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会议主题 [生物化学的新时代]

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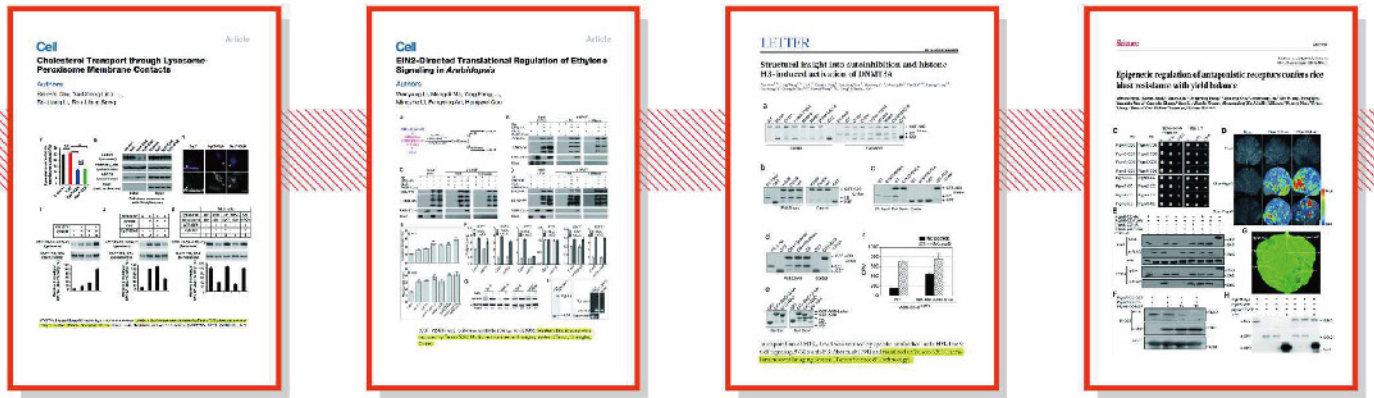

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目录 Contents

Plenary Lectures

Symposia

Contents

Contents

Abstracts

专题一：RNA与生命调控

专题二：代谢网络与稳态

专题三：蛋白质功能与修饰

专题四：糖脂生物学

专题五：应用生物化学与新技术

青年科学家论坛

Plenary Lectures

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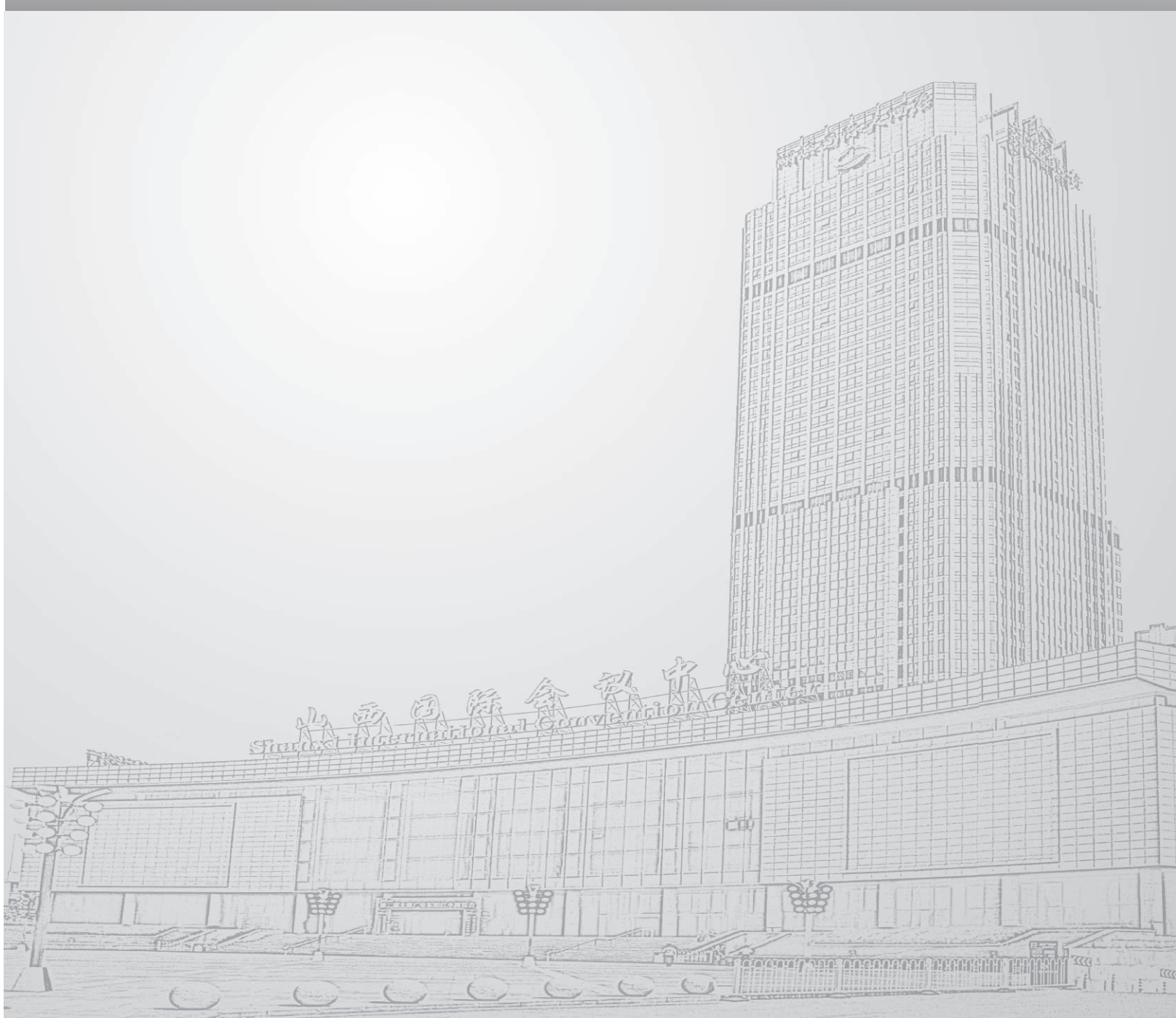

Architecture of ASFV and implications for viral assembly and vaccine design

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冷冻电子显微学（cryo-EM）对于超大蛋白质机器结构研究

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生命活动的很多基本过程是与系统的结构层次密切相关的。较高级的细胞结构层次显示出蛋白质、核酸等生物大分子单独所不具备的生命特征。对于生物超大分子复合物的研究正在成为结构生物学的新前沿。近年来冷冻电子显微学的迅速崛起，特别到2013年图像采集设备和图像处理方法的技术突破引发了结构生物学的革命，在《科学》（Science）评选出的2017全球十大科学突破中，第二项就是冷冻电子显微学将生命科学研究推进到原子级时代（life at atomic level）。

藻胆体是蓝藻和红藻中的超大蛋白复合体，吸收并传递太阳光能给藻类进行光合作用。自发现藻胆体半个多世纪以来，科学家们一直期望知道这种超大蛋白复合体是如何组装、如何高效的传递能量的，但要回答这些问题就必须知道藻胆体的三维结构。我们将报告该领域研究的最新突破。这些研究不仅为揭示藻胆体的组装机制和光能传递途径奠定了基础，同时也为其它超大蛋白质机器的组装机制提供重要启示。

How to Battle Nutrient Excess? -The Biology and Mechanism of Lipid Storage

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Sufficient energy storage in the form of neutral lipid TAG is improtant for survival during evolution. However, excess lipid storage leads to the development of metabolic diseases including obesity, diabetes and fatty liver disease. Lipid droplets (LDs) are dynamic subcellular organelles responsible for lipid storage and control intracellular lipid homeostasis. This seminar will discuss the role of CIDE family in controlling LD fusion and lipid storage. CIDE proteins consist Cidea, Cideb and Cidec (Fsp27) are LD and ER-associated proteins. *CIDEs* deficient animals indicate that these proteins play important roles in controlling lipid storage in adipocytes, hepatocytes, mammary epithelial cells and skin sebocytes. Further molecular and cell biological evidence suggest that CIDE family proteins are highly enriched at LD-LD contact sites (LDCS) and promote atypical form of LD fusion and growth by initiating a directional lipid transfer from smaller to larger LDs. Several regulatory proteins including Perilipin1 (Plin1) and Rab8a are shown to enhance CIDE-mediated LD fusion and growth. Our recent analyses demonstrate that LD fusion was controlled by FA-mediated protein acetylation and degradation. In addition, LD fusion complex formation is potentially mediated by 2-dimensional phase transition. Other factors regulating lipid storage will also be discussed.

RNA methylation in gene expression regulation

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Over 150 types of post-transcriptional RNA modifications have been identified in all kingdoms of life. We have discovered the first two RNA demethylases, FTO and ALKBH5, which catalyze oxidative demethylation of the most prevalent modifications of mammalian messenger RNA (mRNA) and other nuclear RNA, N^6-methyladenosine (m^6A). These findings indicate that reversible RNA modification could impact biological regulation analogous to the well-known reversible DNA and histone chemical modifications. We have also characterized proteins that selectively recognize m^6A-modified mRNA and affect the translation status and lifetime of the target mRNA, as well as molecular machines that deposit the m^6A methylation on mRNA. Functional studies reveal m^6A methylation as a critical mechanism to synchronize groups of transcripts for coordinated metabolism, translation, and decay, allowing timely and coordinated protein synthesis and transcriptome switching during cell differentiation and development. Misregulations of these processes lead to embryo lethality and human diseases such as cancer. I will present effects of m^6A regulation on cancer progress and immune response.

Pyroptosis: from innate immunity to cancer

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Pyroptosis, originally known to be activated by caspase-1/4/5/11, is critical for immune defenses and development of many immunological diseases. While caspase-1 is downstream of the inflammasome complex that senses infections, caspase-11 and its human counterparts caspase-4/5 serve as cytosolic receptors for bacterial lipopolysaccharide (LPS) to activate pyroptosis-mediated immune defenses. These caspases cleave Gasdermin D (GSDMD) to release the autoinhibition on its Gasdermin-N domain that executes pyroptosis via an intrinsic membrane pore-forming activity. *Gsdmd*$^{-/-}$ mice are susceptible to various bacterial infections but also resistant to LPS-induced septic shock. GSDMD belongs to a large Gasdermin family sharing the autoinhibited pore-forming domain. Another family member GSDME harbors a caspase-3-recognition motif also in the middle linker region and can switch caspase-3-induced apoptosis to pyroptosis. Similarly, caspase-3 cleavage releases the pore-forming domain of GSDME, and the resulting pyroptosis also occurs in cells treated with DNA-damaging chemotherapy drugs. GSDME is silenced in most cancer cells but expressed in normal tissues. GSDME-positive cells from normal human tissues undergo caspase-3-dependent pyroptosis in response to chemotherapy drugs. Knockdown of *GSDME* expression in these primary cells converts the death from pyroptosis to apoptosis. Importantly, *Gsdme*$^{-/-}$ mice are protected from chemotherapy drug-induced tissue damage and weight loss. These findings define pyroptosis as Gasdermin-mediated programmed necrotic cell death that shall have important functions in a wide spectrum of biological and pathological processes.

Symposia

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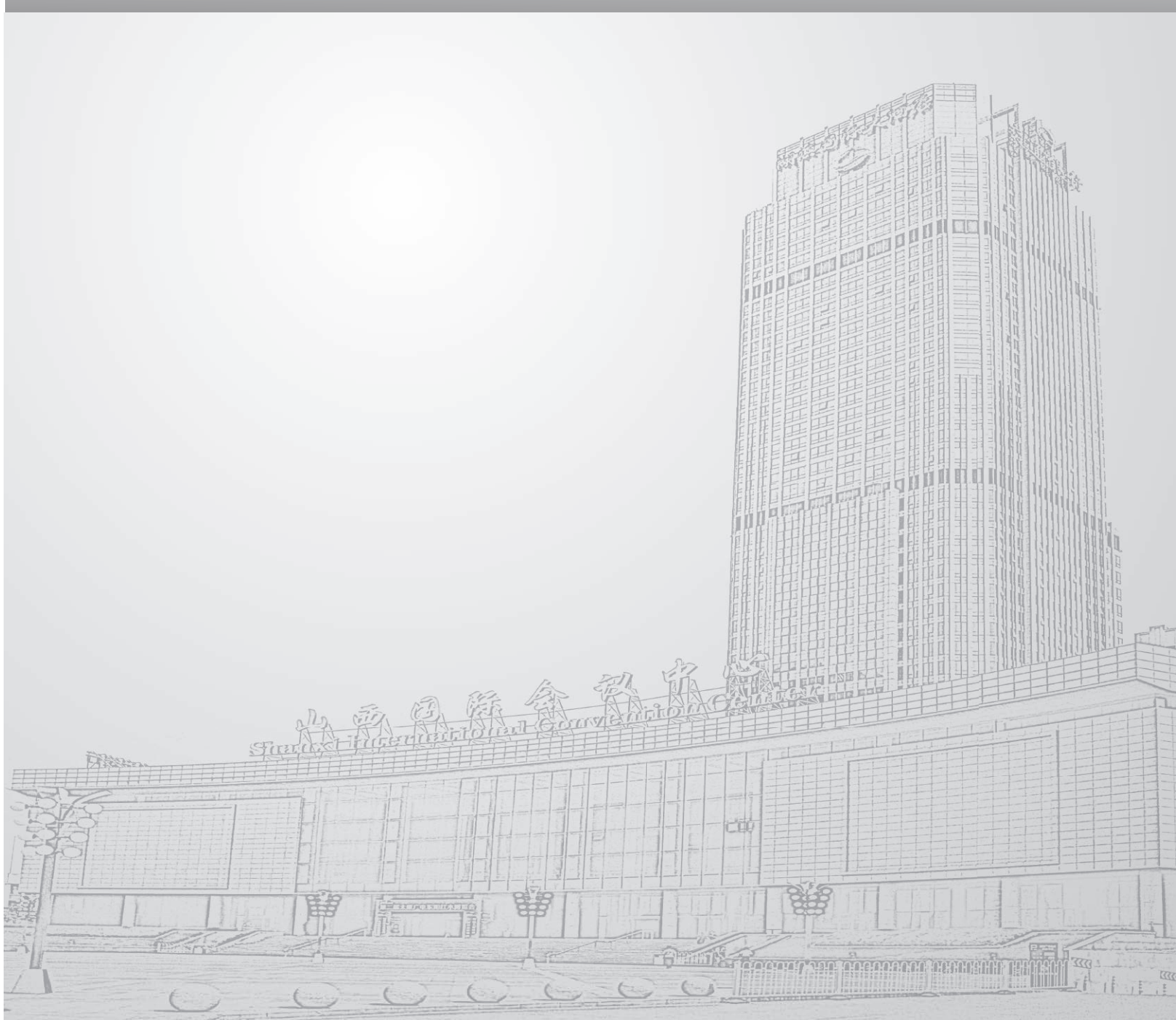

S-1-01 Reporting and harnessing microRNA activity by CRISPR-Cas9

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microRNAs (miRNAs) are an important class of small regulatory noncoding RNAs expressed by plants, animals and viruses. Using a miRNA-mediated sgRNA releasing strategy, we create a miRNA-inducible CRISPR-Cas9 platform that can be used to report miRNA activity and control genome editing tools in a cell type specific manner. In this presentation, I will report our progress on using this system for a variety of applications, including killing cancer cells and monitoring post-transcriptional regulation.

Reference:

[#]Wang, X.W., [#]Hu, L.F., Hao, J., Liao, L.Q., Chiu, Y.T., Shi, M.and [*]Wang Y. (2019) A microRNA-inducible CRISPR-Cas9 platform serves as a microRNA sensor and cell-type-specific genome regulation tool. *Nature Cell Biology* 21, 522-530.

S-1-02 Lnc-ing RNA Processing and Function

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Long noncoding RNAs (lncRNAs) are emerging as new regulators in gene expression networks and exhibit a surprising range of shapes and sizes. Many lncRNAs are transcribed by RNA polymerase II and are capped, polyadenylated, and spliced like mRNAs. By developing methods for genome-wide discovery and characterization of non-polyadenylated RNAs, we have identified several RNA species with unexpected formats. These RNAs are derived from long primary transcripts via unusual RNA processing pathways and are stabilized by different mechanisms, including capping by small nucleolar RNA (snoRNA)–protein (snoRNP) complexes at their ends or forming circular structures. We have shown that some such RNAs are involved in gene regulation and are also implicated in human diseases. I will discuss our most recent

findings of underlying mechanisms related to their formation and function, with a focus on one type of circular RNAs that is produced by pre-RNA back-splicing of exons of thousands of genes in eukaryotes.

Key Words: Long noncoding RNAs, circular RNAs, gene regulation, human diseases, innate immunity

S-1-03 Initiation of Parental Genome Reprogramming in Fertilized Oocyte by Splicing Kinase SRPK1-Catalyzed Protamine Phosphorylation

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The paternal genome undergoes massive exchange of histone with protamine for compaction into sperm during spermiogenesis. Upon fertilization, this process is potently reversed, which is essential for parental genome reprogramming and subsequent activation; however, it remains poorly understood how this fundamental process is initiated and regulated. We report that the previously characterized splicing kinase SRPK1 initiates this life-beginning event by catalyzing site-specific phosphorylation of protamine, thereby triggering protamine-to-histone exchange in the fertilized oocyte. Interestingly, protamine undergoes a DNA-dependent phase transition to gel-like condensates and SRPK1-mediated phosphorylation likely helps open up such structures to enhance protamine dismissal by nucleoplasmin (NPM) and enable the recruitment of HIRA for H3.3 deposition. Remarkably, genome-wide ATAC-seq analysis reveals that selective chromatin accessibility in both sperm and MII oocytes is largely erased in early pronuclei in a protamine phosphorylation-dependent manner, suggesting that SRPK1-catalyzed phosphorylation initiates a highly synchronized genome reorganization program in both gametes.

S-1-04 Increasing the coding complexity of human genome at RNA level

RNA水平的调控提高了人基因组的编码复杂性

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The number of human coding genes is surprisingly small given the biological complexity of the organism, suggesting additional layers of regulation to increase its coding complexity. We found that at the coding complexity of human genome can be increased by multiple pathways at RNA level. First, the majority of human genes undergo alternative splicing to produce multiple isoforms with distinct functions, which is a major mechanism to increase proteome complexity of human genome. This process is tightly regulated, and misregulation of splicing is closely associated with various human diseases. We have been focused on systematic study of splicing regulation, as well as the dysregulation of alternative splicing in cancers. We have developed a series of new methods to identify and study the regulatory *cis*-elements and trans-acting splicing factors, and seek to assemble such information into a predictive "splicing code" to help us understand how the alternative splicing is controlled in different cell types and in different disease stages. In addition, the mRNA translation can be regulated to produce multiple peptides from single mRNA. By using circRNA as a model, we have studied the non-canonical RNA translation and the regulation and new function of circular RNAs. We found that a large fraction of circular RNAs can function as mRNA to code for proteins, and the translation of circRNA can be driven by diverse sequences to produce additional translation isoforms. These findings provided new molecular mechanisms to expand the coding capacity of human genome at RNA levels.

S-1-05 SpyCLIP: An easy-to-use and high-throughput compatible CLIP platform for the characterization of protein-RNA interactions

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Growing numbers of proteins are found to be associated with RNAs and to play critical roles in both

normal and pathological processes within cells. UV crosslinking and immunoprecipitation (CLIP) coupled with high-throughput sequencing is the most powerful technology to accurately identify the binding sites of a given RNA-binding protein (RBP) *in vivo*. Several CLIP varieties, such as PAR-CLIP, iCLIP, eCLIP and irCLIP, have been developed to improve the performance and reduce the technical difficulty of this technology. However, all these CLIP varieties depend on PAGE-membrane purification steps of the captured ribonucleoprotein (RNP), including SDS-PAGE separation and membrane transfer of RNP, labeling of RNA for visualization and recovery of RNA from the membrane. These complex procedures cause tremendous loss of the RNP, frequent experimental failure and non-reproducible results, which not only hampered routing application of this technology in common laboratories, but also render the powerful CLIP technology inapplicable to automation and high-throughput studies. In this study, we report an easy-to-use SpyCLIP platform employing the SpyTag-SpyCatcher chemistry. Due to the irreversible covalent linkage formed between the SpyTag-fused RBPs and the SpyCatcher-coated beads, the RNP can withstand the harshest washing conditions during purification. Such a stringent purification scheme significantly improves the signal-to-noise ratio of obtained protein-RNA interaction maps and thus completely omits any PAGE-membrane purification steps, making SpyCLIP readily amenable to automation and high-throughput applications. We also re-design the library construction strategy such that all of the reaction steps can be continuously performed on beads or in solution, which dramatically reduces the technical difficulty and procedure time. Finally, we introduce a single universal input control to remove background noise, further guaranteeing an excellent specificity and productivity for SpyCLIP data. Using this new tool, we have generated highly complex and reproducible RNA binding maps with the highest specificity and sensitivity so far for diverse RBPs, including RBFOX2, SLBP and AGO2, and revealed novel insights into their functional mechanisms, demonstrating that SpyCLIP represents an robust tool for systematic studies of protein-RNA interactions.

