leveraging the interaction between PYL1 and ABI1 proteins induced by abscisic acid (ABA) and the enrichment of the lipid-anchored protein BASP1 on the inner side of exosomes. This involves constructing fusion proteins by combining the target protein with ABI1 and BASP1 with PYL1, allowing ABA to induce the specific loading of target proteins into exosomes. Using fluorescence microscopy, luciferase assays, and Western blot experiments, we confirmed that various target proteins could be efficiently and specifically loaded into exosomes produced by HEK293T cells. Furthermore, these exosomes successfully delivered the target proteins to recipient cells, demonstrating that the ABA-induced exosome loading and delivery system can effectively load and deliver target proteins.
Key Words: Exosome, Abscisic Acid, BASP1.

P91: Generate A KDM5D Deleted Human Embryonic Stem Cell Line
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Objective: KDM5D is a gene encoding lysine-specific demethylase, located in the azoospermia factor b (AZFb) region on the Y chromosome. Deletion of the AZFb region is associated with oligoasthenoteratozoospermia (OAT syndrome), a common cause of male infertility. The specific mechanism by which KDM5D gene deletion in the AZFb region causes oligoasthenospermia remains unclear. This study utilized CRISPR/Cas9 gene editing technology to construct KDM5D-deleted human embryonic stem cells, providing a foundation for investigating the molecular mechanism of oligoasthenospermia caused by KDM5D deletion.

Materials and Methods: A pair of sgRNAs was designed and delivered into a human embryonic stem cell line via electroporation, along with Cas9. The deletion of the KDM5D region was confirmed by PCR. Immunofluorescence was employed to verify the pluripotency and differentiation capabilities of the KDM5D-deleted embryonic stem cells.
Results: Approximately 35kb of KDM5D genomic DNA was successfully deleted using CRISPR/Cas9 genome editing technology. Off-target effects of the sgRNAs were assessed, and none were detected. The expression of human embryonic stem cell markers, including surface antigens SSEA-4 and TRA-1-60, and transcription factors OCT4 and SOX2, was observed in KDM5D-deleted cell lines. Following the induction of differentiation in KDM5D knockout cell lines, the expression of specific transcription factors PAX6 and Nestin (ectoderm), SOX17 and FOXA2 (endoderm), and Brachyury and CXCR4 (mesoderm) was detected.
Conclusion: Human embryonic stem cells with a KDM5D deletion and a normal karyotype were successfully obtained. This provides a reference and basis for further research into the molecular mechanisms underlying oligoasthenospermia.
Key word: KDM5D，OAT syndrome，human embryonic stem cell

P92: Action Mechanism of Hyperoside Inhibiting Invasion and Migration of Gastric Cancer Cells Based on Regulating IL－6 /STAT3 Signaling Pathway

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ABSTRACT
To study the effect and mechanism of hyperoside （Hyp）on inhibiting the invasion and migration of MGC－803 gastric cancer cells. Results： Compared with those in the control group， the viability of MGC－803 cells decreased， and the migration and invasion of MGC－803 cells were significantly reduced after Hyp intervention（P < 0. 01）. Compared with those in the Hyp group， the migration and invasion of MGC－803 cells were significantly increased in the Hyp plus IL－6 group（P < 0. 01）. Hyp decreased the level of IL－6 and significantly inhibited the phosphorylation of STAT3 mediated by IL－6（P < 0. 01）. Conclusion：Hyp can inhibit the invasion and migration of MGC－803 gastric cancer cells， and the mechanism may be related to regulating IL－6/STAT3 signaling pathway.
Key Words:Hyperoside； Gastric cancer； Invasion； Migration

P93: LncRNA TUG1 in Cancer Cells Regulates the Antitumor Response of CD8+ T Cells and Macrophages

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ABSTRACT:
Immune checkpoints play critical roles in crosstalk of tumor cells and adaptive/innate immune cells, and immune checkpoint inhibitors have achieved promising clinical effects in treating a wide range of cancers. The long non-coding RNA taurine-upregulated gene 1 (TUG1) is upregulated in hepatocellular carcinoma (HCC). However, how TUG1 is upregulated and the effects on tumor immune evasion are incompletely understood. Here, we demonstrated that METTL3-mediated m6A modification led to TUG1 upregulation. Knockdown of TUG1 inhibited tumor growth and metastasis, increased the infiltration of $CD8^+$ T cells and M1-like macrophages in tumors, promoted the activation of $CD8^+$ T cells through PD-L1, and improved the phagocytosis of macrophages through CD47. Mechanistically, TUG1 regulated PD-L1 and CD47 expressions by acting as a sponge of miR-141 and miR-340, respectively. Meanwhile, TUG1 interacted with YBX1 to facilitate the upregulation of PD-L1 and CD47 transcriptionally, which ultimately regulated tumor immune evasion. Clinically, TUG1 positively correlated with PD-L1 and CD47 in HCC tissues. Moreover, the combination of TUG1-siRNA therapy with a PD-L1 antibody effectively suppressed tumor growth. Therefore, we have revealed the mechanism of TUG1 in regulating tumor immune evasion, which could inform existing strategies targeting TUG1 for enhancing HCC immune therapy and drug development.
Key Words: Hepatocellular carcinoma; LncRNA TUG1; PD-L1; CD47; Immune escape.

P94: Synergistic Antimicrobial Mechanism of the Ultra-short Antimicrobial Peptide R3W4V with A Tadpole-like Conformation

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ABSTRACT.
Antimicrobial peptides (AMPs) are promising candidates in combating multidrug-resistant microorganisms because of their unique mode of action. Among these peptides, ultra-short antimicrobial peptides (USAMPs) possess sequences containing less than 10 amino acids and have some advantages over traditional AMPs. However, one of the main limitations of designing novel and highly active USAMPs is that their mechanism of action at the molecular level is not well known. In this article, we report the antimicrobial mechanism of the USAMP verine (R_3W_4V) with a tadpole-like conformation and high antibacterial activity against *Escherichia coli*. Here, by using well-tempered bias-exchange metadynamics simulations and long-time conventional molecular dynamics simulations, we evaluated whether verine exhibits the same antimicrobial mode of action as that of traditional AMPs. The single verine-membrane system exhibited a relatively flat surface with multiple shallow

minima separated by very small energy barriers and adopted highly dynamic structural ensembles, wherein the verine peptide is localized on the surface of the membrane or inserted into the center of the membrane. Although the verine sequence is very short, it can still exist briefly in the center of the cell membrane in a transmembrane state. As the concentration of verine increased, the transmembrane conformation was relatively stabilized in the membrane center or proceeded toward the membrane bottom. The lipid bilayer membrane showed relatively large deformation, including the phospholipid head groups embedded inside the lipid hydrophobic center, accompanied with a flip-flop of some lipids. The results showed that verine may use several mechanisms to disrupt the cell membrane, including the carpet model with the binding of verine to the membrane surface and covering it; the toroidal pore model with insertion of verine into the membrane and formation of a transmembrane peptide; and the lipid flip-flop model. Simulation results indicated that verine has a specific mechanism of action different from that of traditional AMPs. Based on this antimicrobial mechanism of verine, we can design new high-potential USAMPs by reducing the free energy value and enhancing the structural stability of the transmembrane state.

Key Words: ultra-short antimicrobial peptide; antimicrobial mechanism; molecular dynamics simulation; peptide-membrane interaction

P95: MiR-592Targets Glut1 to Regulate Astrocyte Energy Metabolism to Inhibit GBM Malignant Progression

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Glioblastoma multiforme (GBM) is one of the most common primary brain tumors in adults, characterized by a highly infiltrative growth pattern, necrosis, and microvascular proliferation. The pathophysiology of GBM can be influenced by deteriorating glial cells in the brain. Astrocytes, an abundant type of glial cells, are the key cells providing metabolic support in the central nervous system (CNS). In our previous study, we report that microRNA-592 (miR-592) knockout represses IPC-to-mature neuron transition and lead an abnormal increase in astrocytes. According to clinical studies, there was a correlation between increased miR-592 expression levels after treatment and the prognosis of GBM patients. However, the influence of miR-592 in the regulation of GBM in *vivo* remains largely unexplored. An orthotopic glioma model for mouse was generated in this study, and the GBM organoids were used to identify the role of miR-592 in GBM malignant progression. The results showed that miR-592 regulates astrocyte energy metabolism by targeting

glucose transporter type 1 (Glut1). Furthermore, we demonstrate that miR-592 inhibits the progression of GBM malignancy by regulating the energy metabolism of astrocytes by targeting Glut1. It can be exploited as a potential target for novel therapies by regulating metabolic crosstalk between astrocytes and GBM.
Key Words: GBM, astrocytes, energy metabolism, miR-592, Glut1