S-1-06 Transcriptional activation by small RNAs in plants

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Conventional RNA interference (RNAi) pathways suppress eukaryotic gene expression at the post-transcriptional or transcriptional level. At the core of RNAi are small RNAs (sRNAs) and effector Argonaute (AGO) proteins. In plants, AGO1 binds microRNAs (miRNAs) to post-transcriptionally repress target genes, while AGO4 binds 24-nt siRNAs to direct DNA methylation and transcriptional repression. In this talk, I will present our unexpected findings that *Arabidopsis* AGO1 binds to the chromatin of active

genes and promotes their transcription and discuss how the role of AGO1 in gene activation contributes to the regulation of diverse signaling pathways and associated biological processes. I will also present our findings that 24-nt siRNA-directed DNA methylation promotes gene transcription and regulates agronomically important traits in rice.

S-1-07 Detection of epitranscriptomic mark N6-methyladenosine

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The epitranscriptomic mark N^6-methyladenosine (m^6A) is the most abundant post-transcriptional RNA modification in both eukaryotic mRNA and long non-coding RNA (lncRNA). These marks are commonly installed by an m^6A writer complex and erased by AlkB family dioxygenases (e.g., FTO and ALKBH5 in human). m^6A marks mediated by m^6A-binding proteins can regulate RNA processing and metabolism. Owing largely to the inert reactivity of the methyl group of m^6A, transcriptome-wide m^6A detection methods have to date relied on m^6A-antibody immunoprecipitation (m^6A-IP). And the RNase H derived SCARLET method is the only one that can quantitatively detect the m^6A status of a single mRNA or lncRNA locus, but its time-consuming and radioactive labeling requirements have limited its wider application. Thus it is desirable to develop new techniques/tools for detecting locus-specific m^6A and transcriptional-wide m^6A sites. Here we developed two tools: 1) an elongation- and ligation-based qPCR amplification method for the radiolabeling-free detection of m^6A position in a single gene; and 2) antibody-free and chemical-labeling method for mapping m^6A methylome.

Key Words: RNA modification, Epitranscriptomics, N^6-methyladenosine (m6A)

S-1-08 Mapping spatial transcriptome with light-activated proximity-dependent RNA labeling

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RNA molecules are highly compartmentalized in eukaryotic cells, with their localizations intimately linked to their functions. Despite the importance of RNA targeting, our current knowledge of the spatial organization of transcriptome has been limited by a lack of analytical tools. In this study, we develop a novel chemical biology approach to label RNAs in live cells with high spatial specificity. Our method, called CAP-seq, capitalizes on light-activated, proximity-dependent photo-oxidation of RNA nucleobases, which could be subsequently enriched via affinity purification and identified by high-throughput sequencing. Using this technique, we investigate the local transcriptomes that are proximal to various subcellular compartments, including the endoplasmic reticulum and mitochondria. We discover that mRNAs encoding for ribosomal proteins and oxidative phosphorylation pathway proteins are highly enriched at the outer mitochondrial membrane. Due to its specificity and ease of use, CAP-seq is a generally applicable technique to investigate the spatial transcriptome in many biological systems.

Key Words: RNA labeling, photo-oxidation, click reaction, mitochondria

S-1-09 Mapping the functional mammalian epitranscriptome

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More than 100 different types of post-transcriptional modifications to RNA molecules have been characterized so far. Recent transcriptome-wide identification of several RNA modifications has triggered an explosion of new information, leading to the emerging field of epitranscriptomics. Because many RNA modifications form regular base pairs during reverse transcription and are of very low abundance, highly selective and sensitive methods are required for their detection. In my lab, we utilize selective chemical/

biochemical labeling to develop high throughput sequencing methods for these RNA modifications; in particular, we have developed two specific technologies for the transcriptome-wide sequencing of pseudouridine (Ψ) and N^1-methyladenosine in RNA, respectively. With CeU-Seq, we identified thousands of Ψ sites in human cells and mouse tissues, showed that hPUS1 acts on mRNA and revealed inducible and stress-specific mRNA pseudouridylation events. With m^1A-ID-Seq, we identified ~900 m^1A peaks in mRNA and ncRNA, and revealed a prominent feature of enrichment in the 5'-untranslated region of mRNA transcripts. Recently, we have developed a new technology that detects transcriptome-wide m^1A methylome at single-base resolution, and reveal distinct classes of m^1A methylation in the human transcriptome. Such transcriptome-wide sequencing technologies will allow future functional studies of the epitranscriptome.

S-1-10 The RNA structural landscape of RNA-protein interaction

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The mechanistic study of RNA and RBP(RNA binding proteins) interactions is essential to the understanding of RNA regulation and, associated diseases. Here we approach the principles of RBP-RNA interactions by constructing deep generative models that combines information of both RNA sequence and structure *in vivo*. We characterized the sequence and structural determinants of the RNA binding sites from the RNA-RBP binding models. We showed that different proteins have different sequence and structure preferences. We demonstrated that by integrating *in vivo* RNA structural information, the binding model more precisely predicted new bindings in varying cellular conditions. Finally we predicted and validated RNA structure changes that impact RBP binding and could contribute to Hutchinson-Gilford Progeria Syndrome, a rare genetic disorder characterized by premature aging. Our results highlight the basis and the significance of RNA structure in determining RBP binding and its unappreciated role in human disease.

S-1-11 CRISPR-Cas Mediated Cleavage of Invading RNAs

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Bacteria and archaea are protected against invading nucleic acids from phages and plasmids because of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR associated proteins) systems, which are RNA-guided prokaryotic adaptive immune system. CRISPR-Cas systems are found in nearly half of all bacteria studied so far, as well as in the majority of archaea. CRISPR-Cas systems are boardly grouped into two classes and six types. Unlike other CRISPR systems, the crRNAs in type III and type VI recognize complementary target RNA and the effectors complex cleave the associated target RNA. In type III, the crRNA-dependent target RNA binding also activates non-specific single-stranded DNA cleavage and cyclic oligoadenylate (cOA) generation. In type VI, Cas13a exhibits both target and "collateral" cleavage upon target RNA binding. We report a series of structures of Cas13a and Csm in complex with crRNA and its complementary target strand. Our studies revealed how Cas13a of type VI CRISPR systems defend against RNA phages and sent the stage for its development as a tool for RNA manipulation. In addition, our structural studies provide crucial insights into the mechanistic processes required for crRNA-meditated sequence-specific RNA cleavage, RNA target-dependent, non-specific DNA cleavage, and cOA generation by type III-Csm complex.

S-1-12 m^{6}A RNA modification: mechanism, function and social implications

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As the most abundant modification on mRNAs, N^6-methyladenosine (m^{6}A) has been recently identified as an important regulators for many essential biological processes. m^{6}A modification usually occurs on adenosine within RRACH motifs, but usually only a small proportion of adenosine within the RRACH motif are methylated at certain cell stage. We have identified that the selectivity of m^{6}A modification sites are regulated by miRNAs via sequencing pairing, revealing a novel function of miRNAs in regulating

mRNA epigenetic modification. We also systematically characterized the function of m^6A modification in regulating mouse cerebellum development and functions. Intriguingly, we found that the formation of m^6A modification can enhance the efficacy of hippocampus-dependent memory consolidation by regulating early-response genes, yet excessive training can somehow compensate the function of m^6A in regulating long term memory formation.

S-1-13 Decoding the Regulation and Function of RNA modifications from Epitranscriptomic and Epigenomic Data

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N6-methyladenosine (m^6A) is the most prevalent internal post-transcriptional modification in human transcriptome and has been shown to have important roles in various normal and pathological processes. However, the process by which m^6A is deposited on mRNAs is largely unknown. Here we developed a serial of computational and experimental methods to decode the regulation of m^6A methylation from epigenomic and epitranscriptomic data and demonstrated that histone H3 trimethylation at Lys36 (H3K36me3), a marker for transcription elongation, guides m^6A deposition globally. Comparative analyses of ChIP-seq data for H3K36me3 and m^6A-seq data revealed that majorities of m^6A peaks overlapped with H3K36me3 sites and that the overlapping sites were enriched near stop codons. We also found that m^6A sites identified from miCLIP are enriched in the vicinity of H3K36me3 peaks and are reduced globally when cellular H3K36me3 is depleted. Furthermore, we show that a significant genome-wide correlation between chromatin binding of METTL14 to H3K36me3. Mechanistically, H3K36me3 is recognized and bound directly by METTL14, a crucial component of the m^6A methyltransferase complex (MTC), which in turn facilitates the binding of the m^6A MTC to adjacent RNA polymerase II, thereby delivering the m^6A MTC to actively transcribed nascent RNAs to deposit m^6A co-transcriptionally. The discovery of interplay between modified histones and RNA methylation represents a new regulatory layer, and an additional level of complexity, in the control of gene expression.

S-1-14 A novel tRNA modification enzyme plays roles in both transcription and translation

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Abstract

Transfer RNAs (tRNAs) are extensively modified by a variety of tRNA modification enzymes; however, the biology role of tRNA modification and tRNA enzymes in remains poorly explored. Here we identified that coiled coil domain containing 76 (CCDC76) is responsible for 2'-O-methylation at position 4 of five human cytosolic tRNAs. CCDC76 locates to both cytoplasm and nucleus. Knockdown of CCDC76 will decrease the global protein translation level due to loss of enzymatic function of CCDC76. Loss-/gain-of-function studies show that CCDC76 regulates cell migration and lung metastasis of the MDA-MB-231 tumors independent of its enzymatic activity. We further find out that nuclear CCDC76 co-localizes with chromatin and can directly bind to specific DNAs. Transcriptomics studies suggest that CCDC76 promotes Epithelial-Mesenchymal transition by regulating ZEB1/SNAIL1 via the TGF-β1/Smad pathway. Mechanismlly, CCDC76 regulates the transcription of *tgf-b1* through binding to its promoter region and collaborating with some other transcription factors. Clinically, the expression level of CCDC76 is increasing during breast and pancreatic cancer progression. Moreover, the up-regulation of CCDC76 acts as adverse prognosis factors for survival rate of breast cancer patients in stage 2. Our study reveals a novel tRNA modification enzyme plays roles on both translation and transcription through either its classical function or non-canonical function independent of enzymatic activity. A recent pioneer study suggests that many RBPs could regulate transcription through RNAs. However, the mechanism of CCDC76 playing role in transcription is quite special; CCDC76 harbors a zinc finger domain to bind DNA without the participation of RNA. To our knowledge, there are few proteins have been reported to be capable of binding to both RNA and DNA directly.

S-1-15 Identification of Flavin Mononucleotide as a Cell-Active Artificial N^6-Methyladenosine RNA Demethylase

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N^6-Methyladenosine (m^6A) represents a common and highly dynamic modification in eukaryotic RNA that affects various cellular pathways. Natural dioxygenases such as FTO and ALKBH5 are enzymes that demethylate m^6A residues in mRNA. Herein, the first identification of a small-molecule modulator that functions as an artificial m^6A demethylase is reported. Flavin mononucleotide (FMN), the metabolite produced by riboflavin kinase, mediates substantial photochemical demethylation of m^6A residues of RNA in live cells. This study provides a new perspective to the understanding of demethylation of m^6A residues in mRNA and sheds light on the development of powerful small molecules as RNA demethylases and new probes for use in RNA biology.

Key Words: artificial enzymes, flavin mononucleotide, m^6A RNA, RNA demethylase, RNA demethylation

S-1-16 Sorting of Nascent RNAs into the Export or the Degradation Pathway

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In eukaryotes, RNA polymerase II transcribes many different types of RNAs. Although these RNAs share apparent structural similarities, the fates of them can be drastically different. Some are exported to the cytoplasm, some are degraded rapidly after transcription, and some function in the nucleus. Accumulating evidence suggests that nucleocytoplasmic distribution can play a determinant role in RNA functions. Noncoding RNAs can associate with translation machinery when transported into the cytoplasm, whereas nuclear retained mRNAs can regulate gene expression as noncoding RNAs. Thus, understanding the

molecular principles and biological relevance for sorting newly transcribed RNAs into the export or the degradation pathway is of fundamental significance. To understand this, we have been focused on studying two closely related aspects of gene expression: mechanism and regulation of RNA sorting; the complex interconnection between mRNA processing and export. We revealed an important mechanism for nuclear RNA sorting (EMBO J, 2017; Nucleic Acids Res, 2018), and uncovered NRDE2 as a key regulator for this sorting (Genes Dev, 2019) that ensures efficient assembly of export-competent mRNPs in nuclear speckles (J Cell Biol, 2018). Equally importantly, we identified the general roles of export factors in coordinating pre-mRNA processing and nuclear export for both polyadenylated RNAs (Mol Cell, 2019) and non-polyadenylated mRNAs (EMBO J, 2019). Interestingly, we also uncovered a novel mechanism that ensures the balanced pre-mRNA processing and mRNA export (Proc Natl Acad Sci, USA, 2019). In this presentation, I will introduce and discuss these recent work.

S-1-17 RNA Programs to Control Neuronal Reprogramming

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Direct conversion of somatic cells into neurons holds great promise for regenerative medicine. However, neuronal conversion is relatively inefficient in human cells compared to mouse cells. It has been unclear what might be the key barriers to reprogramming in human cells. We recently elucidated an RNA program mediated by PTB to convert mouse embryonic fibroblasts (MEFs) into functional neurons. In human adult fibroblasts (HAFs), however, we unexpectedly found that invoking the documented PTB–REST–miR-124 loop generates only immature neurons. To get mature and functional neurons, it is critical to sequentially inactivate PTB and the PTB paralog nPTB in HAFs. Inactivation of nPTB triggers another self-enforcing loop essential for neuronal maturation, which comprises nPTB, the transcription factor BRN2, and miR-9. I will show our recent progress about how nPTB represses BRN2 transcription to control neuronal maturation, and how to modulate this RNA program to enable deterministic reprogramming of HAFs into functional neurons.

S-1-18　对lincRNAs在动物中生理功能的系统研究

单革

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从一个物种的一类长链非编码RNA生理功能来认知非编码RNA具有一定的意义。我们通过CRISPR-cas9 系统对秀丽线虫中155个基因间的长链非编码RNA(lincRNA)进行逐一敲除（秀丽线虫已知的全部lincRNA共170个），系统地研究了秀丽线虫中lincRNA的功能。这也是第一次在多细胞动物中在全基因组水平上、对一种特定类型长非编码RNA进行敲除并系统分析其生理功能及功能机理的研究。对155个lincRNA敲除突变体进行六个方面表型的筛选发现23个lincRNA突变体分别在其中一个或者两个生理表型上具有不同程度的缺陷。通过对秀丽线虫不同发育时期的转录组测序进行分析，研究了lincRNA 和mRNA共表达情况，同时建立了lincRNA和microRNA共表达及调控网络。对秀丽线虫不同发育时期近300个转录因子的ChIP-seq分析来探究转录因子在线虫不同发育阶段对lincRNA的调控进行了分析。对这23个在本次研究中具有表型的lincRNA如何行使其生理调控功能的机理也进行了研究。本研究系统地探索了lincRNA在多细胞动物中的生理功能，一定程度拓宽了lincRNA研究领域。

S-1-19　Orphan snoRNA SNORA73 links canonical snoRNP NHP2 and non-canonical PARP1 to regulate myeloid cell differentiation

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Small nucleolar RNAs (snoRNAs) has been well established as guiding sequence-specific chemical modification of ribosomal and small nuclear RNAs. However, the targets or ascribed functions of a subgroup of snoRNAs, namely orphan snoRNAs, remain largely elusive. Specifically, orphan snoRNAs with more than 200 nucleotides resemble long non-coding RNAs(lncRNAs), and possess more complex structural features than canonical snoRNAs, suggesting the possibility of alternative cellular function. Here，we reported an H/ACA box snoRNA, SNORA73, which was downregulated in myeloid leukemia patients and promoted the differentiation of myeloid cells. SNORA73 binds chromatin and functions as a

caRNA. The non-canonical structure of its 5'end specifically binds novel small nucleolar ribonucleoprotein (snoRNP) Poly(ADP-ribose) Polymerase-1 (PARP1) and is sufficient for promoting cell differentiation in myeloid cells. Mechanically, SNORA73 regulates differentiation by inhibiting PARP1 self-PARylation, which in turns impairs DNA damage repair. The inhibitory effect on PARP1 self-PARylation is illustrated by enhancing the interaction between canonical snoRNP NHP2 and PARP1, by which NHP2 binds the self-modification domain of PARP1 to exclude the addition of poly(ADP-ribose). The linking non-canonical snoRNP PARP1 to non-canonical PARP1 by SNORA73 suggests more diverse roles of canonical snoRNP than previously thought.

S-1-20 A Novel hMTR4-PDIA3P1-miR-125/124-TRAF6 Regulatory Axis in NF-κB Signaling and Chemoresistance

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We found that lncRNA PDIA3P1 was up-regulated in multiple cancer types and upon treatment with DNA-damaging chemotherapeutic agents, like doxorubicin (Dox). Higher PDIA3P1 level was associated with poorer recurrence-free survival of human hepatocellular carcinoma (HCC). Both gain- and loss-of-function studies revealed that PDIA3P1 protected cancer cells from Dox-induced apoptosis and allowed tumor xenografts to grow faster and to be more resistant to Dox treatment. Mechanistically, miR-125a/b and miR-124 suppressed the expression of TRAF6, but PDIA3P1 bound to miR-125a/b/miR-124 and relieved their repression on TRAF6, leading to activation of NF-κB pathway. Consistently, the effect of PDIA3P1 inhibition in promoting Dox-triggered apoptosis was antagonized by silencing IκB α or overexpressing TRAF6. Administration of NF-κB inhibitor attenuated PDIA3P1-induced resistance to Dox treatment in mouse xenografts. Moreover, up-regulation of PDIA3P1 was significantly correlated with elevation of TRAF6, phosphorylated p65, or NF-κB downstream anti-apoptosis genes in human HCC tissues. These data indicate that enhanced PDIA3P1 expression may confer chemoresistance by acting as a miRNA sponge to increase TRAF6 expression and augment NF-κB signaling. Subsequent investigations into the mechanisms of PDIA3P1 up-regulation revealed that hMTR4, which promotes RNA degradation, could bind to PDIA3P1, and this interaction was disrupted by Dox treatment. Overexpression of hMTR4 attenuated Dox-induced elevation of PDIA3P1, whereas silencing hMTR4 increased PDIA3P1 level, suggesting that Dox may up-regulate PDIA3P1 by abrogating the hMTR4-mediated PDIA3P1 degradation. *Conclusion:* There exists a novel hMTR4-PDIA3P1-miR-125/124-TRAF6 regulatory axis that promotes NF-κB signaling and chemoresistance, which may be exploited for anticancer therapy.

S-1-21　非编码环RNA与血管疾病

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《中国心血管病报告2018》指出我国心血管病死亡率仍居首位，高于肿瘤及其他疾病。动脉粥样硬化性血管疾病仍处于逐年上升趋势。探究血管疾病发生发展的分子机制、发现新的药物靶点仍然是基础研究的热点。非编码环RNA（circRNA）是一类通过mRNA前体剪接、反向连接成环的转录本，在人的血管平滑肌中已经鉴定出7000余种circRNA，但其在血管疾病发生发展中的作用尚不清楚。我们近期研究证实，circRNA是一类新的调节平滑肌细胞表型转化和介导血管细胞通讯的活性因子，以多种不同的分子机制，通过调控mRNA稳定性、转录因子核转位和亲本基因表达活性等生物学过程，进而影响细胞凋亡、增殖和炎症应答活性，参与缺血后动脉生成、血管内膜增生和主动脉瘤形成等重大疾病的发生发展。

我们发现，人SIRT1外显子2-7可共价闭合形成 circSirt1；体外研究显示，无论在哪种表型的平滑肌细胞中，circ-Sirt1的表达均与SIRT1蛋白表达成正相关。circ-Sirt1可吸附miR-132/212发挥分子海绵效应，从而部分解除miR-132/212与SIRT1 mRNA 3’UTR的结合及模板抑制作用上调SIRT1表达，后者可通过使p65脱乙酰化失活，进而抑制炎症关因子表达。另外，circ-Sirt1主要定位于细胞浆中，过表达的circ-Sirt1通过与NF-κB p65结合，从而阻止TNF-α诱导的NF-κB核转位，进而抑制炎症相关因子表达。总之，circ-Sirt1通过两种不同的机制抑制NF-κB激活和血管炎症应答：一方面抑制胞浆p65核转位激活；另一方面，通过上调SIRT1表达，促进核p65脱乙酰化失活，进而抑制炎性因子表达和血管炎症。circ-Sirt1可能是血管损伤的新的循环标志物。

我们还证实，过表达SIRT1的血管平滑肌细胞可通过分泌circZFP609，后者结合并抑制缺氧诱导的HIF1α核转位激活，导致缺血后血流恢复延迟，内皮血管新生和动脉生成障碍。

我们发现炎性细胞诱导平滑肌细胞凋亡的新机制，证实平滑肌细胞可调控巨噬细胞极化。SM22α缺失诱发腹主动脉瘤（AAA）形成，血管炎症加重，VCAM-1表达增加，后者招募并激活巨噬细胞浸润。活化的巨噬细胞分泌circRasGEF1B，后者可进入平滑肌细胞并介导凋亡重编程。circRasGEF1B通过上调ZFP36表达，并引导ZFP36结合并降解Bcl2 mRNA，进而诱导平滑肌细胞凋亡，促进腹主动脉瘤形成。

关键词：环RNA、血管炎症、动脉生成、血管新生、动脉瘤

S-1-22　非编码RNA在脑硫脂调控肝癌细胞整合素αV表达过程中的作用研究

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整合素αV亚基（*ITGAV*）与整合素β3亚基形成αVβ3异二聚体，后者被证明与多种肿瘤的生长、转移、血管新生、表皮细胞间充质转化等恶性表型有关。在研究脑硫脂促进*ITGAV*表达的过程中，我们发现长链非编码RNA（AY）在脑硫脂处理后的肝癌细胞中显著高表达。对含56对肝癌组织的组织中进行AY检测，结果发现37对（占66%）的肝癌病人癌组织中的AY水平显著高于其癌旁组织中AY；此外，在来自TCGA数据库的248对肝癌与癌旁配对的病人组织中，肝癌组织中的AY表达量也显著高于其对应的癌旁组织。进一步对另一组80例肝癌组织和TCGA数据库中肝癌病例的临床数据分析，发现AY高表达与肝癌病人的不良预后紧密相关，与肿瘤大小、分期和转移情况密切相关。为了探究AY调控*ITGAV*表达的机制，我们运用荧光素酶报告实验证明AY能增强*ITGAV*基因核心启动子的转录活性，干扰AY则降低该启动子的转录活性。此外我们运用质谱和蛋白芯片技术鉴定到与AY特异结合的蛋白是组蛋白H1FX。通过RNA Pull-down进一步验证AY与H1FX的确直接结合，不过缺失AY371-522这段序列后， AY就不能与H1FX相互结合，这表明AY与H1FX主要是通过AY371-522这段序列结合，而且缺失AY371-522的AY也丧失了促进*ITGAV*的作用。染色体免疫共沉淀实验结果表明H1FX在*ITGAV*基因上广泛分布（包括启动子区和编码区），其中在编码区的结合总体上高于在启动子区的结合，而在转录核心启动子区（-672~-492及-894~-677）的结合高于更远的启动子区（-1241~-1128），AY过表达后H1FX在*ITGAV*基因上的结合显著降低，而RNA聚合酶II在*ITGAV*基因上的结合丰度显著上调，此外转录激活的表观遗传标志H3K4Me3和H3K9/14Ac在转录核心启动子区的丰度明显升高。我们因此认为AY促进*ITGAV*转录，AY与H1FX结合使H1FX与DNA的结合减弱，染色质的高级结构松弛，H3K4Me3的甲基转移酶和H3K9/14Ac乙酰基转移酶与核心组蛋白结合对H3进行甲基化和乙酰化修饰，从而使DNA与核小体的结合松弛，染色质局部展开，RNA聚合酶II和多种转录因子子结合启动子区从而促进*ITGAV*的转录。

关键词：肝癌；整合素αV；长非编码RNA

S-2-01　核糖代谢失调在糖尿病脑病中的作用

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自从1815 年，法国化学家Chevreul发现糖尿病患者尿液中含有葡萄糖以来，历时200多年，糖尿病被认为是一组以慢性血葡萄糖(简称血糖)水平增高为特征的代谢病群。还原糖对蛋白质的非酶糖化形成具有细胞毒性的糖基化终末产物(AGEs)，是糖尿病并发症发生发展的重要原因。然而，广泛存在于机体内的核糖(D-ribose)，在糖尿病及其并发症的研究中却被忽略。作者在对1型和2型糖尿病临床患者和同龄正常人体内的核糖及葡萄糖进行了比较。结果显示，糖尿病组体内的核糖显著高于正常对照组；糖尿病患者的尿和血核糖与糖化血红蛋白，特别是糖化血清蛋白的相关性高于葡萄糖；在相同条件下，核糖与血清蛋白反应的一级动力学常数大于葡萄糖两个数量级。通过服用或腹腔注射，建立核糖造成小鼠空间认知损伤的模型。蛋白质核糖糖化产物的细胞毒性远较葡萄糖糖化产物显著；核糖容易透过血脑屏障，引起中枢神经系统蛋白质的迅速糖化和AGEs的累积。从而导致神经细胞代谢失调，甚至死亡。动物行为学的研究表明，口服6个月或腹腔注射核糖3个月以上，能够明显导致大鼠空间认知损伤。而葡萄糖对照组，则没有表现出对大鼠的认知损伤。STZ-糖尿病大鼠模型，同样表现出核糖代谢失调和认知损害。这些结果提示，糖尿病不但存在葡萄糖代谢失调，而且存在核糖代谢失调。核糖代谢失调可能是二型糖尿病并发症，特别是糖尿病脑病的重要危险因素。

关键词：核糖，糖尿病，认知损伤，HbA1c, 糖化血清蛋白

S-2-02　一个抑制肿瘤转移的代谢小分子

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代谢异常是恶性肿瘤的重要特征。癌症相关基因的突变造成了细胞内多条信号通路的改变，从而影响肿瘤细胞的代谢，并重塑肿瘤细胞，以增强其存活和生长能力。实际上，肿瘤细胞需要改变代谢的状态来应答癌基因信号通路传递的增殖信号。除此之外，异常的肿瘤微环境也能进一步改变肿瘤细胞的代谢行为，从而影响肿瘤的发生发展及对治疗的反应性。然而，这些异常的代谢如何支撑肿瘤转移却鲜为人知。

UGDH是糖醛酸途径的限速酶，可以催化尿苷二磷酸葡萄糖（UDP-Glc）反应生成尿苷二磷酸

葡萄糖醛酸（UDP–GlcUA）；后者可以作为细胞合成糖胺聚糖的原料。我们研究发现在肺癌细胞中尿苷二磷酸葡萄糖脱氢酶（UGDH）第473位酪氨酸（Y473）发生了磷酸化。磷酸化的UGDH可与HuR结合，并将UDP–Glc转化为UDP–GlcUA，从而削弱了UDP–Glc对HuR与*SNAI1* mRNA结合的抑制，增强了*SNAI1* mRNA稳定性及蛋白表达；*SNAI1*表达的升高增强了肿瘤细胞迁移能力，进而促进了肺癌转移。此外，UDP–Glc水平与肺癌患者的转移复发密切相关。

本研究揭示了UDP–Glc抑制肿瘤的新功能，阐述了代谢小分子调控蛋白质功能的新模式，建立了细胞代谢与RNA稳定性调控的新连接，为肺癌转移的诊疗提供了新的生化标志物及干预策略。

S-2-03 Amino Acids Sensing and the Regulation of the mTOR Signaling

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The mechanistic target of rapamycin complex 1 (mTORC1) is a master regulator of cell growth that responds to a diverse set of environmental cues, including amino acids. Deregulation of mTORC1 has been linked with metabolic diseases, cancer and aging. In response to amino acids, mTORC1 is recruited by the Rag GTPases to the lysosome, its site of activation. We will discuss our recent progress in the identification of new regulators of mTOR pathway, and their functions in mediating amino acids sensing.

Key Words: mTOR, metabolism, nutrient sensing

S-2-04 Skeletal Muscle-Secreted Lipids Regulates Metabolic Homeostasis by Mediating Muscle-Fat Crosstalk

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Abstract

Skeletal muscles play central roles in orchestrating organismal energy homeostasis. However, the molecular mechanisms underlying this globally metabolic regulation of skeletal muscle remain a conundrum. Increasing lines of evidence have demonstrated the functional significance of skeletal muscle-secreted factors in regulating whole body energy homeostasis by actively communicating with other metabolic organs such as fat and liver. Only a few numbers of the the muscle-secreted factors with the metabolicaly regulatory role have been reported. Therefore it is critical to identify the muscle-secreted factors in order to understanding the molecular mechaism of skeletal muscle in orchestrating inter-organ crostalk and developing potential drug for treating metabolic diseases such as obesity and type II diabetes mellitus. Herein, we for the first time demonstrate skeletal muscle-released lipids functionally involve muscle-fat crosstalk to attenuate HFD-induced obesity by inducing white fat browning. The findings uncover the unanticipated role that lipid species serve as a promising agent for preventing and treating obesity in humans.

S-2-05 Chromatin remodeling checkpoint of metabolic regulation in health and disease

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Abstract

Metabolic syndrome has become a global epidemic that adversely affects human health. Both genetic and environmental factors contribute to the pathogenesis of metabolic disorders. However, how these factors

interact and act in concert to control the metabolic gene programs and the development of metabolic disorders remains unclear. Accumulating evidence suggests that ATP-dependent SWI/SNF chromatin-remodeling factors are critical in this process by sensing and integrating the environmental cues and directing metabolic reprogramming and adaptation, thereby maintaining nutrient and energy homeostasis. The SWI/SNF chromatin-remodeling complexes comprise up to 11 subunits, among which the BAF60 subunit serves as a linker between the core SWI/SNF complexes and specific transcriptional factors. BAF60 subunit has three isoforms, BAF60a, b, and c. The distinct tissue distribution patterns and regulatory mechanisms of BAF60 proteins confer each isoform with specialized functions in different metabolic cell types. Today, I will discuss our recent discoveries on the important roles and underlying mechanisms of BAF60 proteins in the regulation of nutrient sensing and energy metabolism under physiological and disease conditions.

S-2-06 Advances on the road map from glucose starvation to AMPK activation

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AMPK and mTOR are the dichotomy of kinase complexes that plays a pivotal role in maintaining metabolic homeostasis. We have delineated the pathway that senses falling levels of glucose and activates AMPK. In low glucose, v-ATPase-bound aldolase senses the absence of the glycolytic product of fructose-1,6-bisphosphate (FBP) and induces conformational changes in v-ATPase, allowing AXIN in complex with the LKB1 kinase to translocate to the surface of lysosomes and activates the lysosome-residing AMPK, and concomitantly switches off mTORC1. More recently, we have identified that the ER-localized TRPV calcium channels relays the FBP-unoccupied state of aldolase to AMPK activation. We have also found that pools of AMPK, localized in different subcellular compartments, are regulated differentially. The seminar will elaborate on the significance of the findings and will discuss about how we shall approach enzymology through new avenues.

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S-2-07 Critical Role of SENP2 in Development of NAFLD

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Non-alcoholic fatty liver disease (NAFLD), ranging from hepatic steatosis to non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and eventually hepatocellular carcinoma, is a major type of metabolic disorders with severe threaten to public health. Aberrant triglycerides (TGs) accumulation in the liver is a hallmark feature of NAFLD, which is tightly regulated by de novo lipogenesis, fatty acid uptake, fatty acid oxidation and fatty acid export. Small ubiquitin-like modifier (SUMO)-specific protease 2 (SENP2) is responsible for protein deSUMOylation. Previous study showed that SENP2 controls adipogenesis and is essential for development of both white and brown adipocytes. Additionally, SENP2 is reported as a master regulator for insulin sensitivity in skeletal muscles. However, effect of SENP2 on hepatic metabolism remains unknow. To address this problem, we generated hepatocellular specific SENP2 knockout mice by crossing $SENP2^{loxp}$ mice with Alb-Cre mice ($SENP2^{LKO}$). We challenged $SENP2^{LKO}$ and WT mice with high fat diet and found that $SENP2^{LKO}$ mice could protect against HFD induced obesity and hepatic steatosis. Further investigation showed that SENP2 ablation inhibited gene expression involved in de novo lipogenesis and promoted genes related with fatty acid oxidation. In addition, SENP2 disruption repressed TG storage in primary hepatocytes induced by FFA treatment. Consistently, SENP2 overexpression in liver by AAV injection facilitated occurrence of NAFLD. Our observation shed light on critical role of SENP2 in development of hepatic steatosis and provided potential target for NAFLD intervention.

S-2-08 Kmt5c is required for adapative thermogenesis in brown and beige adipocytes

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Brown adipose tissue (BAT) and beige adipocytes dissipate energy by producing heat to maintain body

temperature in a process called adaptive thermogenesis. Histone methylation dictates chromatin structure or affects the recruitment of non-histone proteins to chromatin, influencing many fundamental biological processes. Methylation on Histone H3K4, H3K9, H3K27 and H3K36 was reported to modulate brown and beige adipocytes functions, but little is known about the effects of Histone H4K20 methylation on thermogenic adipose program. Here we find the expression of Kmt5c, a H4K20 methyltransferase, is dramatically induced by cold exposure or β3-adrenergic signaling in adipose tissues. Kmt5c is required for Ucp1 and Cidea expression in both brown and beige adipocytes but not for adipogenesis per se, as indicated by the fact that knockdown of Kmt5c significantly downregulates expression of Ucp1 and Cidea. Adipose tissue-specific Kmt5c knockout mice show lower Ucp1 expression in BAT at thermoneutrality and also in iWAT at room temperature. Importantly, Kmt5c KO mice are prone to high-fat-diet-induced obesity. Therefore, our results indicate Kmt5c may work as a new pharmacological intervention target for the treatment of obesity and the related diseases.

S-2-09 Neuronal regulation of adipose tissue metabolism

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Peripheral metabolism is tightly regulated by the nervous system, and dysregulation could lead to metabolic disorders. We have focused our study on neuronal regulation of adipose tissue biology and systemic metabolism. We established and exploited the volume fluorescence-imaging technique to document the neural network at single-fiber resolution on the whole-tissue level for adipose tissues. We have observed high density of sympathetic arborizations in white adipose tissue which play critical roles in metabolic homeostasis. We have optimized and applied the volume imaging technique to visualize the entirety of the vascular network at a single-capillary resolution. We further showed that the vascular remodeling depends on the sympathetic-derived catecholamine signal.

S-2-10　LGR4-Wnt通路与肥胖发生

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Wnt信号通路广泛参与机体发育与代谢过程。LGR4是一个新发现的G蛋白偶联受体，其病理生理功能、临床意义并不清晰。在过去14年的工作中，我们课题组围绕LGR4与内分泌代谢病，借助多种基因修饰小鼠模型，从基础到临床，发现LGR4促参与生殖、血压调控外，还在前体脂肪细胞命运决定中发挥关键性作用，进而影响米色脂肪的形成和能量平衡。有意思的是，通过对大规模人群测序，我们发现了多个人类LGR4突变，其中部分激活突变与人类中心性肥胖、胰岛素抵抗密切相关。结合deCODE研究团队发现的LGR4失活突变个体的临床特征，我们比较明确的阐述了LGR4在体脂调控与肥胖发生中的重要作用。最近的研究表明，LGR4可能参与辅助经典Wnt信号通路，为此，我们在这一方向进行了初步探讨。寄希望，我们的工作能够为未来临床肥胖症、代谢性疾病的治疗提供一个新的精细诊断手段、或潜在的干预靶点。

S-2-11　CDS2 Deficiency Converts Outcome of VEGFa Signaling from Angiogenesis to Vascular Regression

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Response of endothelial cells to signaling stimulation is critical for vascular morphogenesis, homeostasis and function. Vascular endothelial growth factor-a (VEGFa) has been recognized as a pro-angiogenic factor in vertebrate developmental, physiological and pathological conditions for decades. Here we show

that genetic ablation of CDP-diacylglycerol synthetase-2 (CDS2), a metabolic enzyme that controls phosphoinositide recycling, can alter the output of VEGFa signaling from angiogenesis to unexpected vessel regression. Live imaging analysis uncovered the presence of angiogenic endothelium reverse migration in cds2 mutant zebrafish upon VEGFa stimulation, and endothelium regression also occurred in postnatal retina and implanted tumor models in endothelium-specific CDS2 knockout mice. Mechanistically, VEGFa stimulation reduced phosphatidylinositol (4,5)-bisphosphate availability in the absence of CDS2-controlled-phosphoinositide metabolism, subsequently causing phosphatidylinositol (3,4,5)-triphosphate (PIP3) deficiency and FOXO1 activation to trigger regression of CDS2-null endothelium. Thus, our evidence indicates that the effect of VEGFa on vasculature is context dependent rather than always pro-angiogenic as previously believed.

Key words: vessel regression, phosphoinositide, VEGFa, CDS2, FOXO1, zebrafish

S-2-12 The protein phosphatase 1 complex is a direct target of AKT linking insulin signaling to hepatic glycogen deposition

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Insulin-stimulated hepatic glycogen synthesis is central to glucose homeostasis. Here, we identify that PPP1R3g, a regulatory subunit of protein phosphatase 1 (PP1), is directly phosphorylated by AKT. PPP1R3g phosphorylation oscillates with fasting-refeeding cycle, and is required for insulin-stimulated dephosphorylation (hence activation) of glycogen synthase (GS) in hepatocytes. We show that knockdown of PPP1R3g significantly blunted insulin response. Introduction of wild-type PPP1R3g, but not phosphorylation-defective mutant, leads to increase blood glucose clearance, hepatic glycogen deposition, and improved insulin sensitivity *in vivo*. Mechanistically, phosphorylated PPP1R3g displays increased binding for, and promotes dephosphorylation of, phospho-GS. Furthermore, PPP1R3b, another regulatory subunit of PP1 binds to the dephosphorylated GS, thereby relaying insulin stimulation to hepatic glycogen

deposition. Importantly, this PP1-mediated signaling cascade is independent of GSK3. We have thus revealed a novel regulatory axis consisting of insulin/AKT/PPPP1R3g/PPP1R3b that operates in parallel to the GSK3-dependent route, controlling glycogen synthesis and glucose homeostasis in insulin signaling.

Key Words: Glycogen synthesis, hepatocytes, insulin signal, postprandial glucose regulation, protein phosphorylation.

S-2-13 AMPK monitors sufficiency and controls utilization of amino acids

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AMP-activated protein kinase (AMPK) is a crucial cellular energy sensor that is activated by falling energy status. AMPK exists as heterotrimeric complexes comprising each of a catalytic α1 or α2, a regulatory β1 or β2, and a γ1 or γ2 or γ3 subunits. The α1 or α2 subunits are both activated by threonine 172 (T172) phosphorylation and their functions seem redundant. Why AMPK complex needs two redundant catalytic subunits remains unclear. We show that the α2 subunit of AMPK monitors sufficiency and controls utilization of amino acids. We demonstrate that T172 of α2, but not that of α1, is a substrate of general control nonderepressible 2 (GCN2), a serine/threonine-protein kinase that senses amino acid deficiency through binding to uncharged transfer RNA. T172 of α2 can be phosphorylated either by GCN2 under amino acids withdraw even when cells are cultured in glucose-rich media or by nutrients-starvation, whereas T172 of α1 can only be phosphorylated by nutrients-starvation. Deletion of α2, but not α1 resulted in decreased cellular levels of amino acids and increased levels of proteins in cultured cells and in mice. We provide evidence to show that α1 and α2 subunits of AMPK complex distinct in their regulatory roles.