P96: Palmitoylation Facilitates Gasdermin D-mediated Pyroptosis and Cytokine Release

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ABSTRACT
Gasdermin D (GSDMD)-mediated pyroptotic cell death drives inflammatory cytokines release and downstream immune response upon inflammasome and inflammatory caspases activation, which plays an important role in inflammation and host defense. Cleaved GSDMD-NTD induces self-oligomerization and pore-formation upon phospholipids involved, then triggers membrane rupture and cytolysis, however, the biological mechanisms of its membrane translocation and insertion, and pore-formation are still not well understood. Here we report that residue Cys191 of GSDMD is S-palmitoylated and this post-translational modification regulates the dynamic process of pyroptosis-mediated by GSDMD. Mutation of Cys191 or treatment with palmitoylation inhibitors 2-BP or CMA inhibits pyroptotic cell death, IL-1β secretion and membrane localization. By contrast, co-expression of GSDMD with palmitoyltransferases enhanced the palmitoylation of GSDMD and pyroptotic cell death. Other gasdermin family members are also S-fatty acylated, which suggest that S-palmitoylation or S-fatty acylation exists extensively and plays a critical role in regulating subcellular trafficking and membrane association of gasdermin proteins and pyroptosis.

P97: Differential Susceptibility of Pancreatic α- and β-cells to CVB Infection and Its Implication in Type 1 Diabetes

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Type 1 diabetes mellitus (T1DM) is a metabolic disorder caused by autoimmune attack of pancreatic β-cells, which leads to loss of β-cell function and secretion of insulin. Although the autoimmune disorder affects both α- and β-cells and renders them dysfunctional, only β-cells die while α-cells survive even though both cell types are hormone-producing and have a similar embryonic origin. Genetic factors are known to play a role in certain individuals and viral infections, particularly enterovirus infection, is suspected to be an etiology. Prospective epidemiological studies have strongly associated the persistence of enteroviruses, especially coxsackievirus B (CVB), with the appearance of islet autoantibodies and an increased risk of T1DM. Our evidence suggests that α- and β-cells responded to the initial

inflammatory and immune assault distinctly, with α-cells mounting a better response to viral infections and deleterious immune mediators. In addition, enteroviruses exhibited differential tropism to the pancreatic islets because β cells expressed higher level receptors for these viruses. We showed that upon CVB infection, α-cells mounted significantly stronger innate immune response than β-cells, resulting in resistance of α-cells to coxsackievirus infection. The CVB RNA transcription and protein synthesis in β-cells were reduced when co-cultured with α-cells in trans-well in vitro infection assay, suggesting virus-inhibiting soluble factors secreted by α-cells. Further investigation is being conducted to identify the nature of the soluble factors to understand the mechanisms of differential resistance of α- and β-cells to CVB infection. Implication of the CVB association with T1DM and the virus inhibiting factors secreted by α-cells will be discussed.

Key Words: Type 1 diabetes mellitus，coxsackievirus B;，islet β cell，autoimmune

P98: Identifying Formaldehyde-Induced Epigenetic Adducts with Isotope-Labeled Pseudo Neutral Loss Screening

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ABSTRACT

Exposure to genotoxic agents induce DNA lesions, leading to activation of DNA damage response and repair pathways. This process can cause genomic instability and contribute to the development of various diseases. Comprehensive profiling of DNA adducts is essential for understanding the interactions between cells and their environment and for developing interventions for disease prevention and aging. Although liquid chromatography-high resolution mass spectrometry (LC-HRMS) combined with DNA adductomics has been widely used to identify DNA damage, it faces limitations in terms of sensitivity and cost. Here, we developed an Isotope-Labeled Pseudo Neutral Loss (ILPNL) screening method for the highly sensitive and accurate detection of DNA adducts. To enhance the simultaneous detection of various deoxyribonucleosides, we optimized liquid chromatography and mass spectrometry parameters. By incorporating U-13C glucose to label genomic deoxyribose and leveraging neutral losses during mass spectrometry collisions, we detect formaldehyde-induced cytosine and modified cytosine adducts, including 4hmC, 5m4hmC, and 4hm5hmC, in genomic DNA. To mitigate the instability of these adducts, we developed a protective derivatization strategy using β-mercaptoethanol, which facilitated the quantification of intracellular levels of these adducts. With this approach, we detected increased genomic levels of 4hmC and 5m4hmC in cells lacking the formaldehyde-metabolizing enzymes ALDH2 and ADH5. Additionally, these lesions were found to accumulate further in cells lacking Smug1. In summary, our study introduces a highly sensitive method for identifying DNA damage and provide insight into the mechanisms by which formaldehyde exposure induces epigenetic alterations.

Key Words: Formaldehyde, DNA modification, DNA adduct, LC-MS

P99: Reactivating Tumor Suppressive Function of Wild-type p53 by Disulfiram Metabolite

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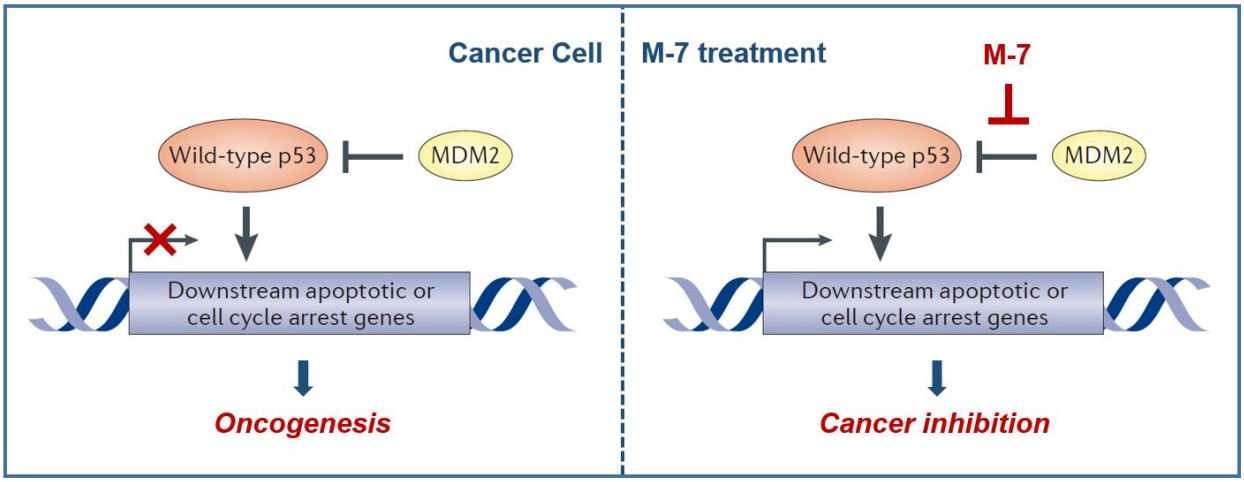
Wild-type p53 (wtp53, encoded by TP53 gene), accounted for about 50% of all cancer cases, significantly regulates cell proliferation and survival via transcriptional and/or non-transcriptional mechanisms, acting as one of the most powerful tumor suppressors within cells. But since the mechanisms including overexpression of MDM2, the E3 ligase of wtp53, wtp53 deficiency is generally occurred in diverse cancer types. Various approaches, such as small-molecule MDM2 inhibitors (e.g., nutlin-3a and Idasanutlin), have been developed to reactivate the anti-cancer function of wtp53. However, limited clinical benefits for patients and severe hematotoxicity and gastrointestinal toxicities led to the failures of these candidates in multiple clinical trials. Thus, developing therapeutic strategy targeting wtp53 is urgently needed. Herein, when performing metabolic analysis of disulfiram, an approved drug for alcoholism treatment, we accidentally discovered M-7 (a secondary metabolite of isulfiram, confirming by 13C-isotope labeling) that specifically suppressed tumors cells without affecting normal cells. To identify the target of M-7, we synthesized Biotin-labelled M-7 (Biotin-M-7), and found that MDM2 was selectively recognized by Biotin-M-7 in Streptavidin-Biotin-based immunoaffinity capture plus nano LC-MS/MS analysis. Biochemical studies indicated that M-7 directly occupied p53-interacting pocket of MDM2 mainly through the hydrogen bond between MDM2's His96 and M-7's hydroxyl group to disturb p53-MDM2 interaction, stabilizing p53 by blocking its proteasomal degradation. Moreover, M-7 triggered the tumorsuppressive signals of wtp53 to induce cell cycle arrest and apoptosis, and specifically inhibited cancer cells expressing wtp53, rather than the cells bearing p53 mutations or deletion. Notably, M-7 administration potently impeding tumor growth in multiple mice models including transgenic, drug-induced and patient-derived tumor xenograft models, and exhibited an improved drug safety over the reported MDM2 inhibitors. Collectively, this work provided an attractive lead compound for the treatment cancers with wtp53 as well as a potential molecular tool to probe p53 biology.