S-2-14 cMyc-mediated epigenetic regulation of cancer metabolic reprogramming

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It is known that cancer cells generally adapt a specific metabolic phenotype that is characterized by the switch to aerobic glycolysis, or Warburg Effect. While this metabolic phenotype is largely believed to be responsible for the growth advantage of various cancers, the underlying mechanisms are not very clear. This talk will be focused on our current progress related to cMyc-mediated epigenetic regulation of cancer metabolic reprogramming.

S-2-15 Exploring the role of p53 in cancer metabolism

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One of the major biochemical hallmarks of cancer cells is a metabolism switch whereby cancer cells consume more glucose and glutamine than normal tissue, and favor a much less efficient but rapid metabolic mechanism for energy production. Abnormal metabolic reprogramming and oncogene/tumour suppressor mutations are primary causes for many human cancer diseases. Of note, the metabolic rewiring of cancer cells can be linked to specific genetic alterations in oncogenes and tumor suppressor genes responsible for cell signaling. However, it still remains a mystery how this might happen and how exactly metabolism is altered by signal transduction changes in cancer cells and what advantages these alterations afford. Here, I will discuss a role of tumour suppressor p53 in metabolic regulation and a role of p53 as a metabolic sensor that integrate metabolic stresses with cell fate decisions. Briefly, we previously found that p53 may be a master regulator of cellular NADPH metabolism (*Nature Cell Biology,* 2011, *Nature* 2013). Loss of p53 results in elevated fluxes of both the pentose phosphate pathway and malic enzyme pathway, leading to increased biosynthesis and tumour growth. Recently, we discovered that urea cycle, a major metabolic pathway responsible for ammonia elimination, is transcriptionally suppressed by p53 (*Nature* 2019). Through this regulation, p53 represses ammonia excretion and tumour growth *in vitro* and *in vivo*. We also have define a role for ammonia in regulating polyamine metabolism via inhibition of ODC mRNA translation.

S-2-16　叶酸代谢稳态的多维度调控及其生理病理效应

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叶酸代谢循环为细胞提供DNA合成和甲基化的原料，是维持细胞生长、增殖和分化的重要代谢通路，其稳态失衡会增加先天性心脏病（先心）等多种疾病的发生风险。我们研究发现，叶酸代谢的稳态受到多维度的精细调控，任一环节的失调均会导致疾病的发生：1）叶酸跨膜转运。与叶酸代谢不直接相关的FIGN蛋白，可通过阻断还原型叶酸受体的蛋白酶体降解来促进叶酸的跨膜转运，在降低血浆叶酸浓度的同时降低先心风险。2）叶酸胞内代谢。叶酸代谢中系列代谢酶的遗传变异均通过抑制叶酸代谢效率、升高同型半胱氨酸（Hcy）来导致先心发生，提示Hcy是叶酸代谢稳态失衡致病的关键调控点。3）叶酸效应的拮抗。Hcy通过修饰蛋白赖氨酸残基来抑制细胞发育相关信号并诱发畸形，同型半胱氨酸修饰的催化酶甲硫氨酰tRNA合酶可在转录上被脂肪酸激活，升高细胞K–Hcy水平，拮抗叶酸对叶酸代谢稳态的维持并诱发类似叶酸缺乏的生理病理效应。上述发现揭示了叶酸代谢稳态的多维度调控，阐明了叶酸缺乏诱发疾病的分子机理，为先心等相关疾病的防控提供了全新思路。

S-2-17　Gut microbiota-bile acid-ceramide signaling orchestrates metabolic diseases

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The gut microbiota is associated with metabolic diseases including obesity, insulin resistance and fatty liver disease, as demonstrated by correlative studies and by transplantation of microbiota from obese humans and mice into germ-free mice. Modification of the microbiota by treatment of high-fat diet (HFD)-fed mice with tempol or antibiotics resulted in decreased adverse HFD-induced metabolic phenotypes. This is due to decrease bile salt hydrolase (BSH) activity. The lower BSH results in increased levels of tauro-β-muricholic acid, a substrate of BSH and a potent antagonist for the farnesoid X receptor (FXR). FXR intestine-null mice have lower serum ceramides, are metabolic fit and resistant to HFD-induced metabolic disease, and this is reversed by injection of C16:0 ceramide. Furthermore, through metagenomics and bacterial transplant studies from humans to mice, the bacterial species *B. fragilis* that is decreased by metformin.

B. fragilis through its BSH activity, was found to carry out deconjugation of glycine-conjugated bile acid metabolites in the gut leading to elevate glycoursodeoxycholic acid (GUDCA) levels. GUDCA is identified to inhibit intestinal FXR signaling and ameliorate insulin resistance; administration of HFD-fed diabetic mice with GUDCA prevents and treats insulin resistance similar to metformin. Lowering ceramide levels through inhibition of intestinal FXR is a feasible means to reduce metabolic diseases.

S-2-18 Neural control of metabolism mediated by a new GPCR messenger

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The autonomous nervous system coordinates metabolic hormone secretion in response to nutrient status, primarily via GPCR-based muscarinic and adrenergic signaling. The signaling network in target tissues/organs remains incompletely understood. We have identified an inositol polyphosphate metabolite synthesized upon GPCR stimulation via a kinase-phosphorylation axis. This metabolite promotes hormone secretion by facilitating SNARE-mediated exocytosis. Disrupting its synthesis has implications in the pathogenesis of obesity and diabetes. Thus, a previously unappreciated GPCR messenger is established which facilitates neural modulation of energy homeostasis.

S-3-01 SIP/CacyBP Promotes Autophagy by Regulating Levels of BRUCE/Apollon, which Stimulates LC3-I Degradation

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BRUCE/Apollon is a membrane-associated Inhibitor of Apoptosis Protein, which is essential for viability and has ubiquitin-conjugating activity. Upon initiation of apoptosis, the ubiquitin ligase, Nrdp1/RNF41, promotes proteasomal degradation of BRUCE. Here, we demonstrate that BRUCE together with the proteasome activator PA28γ causes proteasomal degradation of LC3-I and thus inhibits autophagy. LC3-I on the phagophore membrane is conjugated to phosphatidylethanolamine to form LC3-II, which is required for the formation of autophagosomes and selective recruitment of substrates. SIP/CacyBP is a ubiquitination-related protein that is highly expressed in neurons and various tumors. Under normal conditions, SIP inhibited the ubiquitination and degradation of BRUCE probably by blocking the binding of Nrdp1 to BRUCE. Upon DNA-damage by topoisomerase inhibitors, Nrdp1 caused monoubiquitination of SIP and thus promoted apoptosis. However, upon starvation, SIP together with Rab8 enhanced the translocation of BRUCE into the recycling endosome, the formation of autophagosomes, and BRUCE degradation by optineurin-mediated autophagy. Accordingly, deletion of SIP in cultured cells reduced the autophagic degradation of damaged mitochondria and cytosolic protein aggregates. Thus, by stimulating proteasomal degradation of LC3-I, BRUCE also inhibits autophagy. Conversely, SIP promotes autophagy by blocking BRUCE-dependent degradation of LC3-I and by enhancing autophagosome formation and autophagic destruction of BRUCE. These actions of BRUCE and SIP represent novel mechanisms that link the regulation of autophagy and apoptosis under different conditions.

S-3-02 蛋白泛素化在免疫系统系统的调控机制

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我们过去二十多年的研究主要集中在E3泛素连接酶在免疫系统的调控作用， 特别是在淋巴细胞的发育，活化和耐受诱导的作用机理方面。我们的工作表明E3泛素连接酶VHL–缺氧诱导因子

（HIF）途径通过调节干扰素γ产生来控制调节性T细胞的稳定性和功能（Immunity， Lee, 2015）。我们近来扩展到对于其他细胞类型的研究，包括先天性淋巴样2型（ILC2）细胞，并发现VHL–HIF轴通过平衡细胞内葡萄糖代谢来调节它们的发育和功能， 并对于控制肺部炎症的发生十分重要（Immunity，Li， 2018）。最近，我们也证明了肺泡巨噬细胞中VHL的基因缺失通过减轻ILC2细胞发育而导致2型免疫应答反应的降低（JEM， Zhang，2018）。在本次会议上我们将发表正在进行中的最新研究结果。

S-3-03　泛素化/类泛素化修饰在DNA损伤应答以及肿瘤细胞耐药中的调控作用机制

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哺乳细胞持续暴露在多种能够诱导DNA 损伤产生的因素下，单个细胞每天有多达10 万个DNA损伤位点的产生，其中DNA 双链断裂是各种DNA 损伤中最为严重的类型之一。如果DNA 损伤未被修复或者错误修复，就会导致基因组的不稳定性，甚至肿瘤的发生。为了维持基因组的稳定性，细胞利用DNA 损伤应答（DDR）这个复杂的信号通路来感应并及时修复损伤的DNA。另一方面，肿瘤细胞通过增强DNA 损伤修复的能力逃逸死亡，导致对于放化疗治疗的耐受。因此，DDR 在肿瘤的预防和治疗中发挥着双刃剑的作用。鉴定并阻断肿瘤细胞DDR 通路中的关键调控因子是提高肿瘤放化疗治疗效果的一个重要策略。

泛素化和类泛素化等蛋白质翻译后修饰在DDR调控中发挥重要的调控作用。我们鉴定了在DNA双链断裂修复等DDR通路中发挥作用的调控因子，发现它们分别通过精细调控组蛋白的泛素化和损伤修复蛋白的类泛素化水平来进一步影响下游损伤修复蛋白的招募，从而调控肿瘤细胞中DNA动态修复效率和基因组的稳定性。对临床肿瘤样本的分析发现，所鉴定的DDR调控因子在乳腺癌、白血病等肿瘤中异常表达，与肿瘤病人的耐药和预后具有密切关联。这些研究结果有助于深入了解DDR的精细分子调控机制，也为临床肿瘤的治疗提供了潜在的靶点。

关键词：泛素化修饰、SUMO化修饰、DNA损伤应答、肿瘤细胞耐药

S-3-04　蛋白质翻译后修饰与肿瘤微环境

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靶向肿瘤微环境是目前最有效的肿瘤治疗新手段。围绕肿瘤细胞感应肿瘤微环境中营养、生长、免疫以及机械应激等信号的调控机制，深入解析肿瘤发生发展中的内因和外因相互作用过程，我们发现了多个蛋白质翻译后修饰通过肿瘤微环境调控肿瘤发生发展的分子机制。在肿瘤微环境中，CD47在多种肿瘤细胞中高表达，通过结合巨噬细胞上的SIRPα，向巨噬细胞传递“don’t eat me”的信号从而实现免疫逃逸。靶向CD47– SIRPα的单克隆抗体处于临床研究阶段且具有很好的应用前景，但是对CD47– SIRPα通路的调控机制研究还不是十分清楚。在我们最新的研究中，我们通过CRISPR–Cas9全基因组筛选发现isoQC是CD47– SIRPα通路的重要调节因子。我们进一步发现isoQC可以把位于CD47 N端的谷氨酰胺修饰为焦谷氨酸，我们用特异性识别焦谷酰化的抗体证实了CD47 N端焦谷氨酸的形成。通过敲除及抑制剂处理肿瘤细胞发现isoQC可以调控CD47和 SIRPα的结合能力以及巨噬细胞的吞噬作用。总而言之，我们发现isoQC是CD47–SIRPα通路中重要的调节因子，其在肿瘤的免疫治疗中可以作为一个潜在的药物靶点。

S-3-05　Histone H3Q5 serotonylation stabilizes H3K4me3 and potentiates its readout by TAF3

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Serotonylation of glutamine 5 on histone H3 (H3Q5ser) was recently identified as a permissive posttranslational modification that coexists with adjacent lysine 4 trimethylation (H3K4me3).While the resulting dual modification, H3K4me3Q5ser, is enriched at regions of active gene expression in serotonergic neurons, the molecular basis underlying H3K4me3-H3Q5ser crosstalk remains unexplored. Herein, we examine the impact of H3Q5ser on the readers, writers, and erasers of H3K4me3. We show that the H3Q5ser modification promotes H3K4me3 binding to the PHD finger of TAF3, likely contributing to the potentiating effect of the dual-mark on the interaction with the general transcription factor TFIID. Strikingly, we additionally demonstrate that while the activity of the H3K4 methyltransferase, MLL1,

is unaffected by H3Q5ser, the corresponding H3K4me3 eraser, KDM5B is profoundly inhibited by the presence of the mark. Collectively, this work suggests that H3Q5ser plays distinct roles when coupled with the installation of H3K4me3 to fine tune downstream outputs via chromatin readers and erasers.

S-3-06　果蝇INAD光信号转导机器的组装与调控机制研究

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磷脂酶Cβ介导的光导蛋白质机器，对于非脊椎动物视网膜杆状体感光细胞以及脊椎动物的自主感光神经节细胞的光信号传导都非常重要。在脊椎动物中，自主感光神经节细胞中的磷脂酶Cβ光导蛋白质机器，通过向大脑控制昼夜节律的下丘脑视交叉上核发送光信息，从而对昼夜节律进行调节。研究表明，脊椎动物自主感光神经节细胞的磷脂酶Cβ光导蛋白质机器的组成成分，与非脊椎动物的杆状体感光细胞非常相似，但是目前二者装配与调控的分子机制仍不清楚，对于前者的研究更是处于起步阶段。报告人以果蝇INAD光信号转导机器为基本研究对象，综合运用分子生物学、生物化学、结构生物学、基因敲除动物模型、病毒侵染及膜片钳等技术，对果蝇INAD PDZ45结构域氧化还原反应动态调节光信号转导、INAD/TRP、INAD/NORPA（PLCβ）以及小鼠INADL/PLCβ4等蛋白复合体的互作分子机制进行了一系列探讨，初步揭示了INAD光导蛋白质机器组装与动态调节的分子机制，探讨了该蛋白质机器在模式动物果蝇以及小鼠光信号传导生理过程中的生物学功能。

关键词：光信号转导；蛋白质机器；蛋白质互作；蛋白质结构；INAD；氧化还原反应；PLCβ；TRP通道

S-3-07 The Immunological function of Manganese and its possible applications

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Manganese is one of the most abundant metals in mammals' tissues, and is required for a variety of physiological processes including development, reproduction, neuronal function, immune regulation and antioxidant defenses. Mn exerts its function by regulating various Mn-dependent enzymes, including oxidoreductases, isomerases, transferases, ligases, lyases, and hydrolases. It is also an essential component of some metalloenzymes such as Mn superoxide dismutase (SOD2), glutamine synthetase (GS), and arginase. Although Mn has been implicated in the host-bacteria interface, its function in innate immunity has never been reported. We discover an unexpected role of Mn^{2+} in alarming cells to viral infection by increasing the sensitivity of the DNA sensor cGAS and its downstream adaptor protein STING. Mn^{2+} was liberated from membrane-enclosed organelles and accumulated in the cytosol, and bound to cGAS by increasing both the dsDNA sensitivity and the enzymatic activity of cGAS. Mn^{2+} also significantly promoted STING's activity through the enhanced cGAMP-STING binding affinity. The liberated cytosolic Mn^{2+} thus greatly lowered the detection limit of host cells to dsDNA and virus by several orders of magnitude. Consequently, Mn-deficient mice produced significantly diminished cytokines and were highly vulnerable to DNA virus as $Sting^{-/-}$ mice did. Importantly, Mn-deficient $Sting^{-/-}$ mice displayed no further increased susceptibility to virus compared with Mn-sufficient $Sting^{-/-}$ mice. Reconstitution of cellular Mn in Mn-deficient cells effectively restored cells' responses to DNA virus. In addition, Mn^{2+} itself is a potent innate immune stimulator, inducing a strong type I-IFN and cytokine production in the absence of infection. Our results thus demonstrated for the first time that an element is critically involved and required for the host defense against virus.

Key words: Manganese; innate immunity; cGAS; STING; type-I interferons.

S-3-08 Iron sensitizes melanoma cells to ROS-induced pyroptosis

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Iron has been shown to trigger oxidative stress by elevating ROS and to participate in different modes of cell death, such as ferroptosis, apoptosis, and necroptosis. However, whether iron-elevated ROS is also linked to pyroptosis has not been reported. Here, we demonstrate that iron-activated ROS can induce pyroptosis via the Tom20-Bax-caspase-GSDME pathway. In melanoma cells, iron-enhanced ROS signaling initiated by CCCP causes the oxidation and oligomerization of the mitochondrial outer membrane protein Tom20 to recruit Bax to the mitochondria, which then facilitates cytochrome c release to the cytosol to activate caspase-3, eventually triggers pyroptotic death by inducing GSDME cleavage by caspase-3. Therefore, ROS acts as a causative factor and Tom20 senses ROS signaling for iron-driven pyroptotic death of melanoma cells. Since iron activates ROS for GSDME-dependent pyroptotic induction and melanoma cells specifically expresses high GSDME level, iron may be a potential candidate for melanoma therapy. Based on the functional mechanism of iron shown above, we further demonstrate that iron supplementation at a dosage used in iron-deficient patients is sufficient to maximize the anti-tumor effect of clinical ROS-inducing drugs to inhibit xenograft tumor growth and metastasis of melanoma cells through GSDME-dependent pyroptosis. Moreover, no obvious side effects are observed in the normal tissues and organs of mice during the combined treatment of clinical drugs and iron. This study not only sheds light on iron as a sensitizer switching ROS signaling to drive pyroptosis, but also implicates a novel iron-based intervention strategy for melanoma therapy.

S-3-09 Molecular delineation of BubR1 kinase signaling in cell fate determination

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Error-free mitosis depends on accurate chromosome attachment to spindle microtubules, powered congression of those chromosomes, their segregation in anaphase, and assembly of a spindle midzone

at mitotic exit. The centromere-associated motor CENP-E, whose binding partner is BubR1, has been implicated in congression of misaligned chromosomes and the transition from lateral kinetochore-microtubule association to end-on capture. Although previously proposed to be a pseudokinase, here we report the structure of the kinase domain of Drosophila melanogaster BubR1, revealing its folding into a conformation predicted to be catalytically active. BubR1 is shown to be a bona fide kinase whose phosphorylation of CENP-E switches it from a laterally attached microtubule motor to a plus-end microtubule tip tracker. Computational modeling is used to identify bubristatin as a selective BubR1 kinase antagonist that targets the αN1 helix of N-terminal extension and αC helix of the BubR1 kinase domain. Inhibition of CENP-E phosphorylation is shown to prevent proper microtubule capture at kinetochores and, surprisingly, proper assembly of the central spindle at mitotic exit. Thus, BubR1-mediated CENP-E phosphorylation produces a temporal switch that enables transition from lateral to end-on microtubule capture and organization of microtubules into stable midzone arrays. Given the prevalence of chromosome instability phenotype in BubR1 mutant-elicited gastric tumorigenesis, we developed a strategy to model context-dependent cell division using a combination of light sheet microscope and 3D gastric organoids which revealed that metazoans evolved an elaborate central spindle organization machinery to ensure accurate sister chromatid segregation during cytokinesis. Thus, metazoan BubR1-CENP-E signaling cascade constitutes a novel temporal machinery to determine cell fate plasticity.