P100: Trained Immunity of Intestinal Tuft Cells Enhances Host Defense against Enteroviral Infections in Mice

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Innate immune cells have been acknowledged as trainable in recent years. While intestinal tuft cells are recognized for their crucial roles in the host defense against intestinal pathogens, there remains uncertainty regarding their trainability. Enterovirus 71 (EV71), a prevalent enterovirus that primarily infects children but rarely infects adults. We showed a significant expansion of intestinal tuft cells in an EV71-infected mouse model, which was associated with EV71-induced interleukin-25 (IL-25) production. Further, we found that IL-25 pre-treatment of 2-week old mice enabled tuft cells to acquire immune memory. This was evidenced by the more rapid expansion and stronger response of IL-25-trained tuft cells in response to EV71 infection of 6-week old mice than that of naïve tuft cells in mice without prior IL-25-treatment. Interestingly, IL-25-trained intestinal tuft cells exhibited anti-enteroviral effect via producing a higher level of IL-25. Mechanistically, IL-25 treatment upregulated spermidine/spermine acetyl-transferase enzyme (SAT1) expression, caused intracellular polyamine deficiency, and resulted in the inhibition of enterovirus replication. In summary, our study showed that tuft cells can be trained by IL-25, which supports faster and higher IL-25 production in response to EV71 infection, and exhibits anti-enteroviral effect via SAT1-mediated intracellular polyamine deficiency. Given IL-25 can be induced by multiple gut microbes during human growth and development, including shifts in gut flora abundance, which may partially explain the differential susceptibility to enteroviral infections between adults and children.
Key words: trained immunity, tuft cells, enterovirus, IL-25

P101: A Comparative Study on the Anti-tumor Effects of Polysaccharides from Stems, Leaves, and Flowers of Dendrobium Officinale

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Dendrobium officinale Kimura et Migo, a perennial herb from the Orchidaceae family, is well known for its benefits in strengthening the functions of the stomach and intestines through a long-term use based on the practice of Traditional Chinese medicine (TCM). Recently pharmacological studies have demonstrated that D. officinale possesses anti-tumor activity, and the polysaccharide is one of its main active components. Currently, the different parts of D. officinale, including stems, leaves, and flowers, have been approved as edible materials for consumption. However, it remains unclear whether all these parts own effective anti-tumor properties. The present study aimed at comparing the anti-tumor activities of total polysaccharides extracted from the stems, leaves, and flowers of D. officinale using a tumor-bearing mouse that was established with murine colon carcinoma CT26.WT cells through determining the proliferation, apoptosis and angiogenesis of tumor as well as the immune cells in the tumor microenvironment (TME). The results showed that oral administration of polysaccharides from stems, leaves and flowers could inhibit tumor proliferation and angiogenesis, promoted tumor apoptosis, and altered the balance of immune cells within TME in tumor-bearing mice with varying degrees. These changes contributed to tumor growth inhibition, and stem polysaccharides

demonstrated the strongest anti-tumor activity in a dose-dependent manner. The tumor inhibition rate of stem polysaccharides, after oral administration with 400 mg/kg/day for 30 days, reached 69.15%, which was close to that of the positive control (5-fluorouracil). These findings indicate that the stem is the most effective part for anti-tumor purpose of D. officinale, providing a foundation for the rational use of its stems, leaves and flowers.

Key Words: Dendrobium officinale, Different parts, Polysaccharides, Anti-tumor activity

P102: Schisandra Chinensis Polysaccharides Alleviate Parkinson's Disease via Effectively Activating MCL-1 Expression Regulation of Autophagy Signaling

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ABSTRACT.

Parkinson's disease (PD) is a neurological condition that worsens with time, characterized by tremors and bradykinesia. The disease is a common neurologic ailment, which is a neurodegenerative illness with the greatest rate of growth. The symptoms of PD are exacerbated by various changes in mitophagy. The significance of MCL-1 in PD is crucial for the process of mitochondrial autophagy. Because of the pathogenesis and the drug side effects of PD, there is still a lack of therapeutic drugs with satisfactory efficacy. In the study, the purified neutral polysaccharide fraction, namely SBP-1, was isolated and characterized from Schisandra chinensis (Turcz.) Baill (S. chinensis) crude polysaccharides, which the main chain of SBP-1 was Glcp-(1→, →4)-Glcp-(1→ and →4,6)-Glcp-(1→. And to reveal the effect of SBP-1 on the PD model and their potential underlying molecular mechanism in vivo and in vitro. The results showed SBP-1 administration improved behavioral deficits, increased tyrosine hydroxylase-positive cells, attenuated loss of dopaminergic neurons in MPTP-exposed mice, and reduced cell death induced by MPP+. The MCL-1 was identified as the target of SBP-1 by the combination of docking-SPR-ITC, WB, and IF experiments. Subsequently, the study showed that SBP-1 could target MCL-1 to enhance autophagy with a change in the apoptotic response, which was further demonstrated by a change in LC3/P62, PI3K/AKT/mTOR, and possesses a change in the expression of BCL2/BAX/Caspase3. These results demonstrate that SBP-1 may protect neurons against MPP+ or MPTP-induced damage in vitro and in vivo through enhancing autophagy. In summary, these findings indicate that SBP-1 and S. chinensis show potential as effective candidates for further investigation in the prevention and treatment of PD and associated illnesses, specifically through autophagy apoptotic-based mechanisms.

Key Words: Schisandra chinensis (Turcz.) Baill, Polysaccharide, Parkinson’s disease, Autophagy

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P103: Metabolomics Study on the Effect of the CDK4/6 Inhibitor

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ABSTRACT
Palbociclib, a CDK4/6 inhibitor, is increasingly recognized for its potential to impact tumor metabolism beyond its primary cell cycle inhibition mechanism. This study investigated the metabolic effects of Palbociclib on Hela cervical cancer cells, aiming to provide insights into its mechanism of action and potential for drug development.
The study employed a controlled in vitro approach, treating Hela cells with Palbociclib at specific concentrations and durations. Western blot analysis confirmed the drug's efficacy, followed by high-resolution liquid chromatography-mass spectrometry to analyze metabolic changes.
Metabolomics analysis revealed significant alterations, particularly in the Fatty acid biosynthesis and Dopaminergic synapse pathways. Key findings included increased levels of PC (14:0e/22:1) and Lithocholicacid, while expression of Cys-Gly was significantly reduced. These changes suggest that Palbociclib may disrupt lipid metabolism and neurotransmitter signaling, ultimately contributing to its anti-tumor effects.
This research highlights the importance of studying the metabolic consequences of small molecule drugs like Palbociclib. Understanding these metabolic changes can lead to the development of new strategies to overcome drug resistance and enhance therapeutic efficacy. The study provides a valuable dataset of drug-related metabolites, paving the way for further investigation into the complex interplay between small molecular drugs and tumor metabolism.
Key Words: Metabolomics, Inhibitor, Tumor Metabolism

P104: Cytoplasmic Aggregation of TDP-43 Induced by EV71 Exacerbates the Risk of ALS

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ABSTRACT.
Hand, foot and mouth disease (HFMD) caused by enterovirus 71 (EV71) as the main pathogen has had many outbreaks worldwide, especially in the Asia-Pacific region. And from 2008 to 2012, about 80% of severe cases and 93% of deaths of HFMD patients in China were caused by EV71 infection. EV71 infection more likely to cause

neurological symptoms, including encephalitis, meningitis, and polio-like paralysis. Do children recovering from EV71 infection develop neurologic sequelae and are they at increased risk for amyotrophic lateral sclerosis (ALS)? Our study observed that EV71 infection of mouse motor neuron cells NSC-43 induced nuclear translocation and aggregation in the cytoplasm of TDP-43, a classical pathological protein of ALS. Next, we constructed a mouse model of viral encephalitis by intracranial injection of EV71 and found cytoplasmic aggregation of TDP-43 in motor neuron cells in the brain and spinal cord. Cytoplasmic aggregation of TDP-43 was also observed in autopsy brain slices of patients with severe encephalitis with EV71. In addition, the presence of TDP-43 was still observed in the brain of mice recovering from infection with the EV71, and the morphology of motor neurons was altered after 90 days. Further mechanistic investigations showed that the C-terminus of TDP-43 aggregates in the cytoplasm in response to EV71 infection, and EV71 protease 2A is an important factor in the cytoplasm translocation of the C-terminus of TDP-43. Taken together, our study suggested that EV71 infection of motor neurons triggers the aggregation of TDP-43 C-terminus in the cytoplasm, which exacerbates the risk of ALS in later stages.
Key Words: EV71, TDP-43, ALS

P105: Structure-Guided Discovery of Protein and Glycan Components in Native Mastigonemes

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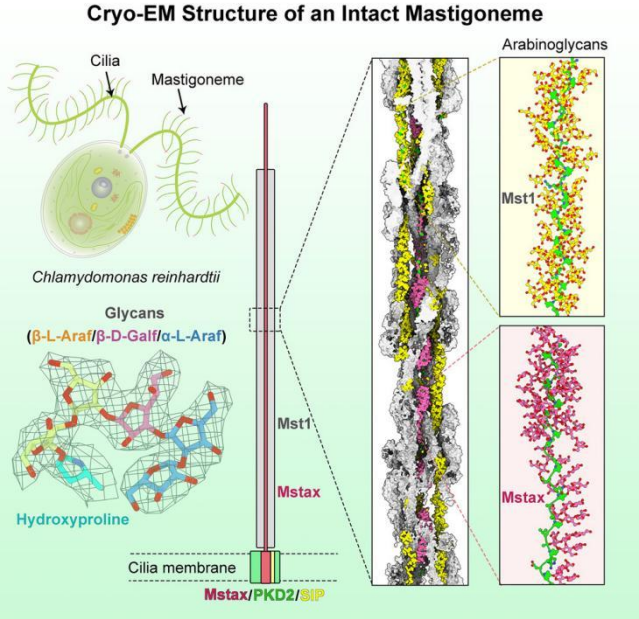
Sugar is essential for life: Carbohydrate molecules represented by glucose are one of the main energy sources for cell metabolism. The cell wall in plants is composed of cellulose. Glycosylation is also one of the major forms of Posttranslational modifications (PTM), which can significantly expand the functional diversity of proteins. However, the intrinsic stereochemical complexity of glycan molecules has hindered the systematic study of them, and the lack of information on the sugar-containing structure has severely limited human understanding of the functions of this important group of biomolecules. In this study, we used a combination of biophysics, cell biology, and bioinformatics methods to elucidate the molecular mechanism of glycans in building biomacromolecules. Plants and algae are known to possess a unique form of Hyp O-glycosylation in which the glycan module consists mainly of arabinose with a small amount of galactose. This Hyp O-glycosylation dependent form is fundamental for plants and algae to exercise normal life activities. Previously, no structural information about glycan-mediated assembly of bio-architectures was available. In this study, a density of more than 1000 sugar molecules was clearly observed, which is the largest complex structure containing

sugar molecules. By analyzing the interaction between polysaccharides and proteins, this study revealed the key role of arabinoglycans in the assembly of biological structures, providing important clues to understand the role of structural glycans in life processes, and reflecting the transformation of modern structural biology from a tool for structural confirmation to a tool for de nove discovery.



Tables and Figures should be included in the text.

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P106: Structural Basis for the Exonuclease-Mediated Termination of mRNA Transcription

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Termination is the last stage of gene transcription, during which RNA polymerases stop RNA extension, release RNA transcript, and dissociate from the DNA template. In eukaryotes, the pathway for transcription termination of mRNAs is conserved in yeast, mammals, and plants, which requires prior cleavage at the polyadenylation site (PAS) of the pre-mRNAs by the endonuclease subunit of the cleavage and polyadenylation-cleavage factor complex to release mRNA and subsequent cleavage of the Pol II-associated RNA by a 5'-3' exonuclease (Rat1 in yeast, XRN2 in humans, and XRN3 in plants). Here, we report cryogenic electron microscopy (cryo-EM) structures of yeast Pol II pre-termination transcription complexes bound to Rat1, as well as plant Pol II pre-termination transcription complexes bound to XRN3. Our structures show that the 5'-3' exonucleases (Rat1 and XRN3) dock at the Pol II-stalk domain, displace the elongation factor SPT5, shield the RNA exit channel of Pol II, and guide the nascent RNA toward its active center. We provide the structural

mechanism for the exonuclease-mediated termination of mRNA transcription by Pol II in yeast and plant.

P107: Pharmacological Suppression of the CD73 Proteolytic Axis Revives Antitumor Immunity against Immune-Suppressive Breast Cancers

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Despite widespread utilization of immunotherapy, challenge to treat immune-cold tumors needs to be resolved. Multiomic analyses and experimental validation identified the OTUD4-CD73 proteolytic axis as a promising target in treating immune-suppressive triple negative breast cancer (TNBC). Mechanistically, deubiquitylation of CD73 by OTUD4 counteracted its ubiquitylation by TRIM21, resulting in CD73 stabilization that inhibits tumor immune responses. We further demonstrated the importance of TGF-β signaling for orchestrating the OTUD4-CD73 proteolytic axis within tumor cells. Spatial transcriptomics profiling discovered spatially resolved features of interacting malignant and immune cells pertaining to expression levels of OTUD4 and CD73. In addition, ST80, a newly developed inhibitor, specifically disrupted proteolytic interaction between CD73 and OTUD4, leading to reinvigoration of cytotoxic $CD8^+$ T cell activities. In preclinical models of TNBC, ST80 treatment sensitized refractory tumors to anti-PD-L1 therapy. Collectively, our findings uncover a novel strategy for targeting immunosuppressive OTUD4-CD73 proteolytic axis in treating immune-suppressive breast cancers with the inhibitor ST80.

P108: *KCTD10* p.C124W Variant Contributes to Schizophrenia by Attenuating LLPS-Mediated Synapse Formation

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Abstract

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

Key words: neuropsychiatric disorder, KCTD10, liquid-liquid phase separation (LLPS), synaptic abnormalities.

P109: α-Glucosidase Inhibitory Activity of 1,3,4-Thiadiazole Derivatives of 3-Aminopyridin-2(1*H*)-ones

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ABSTRACT.
One of the main therapeutic approaches to combatting diabetes is the slowing of postprandial hyperglycemia by reducing glucose absorption through the inhibition of enzymes that hydrolyze carbohydrates in the gastrointestinal tract. α-glucosidase is a key carbohydrate hydrolase that regulates blood glucose levels by specific hydrolysis of the 1,4-α-glycosidic bond, forming α-glucose. Inhibition of α-glucosidase activity can slow down glucose absorption and reduce postprandial blood glucose levels. α-glucosidase is considered a key target for diabetes treatment. α-glucosidase inhibitors can be developed into effective therapeutic drugs for this disease. Currently, only three α-glucosidase inhibitors are used in medical practice: acarbose, miglitol, and voglibose. In recent years, many scientists have been focused on the search for a new class of safe and highly effective inhibitors of α-glucosidase. Many researchers have high hopes for the unique properties of 1,3,4-thiadiazoles, which possess high potential antidiabetic activity.
This abstract reports on the synthesis of promising new 1,3,4-thiadiazole derivatives based on 3-aminopyridones, containing various acidic linkers. The synthesis was carried out by cyclizing the corresponding thiohydrazides and anhydrides of glutaric, maleic, and phthalic acids upon heating in acetic acid solution. The conducted bio-screening of the synthesized new 1,3,4-thiadiazole derivatives containing different acidic linkers (butanoic, acrylic, and benzoic acids) showed that they have significant inhibitory activity against α-glucosidase (up to 95.0%), which is 1.9 times higher than the value for the reference drug acarbose (49.5%). Moreover, one of the 1,3,4-thiadiazole derivatives with a benzoic acid linker -

2-(5-((6-Methyl-2-oxo-4-thiophen-2-yl)-1,2-dihydropyridin-3-yl)carbamoyl)-1,3,4-thiadiazol-2-yl)benzoic acid - showed an IC50 value of 3.66 mM, nearly 3.7 times lower than that of acarbose (IC50 = 13.88 mM). High inhibitory activity was also shown by 1,3,4-thiadiazole derivatives with a butanoic acid linker - with IC50 values of 6.70 and 8.42 mM, respectively. A correlation between the structure of the compounds and their activity was also established. The results of molecular docking correlated well with the bioanalytical data.