S-3-10 The arms race between CRISPR adaptive immune systems of prokaryotes and anti-CRISPRs of phages

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绝大多数古细菌和一半左右的细菌编码编码CRISPR-Cas适应性免疫系统来对抗噬菌体的感染，在几十亿年的进化中，噬菌体进化出Anti-CRISPR系统来拮抗细菌CRISPR-Cas系统，从而感染细菌。Cpf1是目前已知的唯一一个具有核酸序列特异性的，且同时具有DNase和RNase活性的核酸酶。本研究通过结构生物学和生化研究手段揭示了CRISPR-Cpf1识别crRNA以及Cpf1剪切pre-crRNA成熟的分子机制（Nature, 2016），对阐明细菌CRISPR系统抵抗病毒入侵的分子机理具有重要的科学意义，而且为改造Cpf1系统，使之成为特异的、高效的基因编辑系统提供了结构基础（RNA Biology，2017）。通过解析BthC2c1/全长sgRNA/target dsDNA三元复合物的晶体结构，揭示C2c1结合sgRNA的分子机制，以及其与Cas9和Cpf1不一样的严谨型识别PAM DNA的分子机制，为改造C2c1以及其他Cas核酸内切酶，使之成为高效、特异的基因编辑工具提供结构基础(Cell Research，2017)。通过建立生化实验系统发现前噬菌体蛋白AcrIIA4直接结合SpyCas9-sgRNA并抑制SpyCas9核酸酶活

性。进一步结构生物学研究揭示AcrIIA4抑制SpyCas9活性的分子机制（Nature, 2017），这不仅对阐明细菌与噬菌体共进化分子机制具有重要意义，而且为设计时间、空间特异性地，或条件性地精确控制SpyCas9基因编辑活性的工具提供结构基础。通过生物信息学方法寻找到Cas12a的Anti蛋白AcrVA5，进一步生化实验分析表明，AcrVA5蛋白通过乙酰化K635抑制Cpf1/Cas12a活性，首次揭示Anti-CRISPR蛋白通过酶活性修饰Cas蛋白使之失活的分子机制，补充了噬菌体阻止和逃逸细菌CRISPR-Cas免疫防御系统的新策略（Nature Structural & Molecular Biology，2019）。

S-3-11 Pollen Tube Guidance in Flowering Plants: The Interplay between Male and Female Gametophytes

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During evolution, novel reproductive structures and mechanisms have evolved to adapt to terrestrial land environment in plants. In angiosperms, such evolutionary development is manifested by the flower, multicellular gametophyte, double fertilization, loss of sperm motility, and siphonogamy in which the immotile sperm was delivered to the egg by a pollen tube produced by the male gametophyte (pollen), a process named pollen tube guidance (PTG). Previous studies suggested that PTG requires the intimate interactions between the pollen tube and maternal tissue of the pistil and the female gametophyte respectively. Through genetic screen, we isolated a number of Arabidopsis mutants that disrupt PTG processes. *CCG*, a central cell-specifically expressed gene, is required for the female gametophyte to attract the pollen tube. *CCG* encodes a nuclear protein that regulates the expression of a number genes important for PTG via CBP1 which interacts with RNA polymerase II, the Mediator complex and AGL transcription factors in the central cells and also *LURE1* expression in the synergids indirectly. *POD1*, a pollen tube-expressed gene, is required for the male gametophyte to respond to the female signals. *POD1* encodes an ER protein that interact specifically with CRT3 which is implicated to control the folding of LRR-RLKs. These findings suggest that there might be a chaprone complex to monitor the folding of the LRR-RLK proteins in the ER. Recently, two LRR-RLK complexes were identified as *LURE1* receptor in Arabidopsis. Further progresses on the elucidation of PTG mechanisms will be discussed.

Key words: Gametophyte, pollen, pollen tube guidance, Arabidopsis

S-3-12 Myosin-5a motor function is co-activated by two cargo adaptor proteins RILPL2 and melanophilin

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Vertebrate myosin-5a is an ATP-utilizing processive motor associated with the actin network and responsible for the transport and localization of several vesicle cargoes. To transport cargo efficiently and prevent futile ATP hydrolysis, myosin-5a motor function must be tightly regulated. The globular tail domain (GTD) of myosin-5a not only functions as the inhibitory domain, but also serves as the binding site for a number of cargo adaptor proteins, including melanophilin (Mlph) and Rab-interacting lysosomal protein-like 2 (RILPL2). In this study, using various biochemical approaches, including ATPase, single-molecule motility, and GST pulldown assays and analytical ultracentrifugation, we demonstrate that the binding of both Mlph and RILPL2 to the GTD of myosin-5a is required for the activation of myosin-5a motor function under physiological ionic conditions. We also found that this activation is regulated by the small GTPase Rab36, a binding partner of RILPL2. In summary, our results indicate that RILPL2 is required for Mlph-mediated activation of Myo5a motor activity under physiological conditions and that Rab36 promotes this activation. We propose that Rab36 stimulates RILPL2 to interact with the myosin-5a GTD; this interaction then induces the exposure of the Mlph-binding site in the GTD, thereby enabling Mlph to interact with the GTD and activate myosin-5a motor activity.

Key Words: allosteric regulation, melanophilin, melanosome, molecular motor, myosin, small GTPase, vesicle transport

S-3-13 蛋白质组学与精准医疗

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肿瘤防控的关键在于“早发现、早诊断、早治疗”。然而，临床应用的肿瘤诊断标志物寥寥无几，且存在一定的假阳性和假阴性，并不十分理想。同时，肿瘤的治疗效果存在较大的个体差异，临床急需指导个体化精准医疗的标志物。基因组学、转录组学技术的快速发展，推进了肿瘤发生相关基因及重要驱动基因突变模式的研究和规模化发现，推动了精准医学的产生和发展。但是，仅仅从基因层面探讨肿瘤的精准诊疗尚不能满足临床需求。随着蛋白质组学的飞速发展，以蛋白质组为核心的多维度组学在生命科学和生物医药领域的重要作用和应用前景已经日益明晰。技术的最新发展已经引领我们进入一个利用蛋白质组学解决人类健康重大问题的历史时刻。经历了多年的积累，我们在蛋白质组学领域的技术、平台、应用等方面均达到了国际领先水平。以基因功能的执行者——蛋白质及其翻译后修饰调控为核心的肿瘤标志物发现研究，更有可能获得肿瘤发生发展的动态信息，更加精准地指导肿瘤的诊断和预后。

S-3-14 Identification of Ubiquitin Ligase Interacting Proteins with a Novel Tagging System

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The communication between cells and the communication between cellular organelles are often be controlled by the interaction of membrane proteins. Despite of many methods to detect protein-protein interactions (PPIs), there are still challenges in detecting membrane PPIs. Firstly, transient and weak PPIs are mostly associated with membrane receptor-mediated signaling pathways. Secondly, mass spectrometry-based membrane protein interaction studies have not been widely successful due to the poor solubility of membrane proteins or the loss of PPIs in sample preparation. Recent years, protein proximity tagging methods, such as APEX and BioID, have been applied in membrane protein study and showed great results.

Here we developed another method to specifically tag the interacting proteins of MPOI (membrane protein of interest) in cells. This approach transforms transient and weak interactions into covalent binding and the tagged proteins can be enriched by affinity purification under denaturing conditions for mass spectrometry-based identification. We applied this approach to MARCH5, a trans-membrane ubiquitin ligase that is an important regulator for mitochondria fusion and fission. We have identified most known MARCH5 interacting proteins. More interestingly, our data uncover that MARCH5 interacts with several peroxisome biogenesis factors, suggesting a novel role of MARCH5 in regulating peroxisome function.

S-3-15　蛋白质巯基的氧化还原修饰与衰老

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细胞的氧化还原状态直接调控蛋白质等生物大分子功能，介导细胞信号转导以及衰老、神经退行性疾病、代谢病、肿瘤等许多生理和病理过程。细胞的氧化还原平衡为胞内各种生物大分子行使正常功能提供了稳定的微环境，是细胞正常生理功能的重要基础。鉴于上世纪五十年代提出的自由基衰老学说，长期以来氧化应激常与氧化损伤混为一谈，“抗氧化”一度成为“抗衰老”的代名词。进入20世纪90年代，氧化应激参与细胞信号转导的生理功能逐渐被认识，近年来活性氧及活性氮通过对蛋白质巯基的氧化还原翻译后修饰调控蛋白质功能的研究进一步揭示了氧化还原调控的特异性，氧化还原调控机制研究进入全新阶段。

我们利用复制性衰老细胞模型及线虫模型研究了年轻和衰老细胞和线虫对氧化应激刺激的反应，结果发现年轻细胞和线虫比衰老组具有高的产生活性氧能力、氧化还原平衡维持能力及降解氧化损伤蛋白能力，据此我们提出"氧化还原应激反应能力（Redox-stress Response Capacity，RRC）"新概念，发现RRC衰退是衰老的本质特征。进一步研究发现Peroxiredoxin 2 (PRDX2)的巯基修饰异常导致氧化还原信号通路无法传递，是导致RRC下降的关键因素，蛋白质巯基的氧化还原修饰在衰老进程中发挥重要作用。

S-3-16　Neddylation regulates PTEN nuclear import and promotes tumor development

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PTEN tumor suppressor opposes the PI3K/Akt pathway in the cytoplasm and maintains chromosomal integrity in the nucleus. Nucleo-cytoplasm shuttling of PTEN is regulated by ubiquitylation, sumoylation and phosphorylation, and nuclear PTEN has been proposed to exhibit tumor-suppressive functions. Here we show that PTEN is conjugated by Nedd8 under high glucose conditions, which induces PTEN nuclear import without effects on PTEN stability. PTEN neddylation is promoted by the XIAP ligase and removed by the NEDP1 deneddylase. We identify Lys197 and Lys402 as major neddylation sites on PTEN. Neddylated PTEN accumulates predominantly in the nucleus and promotes rather than suppresses cell proliferation and metabolism. Neddylated PTEN stabilizes fatty acid synthase (FASN) and promotes

triglyceride production through interfering the ubiquitylation and degradation of FASN by TRIM21. In human breast cancer tissues, neddylated PTEN correlates with tumor progression and poor prognosis. These results reveal an unexpected tumor-promoting role of nuclear PTEN modified by Nedd8.

S-3-17 Immune signaling and metabolic checkpoints

免疫信号与代谢检查点

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Abstract: T cells are key players in our immune system to fight with cancer cells. However, cancer cells can develop multiple mechanisms to suppress T cell activity to evade immune attack. Signaling suppression and metabolic suppression are the two major parts of immunosuppression, which involves signaling and metabolic checkpoint molecules in T cells. In clinic, blockade antibodies against signaling checkpoints have been successfully applied to treat multiple types of cancer, which highlights the importance of checkpoint biology. In this talk, I will introduce our recent works on the signaling and metabolic checkpoints. The idea of combination of signaling modulation and metabolic modulation for next-generation cancer immunotherapy will also be discussed.

S-3-18 Establishment and maintenance of epigenetic information

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DNA methylation-mediated gene silencing and the establishment of DNA methylation are classic epigenetic events. Despite of a large body of studies in these directions, many unknown regulations exist. Recently, we performed a number of cell-based large scale screenings to identify novel regulators involved in DNA

methylation-mediated silencing and the establishment of DNA methylation. I will report our findings highlighting mechanisms controlling the establishment of DNA methylation during oocyte maturation. In addition, I will present our recent progresses regarding the kinetics, fidelity control mechanisms, and functional implications of maintenance DNA methylation.

S-4-01 The liver-adipose tissue crosstalk in lipid biosynthesis

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Hedgehog (Hh) had been known as the only cholesterol-modified morphogen playing pivotal roles in development and tumorigenesis. We performed an unbiased biochemical screen and identified that SMO was covalently modified by cholesterol on the Asp95 (D95) residue through an ester bond. This modification was inhibited by Patched-1 (Ptch1) but enhanced by Hh. The SMO(D95N) mutation was refractory to Hh-stimulated ciliary localization and failed to activate downstream signaling. Furthermore, homozygous SmoD99N/D99N (the equivalent residue in mouse) knockin mice were embryonic lethal with severe cardiac defects, phenocopying the Smo-/- mice. Together, the results of our study suggest that Hh signaling transduces to SMO through modulating its cholesterylation and provides a therapeutic opportunity to treat Hh-pathway-related cancers by targeting SMO cholesterylation. Metabolism in mammals is regulated by the complex interplay among different organs. The liver and white adipose tissue (WAT) are critically involved in lipid metabolism. Previous studies have shown that fatty acid synthesis is compensatorily increased in WAT when hepatic lipid synthesis is inhibited, implying that factor(s) produced by liver may promote lipogenesis in the adipose tissue. I will report a secreted factor GPNMB coordinating liver-WAT cross talking in lipogenesis.

S-4-02 HSD17B13：A novel therapeutic target for the treatment of non-alcoholic fatty liver disease

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17β-hydroxysteroid dehydrogenases (17β-HSDs) comprise a large family of 14 members that are mainly involved in sex hormone metabolism. Some 17β-HSD enzymes also play key roles in cholesterol and fatty acid metabolism. Recent study showed that 17β-HSD13, an enzyme with unknown biological function, is a novel liver-specific lipid droplet-associated protein in mouse and humans. 17β-HSD13 expression

is markedly upregulated in patients and mice with non-alcoholic fatty liver disease (NAFLD). Hepatic overexpression of 17β-HSD13 promotes lipid accumulation in the liver, while liver-specific deletion of 17β-HSD13 resists high fat diet-induced hepatic steatosis. In this talk, we summarize recent progress regarding the role of 17β-HSD13 in the regulation of hepatic lipid homeostasis and discuss genetic, genomic and proteomic evidence supporting the pathogenic role of 17β-HSD13 in NAFLD. We also emphasize its potential as a therapeutic target and biomarker for advanced liver diseases, such as NASH and liver cancer.

S-4-03 Aldolase B Opposes Hepatocellular Carcinogenesis through Inhibiting Glucose-6-phosphate dehydrogenase (G6PD) and Pentose Phosphate Pathway

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Metabolic reprogramming is a core hallmark of cancer, but its role and the mechanism in human hepatocellular carcinogenesis (HCC) remain poorly defined. Here we show that the glycolytic enzyme Aldoalse B (Aldob) exerts a novel tumour suppressive role in HCC by directly binding to the rate-limiting enzyme of the pentose phosphate pathway (PPP), glucose-6-phosphate dehydrogenase (G6PD), and inhibiting its enzymatic activity. Decreased expression of Aldob and upregulated expression of G6PD in human tumor tissue are correlated to the poor outcome and prognosis of HCC. Global or liver-specific knocking out of Aldob promotes tumorigenesis in a murine HCC model through enhancing G6PD activity to increase metabolic flux to PPP, whereas pharmacological inhibition or genetic knocking down of G6PD suppresses HCC. Consistently, re-expression of Aldob in liver-specific Aldob KO mice attenuates tumor formation. We further demonstrate that Aldob plays a crucial role in forming a stable Aldob-G6PD-p53 complex, which reinforces p53-mediated inhibition of G6PD activity. In summary, our data suggest that Aldob plays an essential role in remodeling glycolysis and PPP metabolism to favor tumor growth, suggesting that targeting Aldob is a potential therapeutic strategy for HCC.

S-4-04　ACAT与固醇代谢平衡

ACAT and sterol homeostasis

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酰基辅酶A:胆固醇酰基转移酶（ACAT）是细胞内唯一催化具有3-β-OH的游离固醇与长链脂肪酸生成固醇酯的酶，是固醇代谢平衡的关键酶，对细胞生命、细胞命运与细胞活动等发挥极重要的功能作用。ACAT家族有ACAT1和ACAT2，至今其详细结构解析尚未有报道。在长期与美国Dartmouth医学院TY & Catherine CY Chang教授合作中，我们实验室着重研究ACAT表达功能，包括探索基因结构、转录剪接、翻译修饰、表达调控、功能模式及其与固醇代谢平衡的生理功能、病理变化等关系。

前期已有研究表明，ACAT1在所有测定的哺乳细胞中均表达，而ACAT2具有细胞、发育、种族特异性表达。我们实验室深入与TY& Catherine CY Chang教授及有关基础、药物、临床等领域合作中，近年在ACAT1基因表达的新型反式剪接、ACAT2的高/低水平表达和肝癌诱导表达机制、ACAT的白细胞表达协同功能及其关联的特异脂蛋白eLDL外排代谢、ACAT表达与细胞质膜的固醇动态平衡、ACAT的脑细胞特征性表达功能、ACAT与固醇代谢平衡及其关联ROS-能量/糖/O2代谢等探索中取得前沿性进展，可促进阐析生理病理机制及提升转化应用与人类健康。

S-4-05　Cancer metabolic reprogramming and oncogenesis.

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Glutamine metabolism plays an important role in cancer development and progression. Glutaminase C (GAC), the first enzyme in glutaminolysis, has emerged in recent years as an important target for cancer therapy and many studies have focused on the mechanism of enhanced GAC expression in cancer cells. However, little is known about the post-translational modification of GAC. Here, we report that the phosphorylation is a crucial post-translational modification of GAC, which is responsible for the higher glutaminase activity in tumor tissues of lung cancer patients and cancer cells than that from the normal individuals. We identify the key phosphorylation site on Ser314 and this phosphorylation is regulated by the NF-κB-PKCε axis. Blocking the phosphorylation on Ser314 by mutating the 314th serine to alanine in lung cancer cells inhibits the glutaminase activity and triggers genetic reprogramming and alleviates tumor malignancy. Furthermore, we find that the highly phosphorylation level of GAC correlated with poorly

survival rate of lung cancer patients. These findings highlight a previously unappreciated mechanism for activation of GAC by phosphorylation and demonstrate that targeting glutaminase activity can inhibit oncogenic transformation.

S-4-06 Advances in lipidomics and its application to investigate functional lipidome

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The diversity and complexity of the biological lipidomes inspired various technical innovation and improvement to meet the needs of various biomedical studies. Lipidomics, which targets at the construction of a comprehensive map of lipidome comprising the entire lipid pool within a cell or tissue, is currently emerging as an independent discipline at the interface of lipid biology, technology and medicine. The recent wave of expansion in the field of lipidomic research is mainly attributed to advances in analytical technologies, in particular, the development of new mass spectrometry-based tools and approaches for the characterization and quantification of the wide array of diverse lipids in biological samples. In this presentation, we will review recent technical advances in lipidomics and present some of our most recent research progress in applications of lipidomics to address the potential biological roles of lipidome in evolution as well as potential functional roles of lipidome in a few metabolic diseases including Type 2 diabetes.

Key Words: Lipidomics, functional lipids, evolution, biomarkers

S-4-07　CD36 棕榈酰化修饰在脂肪性肝炎发生中的作用

CD36 palmitoylation in non-alcoholic steatohepatitis (NASH)

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Fatty acid translocase CD36 (CD36) is a multifunctional immuno-metabolic receptor with many ligands. CD36 expression is abnormally upregulated and it is mainly located at the plasma membrane of hepatocytes in patients with NASH. Palmitoylation has been suggested to regulate subcellular distribution of CD36, but little is known about its significance in NASH.

Human liver tissue samples were obtained from patients undergoing liver biopsy for diagnostic purposes. C57BL/6J and CD36 knockout mice were injected with lentivirus vectors expressing wild type CD36 and palmitoylation sites mutated CD36. Liver histology, immunohistochemistry, mRNA expression profile, subcellular distributions and functions of CD36 protein were assessed.

Hepatic CD36 expression was significantly higher in patients with NASH compared to patients with normal liver and those with simple steatosis. CD36 was predominantly located at the plasma membrane of hepatocytes in subjects with NASH with a strong inflammatory response. Hepatic CD36 palmitoylation was induced in mice with NASH, and the inhibition of CD36 palmitoylation protected mice from developing NASH by inhibiting lipid accumulation and also metabolic inflammation. In addition, blocking of palmitoylation, either by mutation or by pharmacological inhibition, caused intracellular accumulation of hepatic CD36 and decreased its localization on the plasma membrane. A lack of palmitoylation decreased the formation of CD36/Fyn complex, with the consequent activation of the AMPK pathway and inhibition of the JNK pathway. Consistently, inhibition of CD36 palmitoylation ameliorated fatty acids metabolic disorders and inflammatory response in animal models of NASH and HepG2 cells.

Conclusions: Our findings demonstrate the key role of palmitoylation in regulating CD36 distributions and its functions in NASH. Inhibition of CD36 palmitoylation may represent an effective therapeutic strategy in patients with NAFLD/NASH.