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Key Words: diabetes mellitus, antidiabetic activity, monothiooxamides, 3-aminopyridin-2(1*H*)-ones, 1,3,4-thiadiazole derivatives, α-glucosidase inhibition

P110: Concentrates of Polyphenols from Wild Northern Berries (Cranberries, Lingonberries, Blueberries, Bilberries) Have the Potential to Correct Age-Associated Pathology

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ABSTRACT.
Presumably, due to the antioxidant and cytoprotective effect, some isolated polyphenols have the ability to correct pathophysiological processes in age-associated pathology.
Concentrated total extracts of wild northern berries (cranberries, lingonberries, blueberries, bilberries) from the region of Northwestern Siberia were studied. The concentration of polyphenols in the extracts averaged 19 mg/ml. Quercetin 19 mg/ml was used as an isolated polyphenol standard.
The antioxidant effect of polyphenol concentrates of wild northern berries was expressed in antiradical activity *in vitro*, on cell cultures with the SASP (senescence-associated secretory phenotype) phenotype and *in vivo* (model of type II diabetes mellitus and metabolic syndrome), as well as in an increase in the concentration level antioxidant components of blood serum *in vivo*.
The cytoprotective effect of polyphenol concentrates of wild northern berries was established in the MTT test on cell cultures (protective effect in the presence of toxic concentrations of doxorubicin and/or H_2O_2).
Comparison of the effects of polyphenol concentrates of wild northern berries with similar effects of isolated polyphenol quercetin allows us to state a statistically significant advantage in the severity of the antioxidant and cytoprotective effects of blueberries and bilberries.
Concentrates of polyphenols from wild northern berries (especially blueberries and bilberries) have a significant potential for antioxidant and cytoprotective effects and can be considered as candidate agents for the correction of pathophysiological processes in age-associated pathology.
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"Development of new 3-aminopyridone derivatives with potential geroprotective activity."

Key Words: wild northern berry, polyphenol, antioxidant and cytoprotective effect

P111: Synthesis and *In Vitro* α-Glucosidase Inhibitory Action of Thiourea Derivatives based on 3-Aminopyridin-2(1H)-ones

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ABSTRACT.
Functional derivatives of the thiourea class have a wide range of pharmacological effects – antidiabetic, anticonvulsant, anticancer, antiviral, antifungal and antibacterial. In this regard, thiourea derivatives are widely used in the search for new medicines. Another biologically active sulfonamide group is also very close to thioureas. Thiourea and sulfonamides derivatives are also of increased interest as potential antidiabetic agents. This is mainly due to the widespread use of their structural analogues, sulfonylurea derivatives, as effective antidiabetic drugs in the treatment of type 2 diabetes mellitus (CD2). The search for new antidiabetic agents is also being conducted among conventional thiourea derivatives. There is extensive data on the antidiabetic activity of a series of diaryl substituted thioureas. By the time of this study, thioureas obtained on the basis of 3-aminopyridine-2(1H)-one were presented only in isolated examples and practically not studied biologically.
In order to obtain thioureide and thiourea derivatives of pyridones-2 and their subsequent bioscreening for antidiabetic activity, we synthesized them by the interaction of three 4-methyl-, 4-phenyl- and 4-(thiophene-2-yl)-3-aminopyridine-2(1H)-ones with some isothiocyanates (allylisothiocyanate, phenylisothiocyanate, benzoyl- and acetylisothiocyanates). Thus, the corresponding allyl-, phenyl-, acetyl- and benzoylthioureas were obtained. All of the newly synthesized compounds were tested for their *in vitro* α-glucosidase inhibitory activity.Acarbose was used as a positive control. All samples were studied in triplets. Among the studied compounds, allyl- and phenylthioureas have high inhibitory activity against the enzyme α-glucosidase, comparable to the comparison drug acarbose.
This research is funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan Grant No. AP14871433 "Development of new structural analogues of hypoglycemic drugs based on functional derivatives of 3-aminopyridones, and evaluation of their antidiabetic activity".

Key Words: diabetes mellitus, antidiabetic activity, 3-aminopyridin-2(1H)-one; isothiocyanate, thiourea derivative; α-glucosidase inhibition

P112: Targeting GBM Glycolysis with a Monocyte-based Nanoparticle Delivery System

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ABSTRACT.
Glioblastoma (GBM) is the most lethal primary malignant brain tumor in adults, with the development of effective therapeutic agents largely hampered by vast tumor heterogeneity and the impedance of efficient drug delivery by the blood-brain barrier (BBB). Our prior research has demonstrated that adult neural stem cells (NSCs) and oligodendrocyte precursor cells (OPCs) can act as cells of origin for two distinct GBM subtypes in mice and humans, with significant conservation in functional properties and distinct responses to the inhibition of Tucatinib and Dasatinib. Based on these findings, we have established a robust high-throughput screening assay to identify lineage-dependent subtype-specific as well as lineage-independent small molecule inhibitors for therapeutic development. We identified 3PO, PFK-015, and PFK-158 from the Anti-Metabolism Disease Compound Library for their preferential inhibition of Type 1 GBM cells, with PFKFB3 as their molecular target. PFKFB3, a vital regulator of glycolysis, plays an important role in oncogenesis and the survival and proliferation of cancer cells in the tumor microenvironment. Taking advantage of the significant contribution of monocyte-derived macrophages in GBM tumor mass and the ability of monocytes to travel towards chemotactic gradients, traverse tissue barriers, and accumulate precisely at GBM lesions, we are developing an innovative monocyte-based delivery system, termed Monocyte Enabled GBM Targeting (MEGT). This study aims to overcome the biological barriers of GBM, effectively shuttle drugs across the BBB, and maximize the therapeutic efficacy of glycolysis inhibitors. Findings from this work will have a long-term impact on the development of therapeutic agents targeting GBM.

P113: Rewired m^{6}A Epitranscriptomics Networks Link Mutant p53 to Neoplastic Transformation

Li-Fraumeni syndrome (LFS) is a familial cancer predisposition syndrome with germline *TP53* mutations characterized by autosomal dominant inheritance and early onset of multiple primary neoplasms. High tumor incidences in LFS patients provide the strong epidemiological link between p53 mutation and tumorigenesis.
N6-methyladenosine (m^{6}A), one of the most prevalent mRNA modifications in eukaryotes, plays a critical role in modulating biological and pathological processes. However, it remains unclear if dysregulation of m^{6}A epitranscriptomic networks contributes to mutant p53 neomorphic oncogenic functions.
Here, we applied LFS iPSC-derived astrocytes, the cell-of-origin of gliomas, to investigate LFS neoplastic transformation by mutant p53. We found that mutant p53 but not wild type p53 physically interacts with SVIL to recruit the H3K4me3 methyltransferase MLL1 to activate the expression of m^{6}A reader YTHDF2, culminating in an oncogenic phenotype. Aberrant YTHDF2 upregulation markedly hampers expression of multiple m^{6}A-marked tumor-suppressing transcripts, downregulates CDKN2B and SPOCK2 expression, and induces oncogenic reprogramming. Mutant p53 neoplastic behaviors are significantly impaired by genetic depletion of YTHDF2 or by pharmacological inhibition using MLL1 complex inhibitors. Our study reveals how mutant p53 hijacks epigenetic and epitranscriptomic

machinery to initiate gliomagenesis and suggests potential treatment strategies for LFS gliomas.

P114: Functional Specifications of Conserved Histone Variants, H2A.Z to Keep Genomic Stability of Higher Eukaryotic Cells.

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Abstract

Genetic stability of eukaryotic cells is ensured by accurate chromosome segregation in mitosis and genetic mutations causing its dysregulation consequently results in severe health defects of metazoan organism such as birth defects or cancers. Histones, an octamer of H2A, H2B, H3, and H4, play a key role in this process by regulating chromosome compactness, DNA damage repair, and chromosome segregation. Interestingly, new histone variants emerge during evolution, and provide additional functional specifications to higher eukaryotic organisms. H2A.Z is a conserved essential histone H2A variant and plays an important role in gene expression regulation, heterochromatin formation, DNA damage repair, and chromosome segregation. It has two isoforms, H2A.Z1 and H2A.Z2, encoded by two different genes, H2AFZ and H2AFV, respectively. They are different only in three amino acids but play non-redundant roles in chromosome dynamics. Furthermore, their abnormal regulation causing genomic instability was shown to facilitate tumorigenesis.