S-4-08　O-GlcNAc糖基化修饰在肝纤维化中的作用机制研究

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肝纤维化是肝脏对各种慢性肝损伤做出的持续性的损伤修复反应，然而肝纤维化发生发展的机制尚不完全清楚，肝纤维化也缺乏有效的治疗药物。我们发现O-GlcNAc糖基化修饰在肝纤维化组织中增强，同时O-GlcNAc糖基转移酶（OGT）在肝细胞或者肝星状细胞中缺失也促进肝纤维化的发生发展。进一步研究提示I型胶原的关键转录因子Smad2/3被O-GlcNAc糖基化修饰，O-GlcNAc糖基化修饰可以抑制Smad2/3的转录活性和I型胶原表达。在小鼠水平通过靶向增强O-GlcNAc修饰可以抑制肝纤维化的发生发展。综上，这些结果提示O-GlcNAc糖基化是肝纤维化发生发展中的代偿性保护因素。

S-4-09　基于新材料、新试剂和生物质谱技术的糖蛋白质组分析新方法与应用研究

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糖基化修饰是最常见、最重要的蛋白质翻译后修饰之一。但是，由于其复杂程度高、丰度低；难于富集分离；质谱分析灵敏度差等原因，规模化的糖蛋白分析面临巨大技术挑战。

针对上述问题，我们发展了一系列糖蛋白、糖肽富集新材料、新试剂和质谱鉴定新方法，显著提高了富集效率和鉴定规模。（1）合成了多种糖肽和糖簇混合抗原，制备出亲和性高、非肽段序列依赖的O-GlcNAc糖基化修饰广谱性抗体，在HeLa细胞中鉴定超过5000个O-GlcNAc糖肽，获得了目前最大规模的数据集，并成功应用于营养代谢调控转录因子O-GlcNAc修饰及其转录活性的研究。（2）发展了多种新型糖链标记试剂，可对糖肽实现一步法高选择性富集和高效衍生化，在特异性富集糖肽的同时提高了质谱分析灵敏度。（3）针对传统固态富集材料存在固液界面传质阻力，严重抑制富集反应的技术瓶颈，创新性的提出了基于可溶性环境响应材料的类均相反应富集新策略。通过调节溶液体系的温度或pH值，可对该类材料的溶解性实现精确控制，实现了“均相反应”富集和“异相沉淀”回收，该策略成功应用于小鼠脑组织和HeLa细胞的N、O-糖蛋白、糖肽富集。（4）将所发展新方法用于受体蛋白、转录因子糖基化修饰的规模化鉴定，为营养代谢对EGFR、Yap等蛋白的糖基化修饰和功能调控研究提供有力工具。

关键词：糖蛋白质组，分离富集，生物质谱、转录因子、受体

S-4-10 O-GlyNAcylation and Medulloblastoma

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The Hedgehog (HH) pathway plays a pivotal role in diverse aspects of development and postnatal physiology. Perturbation of the HH pathway and activation of glioma-associated oncogene (GLI), a dedicated transcription factor in this pathway, is responsible for approximately 30% of medulloblastomas, a common and aggressive type of pediatric brain tumor. Therefore, HH signaling has emerged as a therapeutic target of interest for medulloblastoma therapy. Despite the relevance of these insights to development and disease, substantial gaps still remain in our knowledge of the mechanisms involved in regulation of response to HH signaling and crosstalk with other pathways. Therefore, elucidating the molecular mechanisms of HH signaling is essential to advance our fundamental understanding of both developmental processes and HH-dependent medulloblastoma. Combining a novel homemade Pan-anti-O-GlcNAc antibody with proteome-wide profiling of O-GlcNAcylated transcription factors by quantitative mass spectrometry, we identified a previously unknown mechanism by which the HH pathway is regulated by glucose-sensing O-GlcNAcylation.

S-4-11 有氧糖酵解关键酶PKM2的O-GlcNAc修饰促进肿瘤细胞增殖

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O-GlcNAc糖基化是一种广泛发生在丝氨酸或苏氨酸残基上的蛋白质翻译后修饰。因其活化供体UDP–GlcNAc与四种生物大分子的代谢密切相关，故这种修饰也被认为是 “营养状态的感受器” (nutrient sensor)。目前已知多种代谢酶、转录因子、信号蛋白、组蛋白等都可以受到*O*-GlcNAc糖基化修饰的调节。我们的前期工作发现，*O*-GlcNAc糖基化修饰可以通过调节糖酵解途径的最后一个限速酶丙酮酸激酶M2 (PKM2) 的活性和功能，促进肿瘤细胞增殖。其分子机制为*O*-GlcNAc糖基化修饰破坏了维持PKM2四聚体稳定的主要作用力，促使PKM2四聚体发生解聚，并通过其在胞质内代谢酶活性的下降与核内非代谢酶功能的获得，共同促进了肿瘤细胞“有氧糖酵解”的发生。在最近的研究中，我们发现PKM2的*O*-GlcNAc糖基化修饰不但受到肿瘤微环境营养波动的影响，而且还

受EGF等多种生长因子的调节。EGF的刺激可以上调OGT的活性，同时促进OGT与PKM2的结合。另外，我们还发现，*O*-GlcNAc糖基化修饰对PKM2四聚体结构的破坏，除了下调其在胞质内的代谢酶活性和促进其入核并获得转录调节因子的功能外，还“赋予”了PKM2在胞质内调节其他糖酵解关键酶的新功能。这种新功能与已知的PKM2功能同向同行，共同促进了肿瘤细胞“有氧糖酵解”的发生，满足了细胞增殖对生物大分子合成和氧化还原平衡的需要，为理解肿瘤细胞“有氧糖酵解”的调控提供了“酶调节”的新视角。

S-4-12 Delineating the role of protein O-GlcNAcylation in cancer

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O-linked N-acetylglucosamine (O-GlcNAc) is a prevalent post-translational modification on serine and threonine residues of proteins. Since O-GlcNAcylation is critical for normal physiology, its aberrant expression is closely associated with a number of diseases, including neurodegenerative diseases, diabetes, cardiovascular diseases, and cancers. Emerging research evidence indicates that O-GlcNAcylation is globally elevated in various cancers, but the mechanisms involved in the tumor pathology remain incompletely understood at the molecular level. Here, we discuss our recent progresses in understanding the molecular mechanisms by which deregulation of O-GlcNAcylation contributes to cancer development and progression.

Key Words: O-GlcNAcylation, cancer, metabolism, translation, chemoresistance

S-4-13 O-GlcNAc糖基化对胚胎干细胞多能性维持的调控作用

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O-GlcNAc (O-linked N-acetylglucosamine) modification is a non-canonical form of protein glycosylation, which occurs intracellular and is dynamically regulated. Our lab has developed chemical labeling methods that allow quantitative profiling of O-GlcNAcylated proteins and the modification sites. Recently, by applying chemical profiling for probing O-GlcNAcylation of pluripotency transcription factors in mouse embryonic stem cells (mESCs), we discovered that ESRRB, a critical transcription factor for establishing self-renewal and pluripotency, was O-GlcNAcylated at Ser 25. ESRRB O GlcNAcylation enhanced the protein stability by inhibiting ubiquitination. Binding of ESRRB to OCT4 and NANOG, two master pluripotency regulators, was augmented by O GlcNAcylation. Ablation of ESRRB O-GlcNAc impaired its function for maintaining mESC self-renewal and pluripotency, both in cell culture and during teratomas formation in mice.

S-4-14 A Perfect Antibody Reveals A New Function of Fsh on Fat Metabolism.

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Obesity is a serious pandemic that increases the prevalence of life-threaten diseases, including coronary and cerebrovascular diseases, various cancers, type 2 diabetes. However, there is a largely unmet need for treatment of obesity. Since the discovery of the brown adipose cells in adult human, it becomes an attractive organ target for obesity treatment by activation of the brown fat and conversion of white adipocytes into beige cells. Clinical studies indicate a close association of the pituitary hormone follicle-stimulating hormone (FSH) with body fat. In previous works, a polyclonal antibody that targets the β-subunit of Fsh increases bone mass in mice. Recently, we report that the same antibody sharply reduces adipose tissue in wild-type mice, phenocopying genetic haploinsufficiency for the Fsh receptor Fshr. The antibody also causes profound beiging, increases cellular mitochondrial density, activates brown adipose tissue and enhances thermogenesis. These actions result from the specific binding of the antibody to the β-subunit of Fsh to block its action. Therefore, FSH not only regulates reproductive system, but also plays a pivotal role in fat metabolism. This may provide a promising intervention by a single antibody to simultaneously combat both obesity and osteoporosis, as well as the related metabolic diseases.

S-5-01　合成生物学与新型蛋白质材料的设计与应用

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A central question facing the bottom-up approach for material design is how to faithfully transfer the function at the molecular level to the material properties at the macroscopic level. In the past years, there has been a growing trend of designing materials with dynamically tunable properties, which necessitate a new level of control over macromolecular system design and engineering. While natural evolution has led to the creation of a vast number of protein molecules with extraordinary structural and functional diversity, this ecological diversity has yet to be fully utilized for material design. Taking advantage of some emerging synthetic biology tools and principles, we focus on the strategies that enable the assembly of engineered protein molecules into functional macroscopic materials for a variety of applications ranging from 3D cell culture, regenerative medicine to environmental remediation.

S-5-02　质谱技术在神经科学中的应用

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尽管种种证据表明，神经细胞内的化学小分子包括神经递质、脂质、激素、代谢产物等在大脑的正常生理功能及疾病发生中起着重要的作用，然而目前大部分研究的对象仅限于神经环路结构以及基因、蛋白质等大分子物质的调控，对于脑功能及脑疾病中化学小分子的变化仍然缺乏深入系统的研究。因此，研发具有高通量、高度特异性、原位检测、低成本等优点的单细胞质谱与脑质谱成像等新型化学小分子检测技术，可以帮助人们从细胞器到单细胞乃至全脑组织水平等多个尺度，全面解析脑功能及脑疾病中全脑化学小分子的变化和分布，并探索这些变化的分子机制以及这种变化在脑疾病中所起的作用，寻找与之相关的早期诊断分子标志物以及干预靶点。

S-5-03 Anti-tumor immunity controlled through mRNA m6A program

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Emerging evidence reveals the important role of tumor neoantigens in generating spontaneous antitumor immune responses and predicting clinical responses to immunotherapies. Despite the presence of numerous neoantigens, complete tumor elimination rarely occurs in many patients, due to failures in mounting a sufficient and lasting antitumor immune response. Here, we show that durable neoantigen-specific immunity is regulated by messenger RNA (mRNA) N6-methyadenosine (m6A) methylation program. Loss of mRNA m6A in dendritic cells (DCs) enhanced the cross-presentation of tumor antigen and the cross-priming of CD8+ T cells in vivo. Mechanistically, transcripts encoding lysosomal proteases are marked by m6A and its translational efficiency was regulated by m6A, thereby accelerating the destruction of tumor antigens. Inhibition of m6A axis DCs, with reduced translation of lysosomal proteases, retain tumor antigens for enhanced cross-presentation, implicating the Ythdf1 as a new potential therapeutic target in anticancer immunotherapy.

S-5-04 时间分辨的蛋白质在体激活新技术

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蛋白质是细胞内含量最多的一类生物大分子，它们的含量、修饰、功能及其动态变化是执行细胞生命活动的重要物质基础。如何对这些蛋白质机器在其所处的天然环境–活细胞内进行原位标记与调控，是极具挑战性的一项科学难题。报告人的课题组系统地发展了适用于活细胞及活体动物内的蛋白质化学技术平台，获得了可遗传编码的光交联探针、特异标记技术和化学脱笼反应等一系列“在体”研究蛋白质结构与功能的化学生物学工具，开启了利用化学方法研究蛋白质及其他生物大分子的新途径。最近，为了对蛋白质在活体环境下的动态功能进行研究甚至操控，报告人率先利用生物正交断键反应和遗传密码子拓展技术，实现了在蛋白质催化位点的生物正交脱笼，进而原位激活其功能。他们更进一步提出并发展了“邻近脱笼”的新策略（CAGE–prox），与合作者一起利用计算机辅助筛选与学习，将蛋白质脱笼技术的适用范围从催化位点拓展至整个活性口袋，获得了具有高时间分辨率和广泛适用性的蛋白质激活新方法。为开展蛋白质动态功能的原位研究提供了关键技术，也为生物正交反应开拓了新的前沿方向。

S-5-05 Structure and functions of higher-ordered chromatin structures in gene regulation and epigenetic inheritance

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Eukaryotic DNA is hierarchically packaged into chromatin to fit inside the nucleus, in which the accessibility of DNA is dependent on the packing density of chromatin. Dynamics of chromatin structures plays a critical role in transcriptional regulation and all other DNA related biological processes. Previously, we reported the 11 Å resolution cryo-electron microscopy (cryo-EM) structures of 30 nm chromatin fibers reconstituted in the presence of linker histone H1, which reveals a left-handed double helix twisted by the repeating tetra-nucleosomal structural units. Recently, we have determined the 3.9 Å resolution cryo-electron microscopy (cryo-EM) structures of 30 nm chromatin fibers with linker histone H5, uncovering that asymmetries and polarities of nucleosomes play important roles in the folding of chromatin fibers. Using single-molecule force spectroscopy, we reveal that the tetranucleosomes-on-a-string appears as a stable secondary structure during hierarchical organization of chromatin fibers. The stability of the tetranucleosomal unit is negatively regulated by the histone chaperone FACT (Facilitates Chromatin Transcription) in vitro. Interestingly, we further revealed that FACT has dual functions in breaking nucleosome and maintaining its integrity during DNA replication and transcription. In addition, we also demonstrated that formation of 30-nm chromatin fibers greatly facilitates the faithful propagation of H2AK119ub1 by RYBP-PRC1 during cell divisions. In summary, our study demonstrates that the tetranucleosome is a novel regulatory structural unit of chromatin fibers beyond the nucleosome, and provides crucial mechanistic insights into functions of chromatin fibers in transcriptional regulation and epigenetic inheritance.

S-5-06　基于生物传感的生化分析新方法

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在医疗诊断和生命科学研究中，生物传感器是应用最为广泛的测试器件之一。近年来，随着生物化学与生物物理学以及化学、材料学、生物医学工程等学科间的交叉渗透，基于生物传感的生化分析新方法研究得到了飞速发展，因此，核酸适配体、多肽等新的分子识别元件取代抗体，不仅提供了更多生物传感的设计机会，而且极大地节约了测试成本，同时，各类材料被用于生物传感的研究，如生物分子的识别、传感界面的构建、信号分子的标记等方面，为基于生物传感的生化分析新方法研究提供了更多可能。此外，各种分子组装、滚环扩增等新技术的应用，极大地提高了传感界面上目标分子的捕获效率，实现了信号放大和靶标分子的可控识别，从而提升了生物传感的性能，实现了更多生物靶标、尤其是疾病标志物蛋白质的分析检测性能，为液体活检及精准医疗的发展创造了条件。

关键词：生化分析；生物传感；分子识别；分子组装；液体活检

S-5-07　基因编辑与基因治疗

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The CRISPR/Cas9 technology provides a promising tool for genetic engineering. It offers an efficient approach to develop genetically modified (GM) animal models and a potential strategy for targeted gene therapies. We previously applied the CRISPR/Cas9 system to generate knockout mice and knock-in mice, although with mosaicism and relatively low efficiency. Recently, we optimized CRISPR/Cas9 system and obtained fully functional knockout mice and monkey in F0, which could be directly used for phenotypical analysis. We also devised a homology-mediated end joining (HMEJ)-based strategy, yielding knock-in mice and monkeys, with an efficiency much higher than other knock-in strategies. For targeted gene therapies, we have recently developed an off-target detection method named “GOTI” and found that cytosine base editor induced substantial off-target single nucleotide variants. Moreover, we rescued *Fah*$^{-/-}$ liver failure mice by correcting *Fah* mutation using microhomology-mediated end joining (MMEJ) and HMEJ-based strategies.

Furthermore, we demonstrate the use of the CRISPR/Cas9 system to eliminate targeted chromosomes via multiple DNA cleavages, offering a new therapeutic strategy for human aneuploidy diseases involving additional chromosomes. Finally, we achieved multiple genes activation *in vivo* using CRISPR–dCas9-activator, leading to observable phenotypic changes in liver and brain. This offers a new approach for developing targeted epigenetic therapies against human diseases.

S-5-08　光活性生物探针分子的构筑及应用

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生物正交反应如点击化学在生物体系的标记和功能调控方面具有重要应用，但由于生物体系的复杂性，在数以万计的化学反应中，能够在生物相容的条件下发生、并用于复杂生物体系研究中的生物正交反应寥寥无几，开发新的生物正交反应一直是化学生物学研究中的重要任务和挑战之一。同时，基于光照可实现时空分辨这一优势，发展基于光诱导生物正交反应的分子工具，可望为解析生物体系内的动态分子事件提供极大的便利。然而光诱导的成键反应大都需要经由高能激发态及高活性中间体，在复杂生物体系内易受到多种活性基团的进攻而发生副反应，难以实现生物正交。针对生物体系富含水这一极性溶剂的特征，我们提出用水作为特定高能激发态的“极性溶剂笼”这一新概念，并借助这一概念开发出了仅通过温和的可见光照射即可诱导发生的生物正交反应。这一新类型的生物正交反应为生物正交工具箱增添了光活性的分子工具，不仅使得生物大分子及活细胞上的时空分辨标记成为可能，而且可与经典的张力促进的点击反应互相正交，用于多组分生物大分子的正交标记。结合我们所开发的可见光诱导生物正交反应及紫外光诱导的生物正交反应可构建系列具有光活性的生物探针，如多肽探针、核酸探针等，实现对生物体系的光调控及发展生物分子靶标鉴定的新化学生物学方法等。

S-5-09 Decode and reprogram the yeast genome

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Facilitated by advances in technologies for DNA synthesis and assembly, the entire genome of an organism, from viruses to bacteria, has now become the target of redesign and reprogramming. The synthesis of first eukaryote, *Saccharomyces cerevisiae*, genome (Sc2.0) is near completion, tackled by an international consortium. Sets of design features were grafted into the Sc2.0 genome to promote deeper understanding of genome function. For example, a system called synthetic chromosome rearrangement and modification by loxP-mediated evolution (SCRaMbLE) is incorporated to probe the structural rule, gene content and plasticity of the yeast genome, which subsequently generated many new discoveries. In this talk, I will review our previous work in synthetic yeast genome and introduce the next version of the designed yeast genome including the construction of a mega-essential chromosome and strategies to build a highly simplified genome.

S-5-10 Hearbal decocotosome is a novel form medicine

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ABSTRACT

Traditionally, herbal medicine is consumed by drinking decoctions produced by boiling herbs with water. The functional components of the decoction are heat stable. Small RNAs (sRNAs) were reported as a new class of functional components in decoctions. However, the mechanisms by which sRNAs survive heat treatment of the decoction and enter cells are unclear. Previous studies showed that plant-derived exosome-like nanoparticles (ELNs), which we call botanosomes, could deliver therapeutic reagents in vivo. Here, we report that heat-stable decoctosomes (ELNs) from decoctions have more therapeutic effects than the decoctions in vitro and demonstrate therapeutic efficacy in vivo. Furthermore, sRNAs, such as HJT-sRNA-m7 and PGY-sRNA-6, in the decoctosome exhibit potent anti-fibrosis and anti-inflammatory effects, respectively. Decoctosome is comprised of lipids, chemical compounds, proteins, and sRNAs. A medical decoctosome mimic is called bencaosome. A single lipid sphinganine (d22:0) identified in the decoctosome

was mixed and heated with the synthesized sRNAs to form the simplest bencaosome. This simple bencaosome structure was identified by critical micelle concentration (cmc) assay that sRNAs co-assembled with sphinganine (d22:0) to form the lipid layers of vesicles. The heating process facilitates co-assembly of sRNAs and sphinganine (d22:0) until a steady state is reached. The artificially produced sphinganine-HJT-sRNA-m7 and sphinganine-PGY-sRNA-6 bencaosomes could ameliorate bleomycin-induced lung fibrosis and poly(I:C)-induced lung inflammation, re-spectively, following oral administration in mice. Our study not only demonstrates that the herbal decoctosome may represent a combinatory remedy in precision medicine but also provides an effective oral delivery route for nucleic acid therapy.

S-5-11 Gene editing: high-throughput and beyond

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We have previously developed a high-throughput screening (HTS) method using CRISPR/Cas9 system, which has been broadly applied in the functional studies of coding genes. We have also established the first high-throughput screening strategy for functional investigation of long non-coding RNAs (lncRNAs) using a lentiviral paired-guide RNA (pgRNA) library. The screen identified novel lncRNAs that can positively or negatively regulate cancer cell growth. We have developed a novel approach for genome-wide screening of lncRNAs, a high-throughput method to study the function of topologically associating domains and active chromatin hubs, a re-designed sgRNA scaffold that greatly boosts the efficiency and data quality for HTS, and, a new approach for mapping of functional sites of protein of interest at single amino acid resolution. Besides various high-throughput strategies, I will also report one of CRISPR-independent editing technologies – LEAPER, which leverages endogenous mechanism for effective RNA editing.

S-5-12 Fluorescence Quenching at Intetfaces for Biomolecular Dynamics at Living Cell Membranes

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Tracking membrane-interacting molecules and visualizing their conformational dynamics are key to understanding their functions. It is however challenging to probe accurately the positions of a molecule relative to a membrane. Here we report on single-molecule methods to assess interplay between molecules and liposomes. It takes advantage of FRET between a single fluorophore attached to a biomolecule and a large number of quenchers in a solution. We applied the method to characterize interactions of α-synuclein (α-syn) with membranes. Our results revealed that the N-terminus of α-syn inserts into the membrane and spontaneously transits among different depths. In contrast, the C-terminal tail of α-syn is regulated by calcium ions and floats in the aqueous milieu in two conformations. LipoFRET is a powerful tool to investigate membrane-interacting biomolecules with sub-nanometer precision at single molecule level.

S-5-13 核糖体的工作机理与人源体外翻译系统的全重构

林金钟

复旦大学

摘要：核糖体翻译合成蛋白质是个极其动态复杂的反应，几乎每一步都需要相应的翻译因子催化进行。过去几十年的结构生物学研究使我们对核糖体的构造在原子分辨率水平有了深刻的理解，但是翻译因子和核糖体协同互作的机理还不是很明朗。我们一直致力于解析核糖体与翻译因子复合物的高分辨率结构，以阐明蛋白质翻译合成与调控的分子机理。这次报告中我将介绍翻译终止后终止因子RRF和EF-G如何催化核糖体回收再循环的分子机制。另外，目前对翻译调控的研究远远落后于转录调控，一个主要原因是缺乏有效的生化研究体系。为了解决这一问题，我们正在构建人源全重组体外翻译系统用于研究mRNA翻译过程，我将介绍我们在这一尝试上的最新进展。

S-6-01　北京大学“生物化学研讨型小班”教学

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为响应北京大学教务部改革要求，增加教师与学生面对面接触交流的机会，充分调动学生在教学过程中的主体作用，2012年的秋季学期我们开展了“研讨型小班教学”的实践。为此，我们组织了一批资深的和新近回国的青年学者为学生增设《生物化学》小班讨论课，围绕大班授课提纲设立了八个讨论主题。我们将学生分为10个班，每个班学生人数不多于15人，由一位教师和一位由高年级本科生助教负责，开展每星期2个小时的讨论。相对应的是，我们选择了8篇生物化学领域的经典文献，发给所有学生，要求所有学生都对这8篇文献进行深度阅读。在这次报告中，我将以其中一篇经典文献为例与大家分享教学心得。回顾7年的教学实践，我们认为“研讨型小班”教学模式着重于学生深度学习的体验和开放式的探索，激发了学生的学习兴趣、求知动力与探索精神，培养和提升了学生自主学习、批判分析、创新思考、交流表达和团队合作等能力，全面提升了学生的学术素养和综合能力。

S-6-02　架构知识传递、能力培养、思维提升和价值塑造一体化的高质量课堂教学

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德国哲学家雅斯贝尔斯在《什么是教育？》中写道：“教育的本质意味着，一棵树摇动另一棵树，一朵云推动另一朵云，一个灵魂唤醒另一个灵魂。” 德国教育学家斯普朗格也曾说过：“教育的最终目的不是传授已有的东西，而是要把人的创造力量诱导出来，将生命感、价值感唤醒。”因此，教育的核心就在于 “唤醒”学生学习的内在动力和“引导”学生建立过硬的学习能力。特别在当今这个科技飞速进步的时代，社会的快速变革也在倒逼教师（特别是高校教师）不能仅仅满足于把现有知识讲授给学生，而是要能够引导和培养真正具有发现问题，解决问题，而且是创新性的解决问题的人才。在当前互联网技术和数据信息产业飞速发展的当下，我们需要利用好各种渠道的教学资源，发挥好新型教学模式和教学手段的作用，真正以学生学习为中心，根据认知神经科学和行为设计学的基本规律，架构出集知识传递、能力培养、思维提升和价值塑造一体化的高质量课堂教学，真正帮助学生唤醒内在的好奇心和求知欲，引导学生掌握和建立探索和改造世界的方法，形成自己看待问题和思考问题的思维模式，唤醒他们对自我价值的认知和追求，激发学生进行自主学习，最终具备终身学习的意识和能力，从而真正实现我们教书育人的目的——不仅是让学生学会

知识，更是习得一种思维方式和学习能力，在这个知识急速膨胀，技术指数发展的时代，让学生能够驾驭学习，掌控学习，真正成为学习的主人。

S-6-03　以代谢整合与调节一章为例浅谈教学凝练

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物质代谢教学难度较大，各种代谢物纷繁琐碎，众多代谢途径错综交织，是公认的老师难教、学生畏学的难点内容。尤其是代谢整合与调节一章，需要总结和提炼前面所学三大代谢的整体联系和动态变化，综合性强，更具挑战性。本文将围绕“如何吃透这一内容，实现有效的教学凝练”，谈谈我自己在教学不同阶段对它理解程度的不断加深，主要包括教学设计优化、科普想象活化、哲思融合深化三个层次。

S-6-04　以培养卓越医师为目标的生物化学课程改革与实践

解军

山西医科大学

《生物化学与分子生物学》是医学院校基础医学的主干课程。学好生化知识，对于医学生认识人体，认识疾病的发生发展机制，找到治疗疾病的钥匙，有极其重要的作用。但是医学生在学习生化课程时存在一些困难，如，生化进展快，知识更新快，建立分子作用的抽象思维难，需要记忆的关键点多，构建生化反应与人体机能的联系思维难等。

山西医科大学生物化学与分子生物学教研室基于建构主义学习理论的“认知陀螺”学习模式，以医学生自主学习能力提高为目标，基于在线开放课程和学校e-Learn教学平台，建设了互联网+课堂教学模式的线上、线下混合的《生物化学与分子生物学》课程。

课程的线上资源包括知识点微视频、现场教学视频、课件PPT、章节思维导图、重点难点知识、教学动画、英文歌曲等。能将课程与思政相结合，以故事形式潜移默化培养学生的高尚医德；

将专业理论知识从单纯的纸质化教材发展为教材与视频、课件、思维导图、动画、故事等多种线上资源结合的立体化知识库；教学手段从单纯的平面化线下教学发展为线下课堂讲授，线上布置作业、答疑、讨论、随机考试相结合的多方位立体化教学；引入临床教师进入教学团队，课程体系中加入疾病与生化知识的相关性，以此提高学生早期接触临床的知识衔接能力，并激发学生对临床医学的兴趣；利用讲座与小组线上讨论等方式将科研新技术和新进展融入传统知识的学习中，培养学生的科研思维；终结性评价与过程性评价相结合：从单一的终结性评价改革为线上与线下考试相结合的多元化评价体系。

该课程的特点：一是基于建构主义学习理论的“认知陀螺”，突出学生“学”的核心作用。二是通过线上延伸教育的生化故事，将“课程思政”融入专业教育。三是充分利用线上平台，边学边测，突出“线上线下”混合教学的优势。四是临床医生参与教学，突出医学基础课的“早临床”意识。

自2018年11月上线以来，课程的访问量达702万余次，注册人数已达9000余人，覆盖了全国387个高校和科研院所。真正实现了“互联网+”环境下学生自主学习平台的需求，推动了信息技术与基础医学教育教学深度融合，激发了学生的学习潜能，有助于培养学生的创新精神和实践能力。

S-6-05　浅谈医学生物化学案例式教学的几点体会

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医学生学习生物化学的目的主要是为学好临床课程奠定基础，以便将来更好地为患者服务。为了配合医学生“早临床、多临床、反复临床”培养方案的要求，我们在医学生物化学教学中尝试了案例式教学。以下是我们开展医学生物化学案例式教学的几点体会。（1）认真遴选案例式教学的老师：遴选教师的基本标准是既懂生物化学，又有临床专业背景（必要时请临床医生参与）；（2）建立和完善案例式教学师资培训体系：包括听相关的医学基础课和临床课、与临床科室主任互聘、请临床教授讲课等；（3）精选案例：案例要经典（但案例≠病例），案例要与学生现有的知识水平相适应，案例要能很好地反映所讲授的理论（案例要有代表性、知识性、针对性、趣味性、启发性和指导性），案例要贴国家执业资格考试和研究生入学考试案例分析的命题方向，案例方式多样（包括问题式、信息式、实录式、条例式、决策式等），案例可来源于临床/科研/生活实际或教师根据教学内容自行设计；（4）加强师生互动以及学生之间的互动（包括课前、课中和课后互动）；（5）案例式教学中应尽量避免的问题：包括案例与有关理论“两张皮”的问题，案例太偏、太难、太复杂的问题，案例中重点内容不突出的问题等；（6）医学生物化学案例式教学应与其他学科的案例式教学协同配合。

关键词：医学生物化学；案例式教学

S-6-06 “生命观念”视域下的生物化学教学思考与探索

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生物化学是研究发生在活细胞与生物体中，各种不同的分子及其化学反应的一门科学。因其提供了在多样性生命模式中的诸多基本原则，故也被广泛认同为生命科学所有相关学科的基础“语言”。虽然生物化学可以提供在多个领域的重要知识及应用价值，但其根本目的还是在于诠释生命的本质。因此，生物化学课程的终极目标应该定位于，帮助学生在对客观事实、概念和规律等的学习中，不断进行深刻反思、提炼和升华，并形成对生命本质的整体理解和认识，即形成生命观念。生命观念是生命科学知识的最高形式，是真正使学生受益终身的教学成果。基于这样的认识，我们在教学实践中尝试从“生命是什么？生命是如何运作的？生命为什么是这样？生命在哪里？生命的未来？”等生命科学的What、How、Why、Where、When的视角持续反思生物化学课程的价值。在实际的教学实施中，我们将生物化学课程的学习分成两个阶段，即基础生物化学和高级生物化学。前者侧重于学生知识体系的建构和技能、能力的培养；后者则更侧重于学生生命观念的生成。本报告拟结合具体案例，介绍我们在致力于学生生命观念生成方面所做的一些初步思考、尝试与探索。

S-6-07 如何在高校生物教学中组织社会热点问题的课堂讨论

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摘要：在课堂上如何组织学生们对社会热点问题进行科学讨论，这是培养学生理性思维，掌握更全面知识体系的重要过程。在教学中，我们通过一般性讨论提出问题，引导学生查阅文献进行深入辩论，对社会热点问题得出科学结论，再通过实地参观巩固辩论成果的三阶段讨论教学法，提高学生科学、综合认识热点问题的能力和正确表达意见的能力。

关键词：热点问题 课堂讨论 科学论证 三段式

S-6-08　医学本科生物化学与分子生物学课程“3+X”多维度精细化新教学模式的探索与实践

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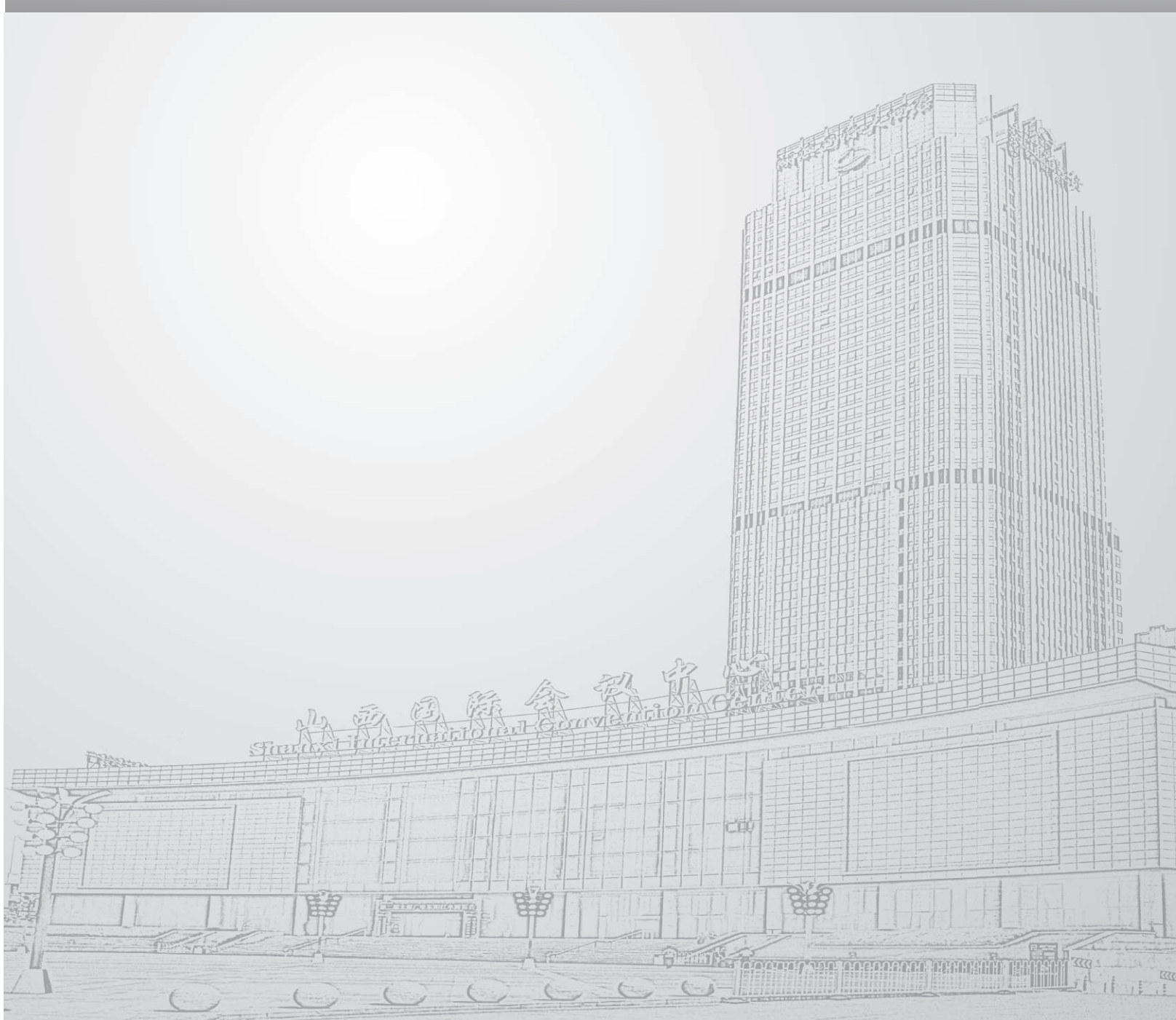
摘要：众所公认，培养造就一批杰出的创新型青年医学人才是当今我国高等医学教育工作的重中之重。其首要环节是医优生（医学优秀本科大学生）科研创新能力的先期培养。以往，此方面的明显乏力，以及包括生物化学与分子生物学课程在内的相关课程教学模式的单一低效，是长期制约我国杰出的创新型青年医学人才培养，以及进一步的医学科学创新体系建设的关键性共性教学问题。始于2002年，基于本团队科研优势（本团队承担国家自然科学基金39项；在Nature等主流学术期刊发表SCI论文238篇，他引6800余次），以科研优势转化促进教学、教学反哺科研、科教平衡发展先进教学思想为指导，以个人兴趣和学业成绩为标准，从医学本科二年级大学生中选拔医优生，以生物化学与分子生物学理论课与实验课教学模式改革为基础，坚持以学生为中心以及以本为本的原则，引入以研究解决临床科学问题为导向的新教学理念，结合系列大学生创新项目，在系列广东省教改项目的支持之下，经多年实践优化，成功创建了此生物化学与分子生物学课程“3+X”多维度精细化新教学模式，为着力培养医优生的科研创新能力提质增效，成效十分显著，为有效解决上述关键性共性教学问题提供了一种有重要借鉴价值的新的教学模式方法。

注释：此文受广东省本科高校高等教育教学改革项目（粤教高函[2016]236号，教改项目题目：医学本科大学生生物化学与分子生物学创新能力培养实验平台建设）和广东省高等院校学科建设教学质量改革工程专项项目（粤财教[2011]473号，教改项目题目：医学分子生物学人才培养模式创新实验区）的资助。

Abstracts

中国生物化学与分子生物学会2019年全国学术会议暨学会成立四十周年



专题一：RNA与生命调控

A-1-001　IGF2BP3在肿瘤中作用的研究进展

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摘要：RNA结合蛋白具有多种功能，其介导的基因表达的转录后调控是决定致癌过程的主要因素。胰岛素样生长因子2 mRNA结合蛋白家族包括3个RNA结合蛋白，IGF2BP1，IGF2BP2和IGF2BP3，参与调控RNA的转运，翻译，并通过结合靶向mRNA如胰岛素样生长因子2（IGF-2），C-Myc等的编码区进行转换。其中，IGF2BP3是一个癌胚蛋白，在胚胎形成期表达最多，而在正常的成年小鼠组织和成年人组织中几乎不表达（除纤维母细胞，淋巴细胞及睾丸）。近年来的研究显示，包括IGF2BP3在内的IGF2BP家族成员作为基因表达的转录后调控因子，与肿瘤细胞的增殖，存活，化疗抵抗以及侵袭相关。同时临床数据也表明，IGF2BP家族成员在侵袭性的恶性肿瘤中表达上调，并与多种癌症的不良预后和转移相关。

关键词： IGF2BP3；肿瘤；侵袭；转移；预后

A-1-002　MIAT silencing inhibited Hepatocellular carcinoma by inducing cellular senescence

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Hepatocellular carcinoma (HCC) is a leading cause of cancer deaths and lacks effective therapies. Cellular senescence acts as a barrier against cancer progression and plays an important role in tumor suppression. Cellular senescence associated long noncoding RNAs (SAL-RNAs) are thought to be critical regulators of cancer development. Here, the long noncoding RNA (lncRNA) myocardial infarction-associated transcript (MIAT) was first identified as an HCC specific senescence-associated long noncoding RNA (SALncRNA) that plays an important role in promoting cellular senescence and inhibiting hepatic progression. In addition, SAL-MIAT acts as a competitive endogenous RNA (ceRNA) that upregulated the expression of SIRT1 by sponging miR-22-3p. MIAT silencing alleviated HCC progression by inducing HCC cellular senescence and stimulating senescent cancer cells to secrete senescence-associated secretory phenotype (SASP) by activating the p53/p21 and p16/pRb tumor suppressor pathway. In summary, we identified a tumor-promoting and senescence-suppressing role of MIAT in HCC tumorigenesis. MIAT silencing-induced HCC cellular senescence served as a typical tumor-suppressive mechanism that restricts the proliferation of malignant cells, and the anticancer function of SASP contributes to tumor cell clearance, which might offer a potential therapeutic strategy for HCC treatment.

Key Words: Long noncoding RNA MIAT, ceRNA, miR-22-3p, SIRT1, hepatocellular carcinoma, cell senescence

A-1-003　Study on goose TLR4、15 and 21 induced by LPS

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ABSTRACT: Toll-like receptors（TLRs） are a class of important pattern recognition molecules, which play an important

role in natural immunity and also mediate adaptive immune response. In this study, the extracellular domain sequences of TLR4, 15 and 21 genes of Northeast domestic Goose（*Anser Cygnoides*） were cloned by RT-PCR and analyzed by bioinformatics. At the same time, some extracellular domain sequences were inserted into prokaryotic expression vector pET32a and optimized expression in E.coli BL21(DE3). The fusion protein was detected by SDS-PAGE and were used to immunize rabbits to prepare polyclonal antibodies. The level of antibody was detected by ELISA and the recombinant protein was identified by Western Blot. Secondly, the inflammation model of Northeast domestic goose induced by LPS was established. Real-time PCR and immunohistochemical techniques were used to detect the expression of TLR4, 15 and 21 in goose tissues. The results showed that TLR4, 15 and 21 part had 8, 9 and 9 leucine enrichment regions, the size of recombinant protein was 40 ku, 43 Ku and 42 ku, respectively, and the titer of antibody was above 1:20000. Under LPS induction, TLR4, 15 and 21 were the highest expression in bursa of Fabricius, followed by thymus and spleen. IL-1β, IL-6, IFN-α and IFN-γ were up-regulated in immune organs. It provided a theoretical basis for the study of natural immune regulation in poultry, and also provided a basis for the development of natural adjuvants.

Key words: *Anser Cygnoides*; Toll-like receptors; Prokaryotic expression; tissue distribution

A-1-004 Mapping spatial transcriptome with light-activated proximity-dependent RNA labeling

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RNA molecules are highly compartmentalized in eukaryotic cells, with their localizations intimately linked to their functions. Despite the importance of RNA targeting, our current knowledge of the spatial organization of transcriptome has been limited by a lack of analytical tools. In this study, we develop a novel chemical biology approach to label RNAs in live cells with high spatial specificity. Our method, called CAP-seq, capitalizes on light-activated, proximity-dependent photo-oxidation of RNA nucleobases, which could be subsequently enriched via affinity purification and identified by high-throughput sequencing. Using this technique, we investigate the local transcriptomes that are proximal to various subcellular compartments, including the endoplasmic reticulum and mitochondria. We discover that mRNAs encoding for ribosomal proteins and oxidative phosphorylation pathway proteins are highly enriched at the outer mitochondrial membrane. Due to its specificity and ease of use, CAP-seq is a generally applicable technique to investigate the spatial transcriptome in many biological systems.