In this study, we aimed to characterize how H2A.Z regulates chromosome segregation in mitosis. To investigate isoform-specific functions of H2A.Z in mitosis, we first developed a genetic approach to deplete each isoform of H2A.Z, H2AFZ or H2AFV, specifically in Hela cells. Our studies with this approach found out that depletion of H2AFZ slightly increased G1 fraction but overall cell cycle profiles were not changed much. We then examined if chromosome segregation fidelity was interrupted by H2A.Z isoform depletion. Since daughter cells experiencing chromosome mis-segregation often contain micronuclei in addition to major nucleus, we used the fraction of micronuclei cells as chromosome mis-segregation index. Consistently, H2AFV depletion increased micronuclei fraction significantly, suggesting defects in chromosome segregation, but H2AFZ depletion didn't. H2AFV depletion also exhibited defects in mitotic checkpoint maintenance. Furthermore, H2AFV deleted cells accumulated DNA damage in many different loci including centromeres. Centromere of the chromosome was known to be heterochromatin, where RNA transcription is repressed. Earlier study showed that H2A.Z functions in heterochromatin formation of the yeast centromeres. Thus, we examined this in human cells and discovered that H2AFV or H2AFZ depletion also generated heterochromatin formation defects in centromere, shown by increased centromeric transcription. Moreover, we discovered that mitotic kinases such as Aurora B and MPS1 bound to H2AFV and that their inhibition also deregulated centromeric transcription.

In conclusion, we unveil the specific roles of histone H2A.Z isoforms in chromosome segregation of human cells and the active kinases that may regulate H2AFV functions.

P115: Microarray Analysis of Oxidative Stress-Related Gene Expression Profile and Identification of Oxidative Stress Status in Chronic Rhinosinusitis without Nasal Polyps

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ABSTRACT
OBJECTIVES-Chronic rhinosinusitis without nasal polyps (CRSsNP) is a chronic inflammatory disease lacking a clear pathogenesis and a systemic evaluation of its oxidative stress status and related gene expression remains to be explored. This study aimed to identify the oxidative stress-related gene expression profile in CRSsNP and its association with CRSsNP pathophysiology and progression.
MATERIALS AND METHODS-A total of 7 control patients with nasolacrimal duct obstructions and 7 patients with CRSsNP were recruited and the 84 oxidative stress-related gene expression was analyzed by real-time PCR array in the nasal mucosa tissues of control and CRSsNP groups. Changes in the mRNA and protein levels of these redox proteins were verified by a customized real-time PCR array in an additional 18 patients and RT-PCR and Western blotting. The overall oxidative stress status in CRSsNP including 4-hydroxynonenal and 3-nitrotyrosine expression (lipid peroxidation and protein nitrotyrosination) was examined by immunohistochemistry.
RESULTS and CONCLUSIONS: Twenty-four genes were significantly upregulated, whereas 4 genes were downregulated in CRSsNP. Among them, nitric oxide synthase 2, NAD(P)H dehydrogenase-quinone 1, aldo-keto reductase family 1-member C2, glutathione peroxidase 2, glutamate-cysteine ligase-modifier subunit, and heme oxygenase-1 were upregulated more than 3 folds. In contrast, lactoperoxidase and myeloperoxidase were two notable downregulated genes. We confirmed the changes in these redox genes and demonstrated that the overall oxidative stress was apparently increased in CRSsNP nasal mucosa.

Key Words:
Chronic rhinosinusitis without nasal polyps; CRSsNP; gene expression; microarray; oxidative stress; regulation

P116: Development, Synthesis, and Characterization of Novel Monothioxamides and 1,3,4-Thiadiazoles from 3-Aminopyridin-2(1H)-Ones, with in Vitro A-Amylase and A-Glucosidase Activity Analysis

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ABSTRACT.
With the increasing prevalence of diabetes mellitus worldwide, the search for effective and safe inhibitors of α-amylase and α-glucosidase has become imperative. This study focuses on the design, synthesis, and comprehensive analysis of novel derivatives of monothioxamides based on 3-aminopyridin-2(1H)-ones. For the first time, these

biologically active compounds were chemically modified to create new monothioxamides, which then underwent transamidation with hydrazine hydrate, resulting in the formation of thiohydrazides. Further reactions with chloroacetyl chloride and succinic anhydride led to the cyclization of these thiohydrazides into 1,3,4-thiadiazole derivatives.
The biological activity of these new compounds was assessed through molecular docking studies using the AutoDock Vina program. Notably, compounds 7a and 8b demonstrated higher binding affinities to target proteins compared to standard antidiabetic drugs like Acarbose and TAK-875. These new 1,3,4-thiadiazole derivatives exhibited significant inhibitory activities against α-amylase and α-glucosidase enzymes in vitro, with compound 8b showing an IC50 value of 122.2 μM, significantly lower than Acarbose's 998.3 μM. Cytotoxicity tests using the MTT assay revealed that these compounds do not exhibit cytotoxic effects at physiological concentrations and some even displayed cytoprotective properties.
Further computational screening confirmed that all synthesized compounds adhered to Lipinski's rule of five, suggesting their potential as orally active drugs with good absorption and moderate lipophilicity. The promising results from both computational and in vitro biological screenings highlight the potential of these newly synthesized compounds for further studies, including in vivo investigations, as potential antidiabetic agents.

Key Words: antidiabetic activity; hydrogen bonding; tosylate; 2-aminospiropyrazolinium salts.

P117: Development of a Multiplex Digital PCR Method for Sensitive and Accurate Detection of Monkeypox Virus Branches

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Abstract.
Background: The current outbreak of monkeypox has been declared a public health emergency of international concern by the World Health Organization. However, distinguishing symptoms of monkeypox virus (MPXV) infection from other orthopox viruses is atypical, necessitating laboratory confirmatory tests to aid in clinical diagnosis. Therefore, rapid and accurate detection and differentiation of various strains of MPXV are imperative.
Objective: A multiplex digital PCR method was developed to detect and differentiate various strains of MPXV using multiple ddPCR assays, with subsequent evaluation of its sensitivity and accessibility through the analysis of 17 clinical samples.
Methods: Primers and probes for multiple digital PCR were designed by comparing multiple complete genomes of poxvirus. Primer and probe concentrations, reaction conditions were optimized on Biorad QX200 platform. 17 clinical samples of MPXV were detected and verifed by the Sanger sequencing genotypes.
Results: The established ddPCR method could detect and differentiate MPXV and the results were consistent with those of Sanger sequencing.
Conclusion: Multiple ddPCR could be used todetect and distinguish different branches of MPXV rapidly and accurately.

Key Words: ddPCR; MPXV; SNPs; orthopox viruses

P118: Soluble Protein Post-Translational-Proteins as a Novel Mechanism to Regulate the Transmission of Pathological Proteins in Neurodegenerative Diseases

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Cell-to-cell transmission of pathological proteins, such as α-synuclein (α-syn) and tau, are critical for the progression of many neurodegenerative diseases including Alzheimer's disease (AD). Reagents that block the transmission of pathological protein are promising therapies for AD. However, the molecular mechanisms that modulate pathological protein transmission remains largely unknown. In this study, we illustrate a novel mechanism that modulates pathological α-syn and tau transmission.

Previous studies of pathological protein spreading have focused on the pathological seeds themselves; in particular, the release, uptake, transport, and degradation of pathological α-syn and tau have been investigated extensively. However, successful amplification of pathological α-syn and tau involves two components: the formation of pathological α-syn and tau (i.e., the seeds) and the transformation of corresponding normal, soluble α-syn and tau (i.e., the substrate). What has been generally ignored is the potential effects of soluble α-syn and tau, as a substrate for amplification, on pathological α-syn and tau spreading.

We showed, for the first time, that phosphorylation of soluble, non-pathological α-syn dramatically changes pathological α-syn amplification, in a site- and conformation-specific manner. We performed LC-MS/MS to systematically identify novel PTMs on soluble α-Syn purified from diseased brains. In addition to phosphorylation, acetylation on soluble α-syn also modified pathological α-syn transmission. Moreover, phosphorylation of soluble α-syn could modulate the seeding properties of pathological α-syn. Finally, similar to α-Syn, we found that soluble tau PTMs could also modulate the amplification of pathological tau in a conformation- and site-specific manner.

Our study represents the first analysis of how soluble protein PTMs affect the spreading of corresponding pathological protein, which represents a novel mechanism that regulates pathological protein transmission in AD. Soluble protein PTMs could also be novel drug targets to block AD progression.

P119: Identification of Aneuploidy Sensing Genes

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Aneuploidy is the presence of an abnormal number of chromosomes caused by mitotic chromosome missegregation as a result of chromosome instability. It is often associated with various cellular stress due to an unbalanced global gene expression, including proteotoxic stress, replication stress, mitotic stress, and oxidative stress. Aneuploidy is a hallmark of cancer. Unlike oncogenes and tumor suppressor genes

which have unique functions in cancer progression, aneuploidy's role highly depends on the genetic contexts of the cells. In most circumstances, the stress associated with aneuploidy will induce substantial fitness cost, which can be detected by the cell's internal sensing machinery to trigger apoptosis or senescence. However, if the sensing machinery is defective, aneuploid cells will gain proliferation advantages and eventually become cancerous. The molecular mechanism of aneuploidy sensing is not well understood. Here, we carried out a genome-wide CRISPR/Cas9 knockout screen to identify 17 putative sensing genes. We discovered that *atpaf1* knockout cells exhibit a proliferation advantage upon aneuploidy induction; they also develop higher aneuploidy and polyploidy levels over time. Mechanistically, we showed that *atpaf1* knockout increases the actively dividing polyploid cells while decreases the G1-arrested polyploid cells in the cell cycle. We also confirmed that apoptosis is reduced in *atpaf1* knockout cells. This research investigated the molecular link between aneuploidy and cancer progression. It may reveal new therapeutic targets or advise the development of predictive biomarkers for cancer.

P120: Molecular Mechanism of PP2A/B55α Inhibition by IER5

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PP2A serine/threonine protein phosphatases are heterotrimeric complexes that have a wide range of essential physiologic functions. The B55α form of PP2A has critical roles in cell cycle regulation, mitotic exit, and the DNA damage response. Its activity is modulated by additional regulatory proteins, such as ARPP19, FAM122A, and IER5. Here, we show that IER5 inhibits pTau dephosphorylation by PP2A/B55α in biochemical assays and report a cryoelectron microscopy structure of the PP2A/B55α-IER5 complex, which reveals that IER5 occludes a surface on B55α used for substrate recruitment, and unlike FAM122A and ARPP19, does not contact the catalytic subunit. Mutation of interface residues on IER5 interferes with recovery of B55α in co-immunoprecipitation assays and suppresses events in squamous carcinoma cells, such as KRT1 expression, that depend on inhibition of PP2A/B55α by IER5. These studies define the molecular basis for PP2A inhibition by IER5 and suggest a roadmap for selective pharmacologic modulation of PP2A/B55α complexes.

Key Words: protein phosphatase; IER5; Cryo-EM;

P121: Estrogen Counteracts Age-Related Decline in Beige Adipogenesis through the NAMPT-Regulated ER Stress Response

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Abstract
Thermogenic beige adipocytes are recognized as potential therapeutic targets for combating metabolic diseases. However, the metabolic advantages they offer are compromised with aging. Here, we show that treating mice with estrogen (E2), a hormone that decreases with age can counteract the age-related decline in beige adipogenesis when exposed to cold temperature, while concurrently enhancing energy expenditure and improving glucose tolerance in mice. Mechanistically, we find that nicotinamide phosphoribosyl transferase (NAMPT) plays a pivotal role in facilitating the formation of E2-induced beige adipocytes, which subsequently suppresses the onset of age-related ER stress. Furthermore, we found that targeting NAMPT signaling, either genetically or pharmacologically, can restore the formation of beige adipocytes by increasing the number of perivascular adipocyte progenitor cells. Conversely, the absence of NAMPT signaling prevents this process. Together, our findings shed light on the mechanisms regulating the age-dependent impairment of beige adipocyte formation and underscore the E2-NAMPT-controlled ER stress pathway as a key regulator of this process.

Keywords: Aging, Beige adipocyte formation, Estrogen, ER stress, NAMPT

P122: Structures and Mechanisms of Respiratory Syncytial Virus RNA Synthesis

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Abstract
Respiratory syncytial virus (RSV) is a nonsegmented negative-sense (NNS) RNA virus and the leading cause of respiratory infections, especially in infants and older

adults. There is an urgent need to understand the RSV RNA synthesis mechanism and facilitate the development of effective antiviral treatments. The RSV polymerase is a multifunctional RNA-dependent RNA polymerase composed of the large (L) protein and the phosphoprotein (P). It transcribes the RNA genome into ten viral mRNAs and replicates full-length viral genomic and antigenomic RNAs. We have determined the cryo-electron microscopy (cryo-EM) structures of the RSV polymerase alone (Cao et al. *Nat Comm* 2020) and its complex with genomic and antigenomic RNA promoters (Cao et al. *Nat* 2023). Our findings offer critical mechanistic insights into the RNA synthesis mechanism of RSV and other NNS RNA viral polymerases, such as those of rabies (RABV), Nipah (NiV), and Ebola (EBOV).

P123: Signaling Regulation of Non-neural Surface Ectodermal Dynamics in Neural Tube Closure

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The cellular and molecular mechanisms underlying mammalian neural tube closure remain poorly understood. We have demonstrated that the non-neural surface ectodermal cells form multicellular rosette structures with convergent cellular protrusions that are connected with an F-actin cable network encircling the pending closure site of the posterior neuropore, which are severely disrupted in the surface ectodermal transcription factor Grhl3 mutants that exhibit fully penetrant spinal neural tube defects. We proposed a novel model of mammalian neural tube closure guided by non-neural surface ectodermal cell dynamics. We also demonstrated that canonical Wnt/beta-catenin is required for caudal neural tube closure by regulating crucial downstream transcription factors in the dorsal neural folds of mice. However, the signaling mechanisms regulating the non-neural surface ectodermal dynamics for neural tube closure remain unknown. To address this, we have generated novel mutants by conditional gene-targeting of representative signaling genes in the non-neural surface ectodermal cells. Our preliminary results demonstrate that canonical Wnt signaling and planar cell polarity (PCP) signaling pathways in the non-neural surface ectoderm are required for initiating neural tube closure and that the canonical Wnt signaling is also required for activation of the surface ectodermal transcription factors to promote multicellular rosette formation and polarized cellular protrusions for neural tube closure.

P124: Engineered CRISPR Systems for Disease Treatment and Diagnostics

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The recent discovery of the CRISPR genome editing systems has been revolutionizing both basic biological research and the treatment of human genetic disorders. However, there are remaining challenges in improving the precision and multiplexity of the current CRISPR systems for genome manipulation. In this seminar, I will overview

our recent development of highly specific and powerful genome-editing tools for the treatment and diagnostics of diseases and the discovery of new potential small-molecule drugs. First, I will introduce the development of high-precision and multiplex CRISPR genome-editing strategies for safe and effective molecular therapy to treat genetic disorders; Second, I will describe the application of these advanced CRISPR tools for fungi genome engineering to enable the production of novel small molecules with therapeutic potential; Finally, I will present the improved ultrasensitive viral detection by using the engineered CRISPR systems to advance timely and accurate diagnostics for the current and future pandemics and epidemics.

P125: Synthesis, Structural Elucidation, and Biological Evaluation of N-p-(Dimethylamino)-N′-(p-Dimethylaminobenzylidene)-N,N′′-Diphenylbenzohydrazonohydrazide

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ABSTRACT.
The synthesis of N-p-(dimethylamino)-N′-(p-dimethylaminobenzylidene)-N,N′′-diphenylbenzohydrazonohydrazide, a bis (biarylhydrazone) derivative, was achieved via a three-component reaction involving 3,5-diacetyl-2,6-dimethylpyridine, p-N-dimethylaminobenzaldehyde, and phenylhydrazine in an ethanolic KOH solution. The resultant compound's structure was confirmed using 1H and 13C NMR spectroscopy and X-ray diffraction analysis. This bis (biarylhydrazone) exhibited notable antiradical and cytoprotective activities. The synthesis process utilized 3,5-diacetyl-2,6-dimethylpyridine, an accessible synthon, allowing for various chemical modifications due to its reactive acetyl groups. These modifications are pertinent for creating heterocyclic compounds with significant biological properties, such as antibacterial and antituberculous activities. The reaction in question yielded a crystalline product, identified via 1H NMR spectroscopy as not including the pyridine fragment but comprising two molecules each of p-N-dimethylaminobenzaldehyde and phenylhydrazine. X-ray diffraction data elucidated the structure of the bis (biarylhydrazone), revealing standard bond lengths and angles, with slight deviations in the configurations of the dimethylamine nitrogen atoms and the central nitrogen connecting the molecule's two parts. The dihedral angle between the key planes in the structure was determined to be 98.2°. Comparisons with the Cambridge Structural Database indicated structural similarities with known compounds, with analogous bis (biarylhydrazones) previously observed as intermediates in synthesizing 1,2,4-triazole derivatives. Biological activity assays demonstrated that this compound had a higher antiradical activity than ascorbic acid, with an IC50 (DPPH) value of 11.0 μM compared to 19.9 μM for ascorbic acid. Moreover, cytoprotective activity was evaluated using the MTT test on the MCF-7 breast cancer cell line. The compound significantly increased cell viability up to 458% compared to the control. Similar results were obtained in the neutral red uptake assay. These findings suggest that N-p-(dimethylamino)-N′-(p-dimethylaminobenzylidene)-N,N′′-diphenylbenzohydrazonohydrazide holds significant potential as an antioxidant and cytoprotective agent, warranting further investigation into its therapeutic applications. The structural characterization and biological activity assays underscore its promise in medicinal

chemistry, particularly for developing new treatments with antioxidant and cytoprotective properties.
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Key Words: 3,5-diacetyl-2,6-dimethylpyridine, 4,5-dihydro-1H-pyrazole derivatives, bis(biarylhydrazone), X-ray diffraction analysis, antiradical and cytoprotective activity

P126: Thyroid Hormone Stimulates Non-Canonical Energy Expenditure via Inducing Sodium Potassium Futile Transport Cycle in Beige Adipocytes

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Uncoupling protein 1 (UCP1)-independent heat generation has been implicated in beige adipocytes upon low temperature stimulation. However, the physiological cue mediating cold-induced UCP1-independent heat generation, and the detailed mechanism is less clear. We found that cold exposure robustly stimulates sympathetic nerve growth in the inguinal white adipose tissue (iWAT) in wildtype (WT) and *Ucp1* knockout (KO) mice. This in turn strongly induces the expression of *Dio2*, the enzyme for bioconversion of T4 to T3. Depletion of endogenous thyroid hormone almost completely abolishes UCP-1 independent heat generation in beige adipocytes. Furthermore, beige adipocytes engages an elevated influx and efflux rates of Na+ and K+, particularly in UCP1 deficient condition. Mechanistically, *Atp1a2*, the gene encoding the key subunit of Na+ /K+ ATPase, as well as the Na+ and K+ channels, were simultaneously induced upon cold exposure or after T3 treatment in beige adipocytes. Inhibition of Na^+ /K^+ ATPase abolishes beige adipocyte thermogenesis in both mouse and human beige adipocytes in vitro. Importantly mice with adipocyte selective deletion of *Atp1a2* are more susceptible to diet-induced obesity and are cold intolerant. ATP1A2 expression in human adipose tissue is negatively corelated with BMI. Furthermore, study based on a cohort including 11,022 Chinese adults reveals a missense variant Arg65Leu in the ATP1A2 gene which is linked with glucose and lipid metabolism. Our study uncovers a plasma membrane futile cycle involving sodium ion (Na+) and potassium ion (K+) transport in beige adipocytes which is orchestrated by Sympathetic nerves system-local biogenesis of thyroid hormone axis. This pathway dissipates energy in a non-canonical, UCP1-independent manner to combat obesity and metabolic disorders.
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P127: Integrative Analysis of Single-Cell and Bulk Transcriptome Data Reveals Novel Biomarkers for Diagnosis and Prognosis of Nasopharyngeal Carcinoma

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The metastasis and recurrence of nasopharyngeal carcinoma remain significant risks for mortality, particularly in the South China. We integrated numerous multi-omics data to identify a batch of consistently dysregulated genes and pathways in nasopharyngeal carcinoma. Further, a diagnostic model was developed by calculating the disorder scores of dysregulated genes, which can distinguish malignancy from benignancy with high accuracy at both the bulk and single-cell levels. Besides, we constructed an interpretable prognostic model, which consisted of four genes (*BUB1B*, *DMD*, *KLF2*, and *VILL*). In sum, our current stdy reveals novel biomarkers for diagnosis and prognosis of nasopharyngeal carcinoma.

P128: TERRA-LSD1 Phase Separation Promotes R-loop Formation for Telomere Maintenance in ALT Cancer Cells

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Chromatin-associated RNAs play essential roles in maintaining genome stability. For example, telomere repeat-containing RNA (TERRA), a long non-coding RNA transcribed from chromosome ends, forms R- loops to promote homology-directed DNA synthesis in the alternative lengthening of telomere (ALT) pathway. Here we report that TERRA contributes to ALT via interacting with the lysine-specific demethylase 1A (LSD1 or KDM1A). We show that TERRA is required for the telomeric localization of LSD1, whose deficiency leads to a loss of ALT signatures. In addition, LSD1 function in ALT is largely independent of its demethylase activity. Instead, LSD1 promotes TERRA localization to ALT telomeres via its interaction with TERRA. Importantly, chemical dimerization-mediated recruitment of LSD1 to telomeres is sufficient to enrich TERRA on telomeres. Additionally, LSD1 recruitment triggers LSD1 phase separation at telomeres, which appears to depend on LSD1's TERRA binding capacity. In vitro reconstitution studies show that TERRA indeed promotes LSD1 phase separation in a manner that is dependent upon TERRA length and its G-quadruplex structure. Importantly, the formation of TERRA- LSD1 condensates enriches the R-loop stimulating protein Rad51AP1 and increases TERRA-containing R- loops at telomeres, suggesting that phase separation may concentrate R-loop regulatory molecules to stimulate DNA synthesis on ALT telomeres. Our findings reveal that mutual enrichment of LSD1 and TERRA at telomeres can function to promote ALT telomere maintenance, suggesting a mechanism for how biophysical properties of histone modification enzyme-RNA interactions impact chromatin function.

P129: Single-cell and Single Myofiber RNA Sequencing Reveals Senescent Cells in Skeletal Muscle and Alterations in Cell-cell Communication Caused by Cellular Senescence

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Background: Cellular senescence is an irreversible growth arrest that occurs when cells are stressed. Senescent cells secrete proinflammatory cytokines and chemokines that contribute to tissue dysfunction and aging. Senescent cells accumulate with age and in most tissues, while clearance of senescent cells alleviates several pathologies in mouse models of aging and age-related diseases. The progressive loss of skeletal muscle mass and strength with age is a major threat to independence and quality of life. Despite profound age-associated changes in muscle, whether its constituent cells are prone to senesce and their physiological function is not clear.

Objective: To comprehensively characterize senescent cells in skeletal muscle and study how age-related changes in senescent cell contribute to muscle aging phenotypes like inflammation.

Methods: Single-cell RNA-sequencing (scRNA-seq) and single myofiber RNA-seq were used to explore the senescent cell types in skeletal muscle from young and old mice. Magnetic-activated cell sorting, qPCR, immunofluorescence, immunohistochemistry, and RNA in situ hybridization (RNA-ISH) were used to confirm the senescence features. Cell-cell communications between cell populations were analyzed using CellChat. A senotherapeutic drug-treated mouse model was used to study the physiological role.

Results: Through scRNAseq, we demonstrated that a subpopulation of old fibroadipogenic progenitors (FAPs) highly expresses $p16^{Ink4a}$ together with multiple senescence-related genes and exhibits DNA damage and chromatin reorganization. Analysis of isolated myofibers detailed a senescence phenotype within a subset of old cells, governed by $p21^{Cip1}$. We discovered a diverse communication network, with FAPs actively crosstalking with macrophages, which were further validated *in vitro*. Furthermore, senotherapeutic intervention in old mice countered age-related molecular and morphological changes and improved skeletal muscle strength. Finally, we found that the senescence phenotype is conserved in the skeletal muscle of older humans.

Conclusion: Collectively, our data provide compelling evidence for cellular senescence as a hallmark and potentially tractable mediator of skeletal muscle aging. The physioloigical role of senescent FAPs and myofibers will be investigated in our futhre study.

P130: Protein Arginylation: a tRNA-dependent Mechanism Linking Translation, Stresses, and Homeostasis.

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The arginyl-transferase ATE1 is a tRNA-dependent enzyme that covalently attaches an arginine molecule to its protein substrates, a process best known for promoting their degradation through autophagy and the proteasome. Conserved from yeast to humans, ATE1 deficiency in mice results in embryonic lethality and defects in cardiovascular development and angiogenesis, while conditional knockouts exhibit reproductive, developmental, and neurological issues. Accumulating evidence suggests that ATE1 is a master regulator of protein homeostasis, stress response, cytoskeleton maintenance, and cell migration. These diverse functions arise from its unique enzymatic activity and diverse protein substrates. However, how ATE1 hijacks tRNA from the highly efficient ribosomal protein synthesis pathways and catalyzes the arginylation reaction remains a mystery. Here, we describe our recent efforts in determining the structure-function relationship of ATE1 and identifying degradative ATE1 substrates in human cells. Structurally, ATE1 adopts a previously uncharacterized fold containing an atypical zinc-binding site and recognizes tRNA through a novel mechanism. Key residues of ATE1 in the substrate binding sites were also identified, mutations of which abolished its enzymatic activity, leading to slower turnover and the accumulation of ATE1 substrates in human cells. Functionally, ATE1 knockout led to substantial changes in the human proteome and transcriptome, hinting at new mechanisms regulating protein secretion, oxygen sensing, and mitochondrial health.

Reference

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