Key Words: RNA labeling, photo-oxidation, click reaction, mitochondria

A-1-005 Focused piRNA pathway screens identified multiple pathways involved in heterochromatin formation

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Piwi-interacting RNA (piRNA) pathway is a small RNA based “innate immune”” system to defend transposons in animal germlines. About 200~300 genes have been identified as piRNA pathway candidate genes in *Drosophila.* Panoramix (Panx) links Piwi:piRNA complexes and general heterochromatin machinery. However, the mechanism of Panx-mediated

heterochromatin formation is unclear. Here, we report a small scale RNAi screen to identify factors required for Panx induced heterochromatin formation. We identified ~40 genes that act downstream of Panx. dNxf2 is one of top candidates in the screen. It was found that dNxf2 functions to silence piRNA guided transposon as a complex with Panx and dNxt1. CG14438 is another top candidates whose function in heterochromatin formation is unknown, although it has been copurified with HP1a. Loss of CG14438 leads to animal sterility and transposon overexpression, similar to other core piRNA pathway components. Further detailed dissection about the mechanism of CG14438 mediated heterochromatin formation will be presented.

Key Words: piRNA, RNAi screen, Heterochromatin

A-1-006 A Pandas complex adapted for piRNA-guided transposon silencing

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The repression of transposons by the Piwi-interacting RNA (piRNA) pathway is essential to protect animal germ cells. In Drosophila ovaries, Panoramix (Panx) enforces transcriptional silencing by binding to the target-engaged Piwi-piRNA complex, although the precise mechanisms by which this occurs remain elusive. Here, we show that Panx functions together with a germline specific paralogue of a nuclear export factor, dNxf2, and its cofactor dNxt1 (p15), as a ternary complex to suppress transposon expression. Structural and functional analyses demonstrate that dNxf2 binds Panx via its UBA domain, which plays an important role in transposon silencing. Unexpectedly, dNxf2 interacts directly with dNxf1 (TAP), a general nuclear export factor. As a result, dNxf2 prevents dNxf1 from binding to the FG repeats of the nuclear pore complex, a process required for proper RNA export. Transient tethering of dNxf2 to nascent transcripts leads to their nuclear retention. Therefore, we propose that dNxf2 may function as a Pandas (Panoramix-dNxf2 dependent TAP/p15 silencing) complex, which counteracts the canonical RNA exporting machinery and restricts transposons to the nuclear peripheries. Our findings may have broader implications for understanding how RNA metabolism modulates epigenetic gene silencing and heterochromatin formation.

A-1-007 RNase L参与DNA双链断裂损伤修复

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DNA损伤修复对维持细胞正常功能具有重要的作用。近来发现RNA分子在DNA双链断裂损伤修复中起重要作用，但确切的作用机制仍不清楚。本研究旨在阐明脊椎动物免疫系统中的重要核糖核酸酶RNase L在DNA双链断裂损伤修复中的作用。研究发现通过电离辐射或喜树碱诱导细胞发生DNA双链断裂，RNase L的敲减降低了细胞存活率，组蛋白γ–H2AX磷酸化检测结果表明DNA双链断裂修复水平显著降低。进一步研究结果表明RNase L与DNA末端连

接中涉及的核心因子XRCC4和Lig4相互作用，并通过非同源末端连接（NHEJ）途径促进DNA双链断裂修复。我们的研究结果揭示了RNase L在NHEJ修复途径中的作用，RNA和RNase L参与NHEJ修复途径的详细分子机制尚需深入研究。

关键词：RNase L；DNA损伤修复；非同源末端连接

A-1-008 Genome-wide identification of protein binding sites on RNAs in mammalian cells

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RNA-binding proteins (RBPs) are proteins that bind to the RNA and participate in forming ribonucleo-protein complexes. They have crucial roles in various biological processes such as RNA splicing, editing, transport, maintenance, degradation, intracellular localization and translation. The RBPs bind RNA with different RNA-sequence specificities and affinities, thus, identification of protein binding sites on RNAs(R-PBSs) will deeper our understanding of RNA-protein interactions. Currently, high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP, also known as CLIP-Seq) is one of the most powerful methods to map RNA-protein binding sites or RNA modification sites. However, this method is only used for identification of single known RBPs and antibodies for RBPs are required. Here we developed a novel method, called capture of protein binding sites on RNAs (RPBS-Cap) to identify genome-wide protein binding sites on RNAs without using antibodies. Double click strategy is used for the RPBS-Cap assay. Proteins and RNAs are UV-crosslinked in vivo first, then the proteins are crosslinked to the magnetic beads. The RNA elements associated with proteins are captured, reverse transcribed and sequenced. Our approach has potential applications for studying genome-wide RNA-protein interactions.

Key Words: RNA-Binding Proteins (RBPs), RNA-Protein interactions, Protein binding sites on RNAs (R-PBSs), High-throughput sequencing

A-1-009 miRNA-141-3p在HELF细胞衰老进程中作用的研究

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摘要：目的： 探讨miRNA-141-3P在人胚肺成纤维细胞HELF衰老过程中的作用及其相关通路的作用机制。通过观察HELF原代细胞不同代龄中miRNA-141-3P的表达探讨miRNA-141-3P在人胚肺成纤维细胞HELF衰老中的作用；检测SA-β-Gal活性和细胞增殖情况，检测p53、CDK1、Keap1的mRNA表达变化，通过相关性分析，提出miRNA-141-3P相关的衰老机制科研假设；为人类衰老基因及衰老相关疾病的研究提供理论参考。**方法：**培养人胚肺成纤维细胞HELF原代细胞，通过细胞传代培养的HELF直至衰老，建立复制性衰老模型。将HELF原代细胞分为青年组（2代）、老年组（9代）。采用CCK-8法测定细胞增殖情况；采用细胞衰老β-半乳糖苷酶染色法检测SA-β-Gal活性；通过形态学观察判断衰老细胞形态变化；采用实时荧光定量PCR法测定各代龄细胞miRNA-141-3p表达水平以及p53、CDK1、Keap1的mRNA表达情况，并比较不同代龄细胞上述指标的差异。实验数据用SPSS17.0统计学软件分析，计数资料以频数或百分率表示，两组正态分布的计量资料采用Student’s t-test 进行检验，组间差异采用单因素方差分析进行处理，相关性分析采用Pearson直线相关分析，对于不满足应用条件的变量的分析采用非参数法分

析，所有统计分析方法均以α=0.05作为检验水准，以P < 0.05为统计学差异，P < 0.01为显著性差异。**结果：**与青年组比较，衰老组HELF细胞数量稀少，细胞核边缘模糊，细胞呈针状或拉丝状，失去成纤维细胞特有的星型形状形态改变明显，增殖能力明显下降（P<0.05），SA-β-Gal染色青年组着色较浅，着色细胞数较少；老年组细胞SA-β-Gal染色反而呈阴性。与青年组相比，老年组miRNA-141-3p表达下降（P<0.05）、p53表达上升（P<0.05）、CDK1表达下降（P<0.05）、Keap1表达下降（P<0.05）。相关分析结果表明miR-141-3p与细胞增殖能力显著正相关（P<0.01），miR-141-3p与P53表达显著负相关（P<0.01），与CDK1表达正相关（P<0.05）；P53与CDK1表达负相关（P<0.05）。**结论：**miRNA-141-3p在人胚肺成纤维细胞HELF衰老中表达下降，miRNA-141-3p/Keap1通路与衰老密切相关。miRNA-141-3p在人胚肺成纤维细胞HELF衰老中与p53显著负相关；原代培养HELF细胞到第9代，即HELF衰老时β-半乳糖苷酶阴性染色可能由于衰老细胞休眠不再表达此酶有关，不再适宜继续培养，不适宜于作为衰老干预研究使用。

关键词：人胚肺成纤维细胞HELF；miRNA-141-3p；p53；Keap1

A-1-010 *Musa balbisiana* genome reveals subgenome evolution and functional divergence

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Banana cultivars (*Musa* ssp.) are diploid, triploid and tetraploid hybrids derived from *Musa acuminata* and *Musa balbisiana*. We presented a high quality draft genome assembly of *M. balbisiana* with 430 Mb (87%) assembled into 11 chromosomes. We identified that the recent divergence of *M. acuminata* (A-genome) and *M. balbisiana* (B-genome) occurred after lineage-specific whole genome duplication (WGD) and that the B-genome may be more sensitive to the fractionation process compared to the A-genome. Homoeologous exchanges occurred frequently between A- and B-subgenomes in allopolyploids. Genomic variation within progenitors resulted in functional divergence of subgenomes. Global homoeolog expression dominance occurred between subgenomes of the allotriploid. Gene families related to ethylene biosynthesis and starch metabolism exhibited significant expansion at the pathway level and wide homoeolog expression dominance in the B-subgenome of the allotriploid. The independent origin of 1-aminocyclopropane-1-carboxylic acid oxidase (*ACO*) homoeolog gene pairs and tandem duplication-driven expansion of *ACO* genes in the B-subgenome contributed to rapid and considerable ethylene production after harvest in allotriploid banana fruits. The findings of this study provide greater context for understanding fruit biology and aid the development of tools for breeding optimal banana cultivars.

Key Words: Banana, Genome, Subgenome, Evolution, Functional divergence

A-1-011 短时效七氟醚处理对高糖损伤的内皮细胞增殖和凋亡的影响

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目的：研究七氟醚对高糖损伤的血管内皮细胞的影响，并探讨PI3K/AKT信号通路在其中的作用。方法培养脐静脉内皮细胞，分为6组：A组，正常对照组（5.5mmol/L葡萄糖）；B组，甘露醇组（5.5mmol/L葡萄糖+27.5mmol/L甘露醇）；C组，高糖损伤组（33mmol/L葡萄糖）；D组，高糖损伤（33mmol/L葡萄糖）+七氟醚预处理组。D组建立高糖损伤模型前以2.5%七氟醚2L/min预处理2小时。损伤24小时后，MTT法和流式细胞术PI/Annexin双染法分别检测细胞增殖和凋亡水平；Western Blot和qPCR法分别检测PI3K、Akt、Caspase-3、Bcl-2、Bax的蛋白和mRNA表达

水平。结果表明，A、B、C、D组细胞增殖水平差异无统计学意义。与A组相比，C、D组凋亡增多，PI3K、Akt、PDK1、Bcl-2蛋白水平降低，Caspase-3、Bax蛋白水平升高（P<0.05）；与C组相比，D组凋亡增多，PI3K、Akt、PDK1、Bax蛋白水平下降，Caspase-3、Bcl-2蛋白水平上升（P<0.05）。结论：短时效七氟醚处理不可逆转高糖引起的血管内皮损伤，其机制可能与PI3K、AKT信号通路被抑制有关。

关键词：七氟醚；高糖损伤；血管内皮细胞；PI3K

A-1-012 miR-520c-3p调控LPS诱导的巨噬细胞吞噬和氧化应激功能的研究

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炎症反应是机体免疫系统对感染和组织损伤的复杂反应，巨噬细胞是炎症反应的主要效应细胞，应对外界刺激可以产生细胞因子和趋化因子以限制感染。MicroRNA（miRNA）是内源性非编码单链RNA分子，长度约为18-25nt，研究显示miRNA可以通过靶基因发挥功能来调节基因表达。研究表明，多种miRNAs参与炎症的调节，由文献可知miR-520c-3p在多类肿瘤中被广泛研究，并且通过荧光素酶实验证实RELA是miR-520c-3p的一个靶基因，那么在肿瘤中的相关性是否也体现在炎症反应中呢，尚未见报道。

目的： 本研究课题以脂多糖（LPS）刺激THP-1建立炎症模型，探究miR-520c-3p在THP-1巨噬细胞炎症反应和氧化应激中的作用，宗旨是：miR-520c-3p和RELA调控THP-1巨噬细胞释放炎症因子（IL-1β、TNF-α、IL-6），吞噬荧光标记的氧化低密度脂蛋白（Dil-ox-LDL），调节丙二醛（MDA）和活性氧（ROS）的表达。

方法：实时荧光定量PCR（qPCR）、ELISA、免疫荧光技术、Western blot等等。

结果：miR-520c-3p在不同浓度LPS刺激的THP-1巨噬细胞中表达下调；Pre-miR-520c使miR-520c-3p的表达量增加，Anti-miR-520c可以抑制miR-520c-3p的表达量；Pre-miR-520c抑制细胞炎症因子IL-1β、TNF-α、IL-6的表达，Anti-miR-520c促进细胞炎症因子IL-1β、TNF-α、IL-6的表达；Pre-miR-520c可以抑制THP-1巨噬细胞摄取Dil-ox-LDL，Anti-miR-520c可以促进THP-1巨噬细胞摄取Dil-ox-LDL；Pre-miR-520c下调THP-1巨噬细胞氧化应激标志物MDA和ROS的表达量，Anti-miR-520c效果相反。

结论： miR-520c-3p在不同浓度LPS刺激的THP-1巨噬细胞中表达下调，并调控IL-1β、TNF-α、IL-6的表达，调控THP-1吞噬Dil-ox-LDL和氧化应激标志物MDA和ROS的表达

关键词：巨噬细胞；miR-520c-3p；炎症反应；氧化应激

A-1-013 Structure and degradation of circular RNAs regulate PKR activation in innate immunity

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Summary: CircRNAs produced from back-splicing of exons of pre-mRNAs are widely expressed, but current understanding of their functions is limited. These RNAs are stable in general and are thought to have unique structural conformations distinct from their linear RNA cognates. Here we uncover that endogenous circRNAs tend to form 16-26 bp imperfect RNA duplexes and act as inhibitors of double-stranded RNA (dsRNA)-activated protein kinase (PKR) related to innate immunity. Upon poly(I:C) stimulation or viral infection, circRNAs are globally degraded by RNase L, a process required for PKR activation in early cellular innate immune responses. Augmented PKR phosphorylation and circRNA reduction are found in peripheral blood mononuclear cells (PBMCs) derived from patients of autoimmune disease systemic lupus erythematosus (SLE). Importantly, over-expression of the dsRNA-containing circRNA in PBMCs or T cells derived from SLE can alleviate the aberrant PKR activation cascade, thus providing a connection between circRNAs and SLE.

Keywords: Circular RNAs (circRNAs), circRNA structure, circRNA degradation, double-stranded RNAs (dsRNAs), RNase L, PKR, innate immune responses, autoimmune disease, SHAPE-MaP, systemic lupus erythematosus (SLE)

A-1-014 LncRNA-guided chromatin remodeling and gene regulation

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Residing in the physical heart of the cell, the nucleus has now fully shed its once one-dimensional reputation as the repository for genetic information toward a more dynamic three-dimensional view of chromatin biology. As an important regulatory factor, long non-coding RNAs (lncRNAs) has exhibited its critical role in chromosomal architecture and gene expression, we thus carried out a series of research to shed light on lncRNA-guided chromatin remodeling and gene regulation. We firstly delineated a novel mechanism by which a kcnq1ot1 lncRNA directly builds an intrachromosomal interaction complex to establish allele-specific transcriptional gene silencing over a large chromosomal domain. In addition, we also proposed a novel mechanism by which *ROR* lncRNA may serve as a decoy oncoRNA that blocks binding surfaces, preventing the recruitment of histone modifying enzymes, thereby specifying a new pattern of histone modifications that promote tumorigenesis. Moreover, we further revealed a novel *GAU1* lncRNA was initially activated by an open chromatin status, triggering recruitment of the transcription elongation factor TCEA1 at the oncogene *GALNT8* promoter and cis-activated the expression of *GALNT8*. Collectively, these studies will provide better understanding of a novel lncRNA-guided dynamic mechanism of chromatin tuning.

Key Words: lncRNA, chromatin remodeling, gene regulation

A-1-015 A microRNA processing mechanism targeting cryptochrome circadian regulator 2 modulates myogenesis

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Cry2 (Cryptochrome Circadian regulator 2) is an important circadian regulator. It has a wide range of functions in regulating sleep, body temperature, metabolism. It is also a critical player in adult myogenesis. We found that Cry2 was significantly upregulated in aged skeletal muscles and driving muscle stem cells moving towards differentiation. Further analysis indicates that Cry2 is the target of miR7 and negatively regulated by the microRNA. Though microRNA metabolism and processing has been shown to be critical in many physiological processes, its functions in myogenesis remains to be elusive. We explored the regulatory mechanism of miR7 processing and found that a pair of RNA binding proteins, Msi2 and HuR, served as negative regulators of the maturation of miR7 and therefore improve the expression of Cry2 in aged muscles. In summary, we identified a new signaling pathway modulating microRNA processing and circadian gene expression to regulate myogenesis.

Key Words: Msi2, RNA binding proteins, myogenesis, circadian regulator 2, miR-7a

A-1-016 Circular RNA CircTET3 Mediates Migration of Vascular Smooth Muscle Cells in Vein Graft by Targeting MiR-351-5p

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MicroRNAs (miRNAs) regulate gene expression by repressing translation of target genes. Our previous work has established a role for miR-33/BMP3 protecting against abnormal proliferation of vascular smooth muscle cells (VSMCs) which is the pathological basis of neointimal hyperplasia in the grafted vein. We further detected whether circular RNAs (circRNAs) interacting with miRNAs are involved in the dysfunction of VSMCs in graft vein. Grafted veins were generated by the 'cuff' technique in rats and the whole transcriptome deep sequencing was applied to identify differential circRNAs in the graft vein compared with the self-control vein. CircTET3 was increased in the graft vein compared with the control vein, and the circular structure was verified by PCR and RNase R digestion. In VSMCs, circTET3 was transcribed from the 4th exon of *tet3* and stably located in cytoplasm. Based on the miRNA-sequencing and bioinformatics, circTET3 was predicted to bind with miR-351-5p, which was confirmed by RNA pull-down and dual-luciferase reporter assay. Specific siRNA of circTET3 suppressed VSMC migration by acting as an endogenous miR-351-5p sponge. Then, a differentially expressed circRNA - miRNA -mRNA triple network was constructed. Ingenuity pathway analysis showed the genes function in the network. PTPN1 were the targeted genes due to the competitive binding of circTET3 to miR-351-5p. Our findings reveal a novel circular RNA circTET3 acts as a nature sponge for miR-351-5p to modulate VSMC migration in graft vein. This regulatory pathway may serve as a potential therapeutic avenue against neointimal hyperplasia. (NSFC, Nos. 11625209, 11772198)

Key Words: Vascular smooth muscle cells, Circular RNAs, Vein graft, miR-351-5p, Migration.

A-1-017 CircTulp4 functions in Alzheimer's disease pathogenesis by regulating its parental gene, *Tulp4*

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Alzheimer's disease (AD)—one of the most common neurodegenerative diseases worldwide—impairs cognition, memory, and language ability and causes dementia. However, AD pathogenesis remains poorly elucidated. Recently, a potential link between AD and circular RNAs (circRNAs) has been uncovered, but only a few circRNAs that might be involved in AD have been identified. Here, we systematically investigated circRNAs in the APP/PS1 model mouse brain through deep RNA-sequencing. We report that circRNAs are markedly enriched in the brain and that several circRNAs exhibit differential expression between wild-type and APP/PS1 mice. We characterized one abundant circRNA, circTulp4, derived from Intron1 of the gene Tulp4. Our results indicate that circTulp4 predominantly localizes in the nucleus and interacts with U1 snRNP and RNA polymerase II to modulate the transcription of its parental gene, Tulp4, and thereby regulate the function of the nervous system and participate in the development of AD.

Keywords: CircTulp4, Alzheimer's disease (AD), Tulp4, Neurite length, U1 snRNP

A-1-018 LncRNA HOXA-AS2在血管内皮细胞中的功能及分子机制研究

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心血管疾病是在全球范围内严重危害公众健康的重要疾病之一，其中动脉粥样硬化（Atherosclerosis，AS）是一种由脂质代谢异常、慢性炎症、免疫紊乱及遗传等多种因素互相作用下发生的一种慢性代偿性炎症反应，是许多心血管疾病的病理基础。近几年的研究表明，长链非编码RNAs主要是从表观遗传学、转录水平及转录后水平等多个层次调控基因的表达，参与机体的生长发育、细胞凋亡、增殖、分化等过程，并与许多疾病的发生有密切关系。既往研究发现lncRNA HOXA-AS2在各种恶性肿瘤中表现出异常表达，其致癌性主要通过直接或间接途径抑制或促进相关基因的表达，但HOXA-AS2与心血管疾病的关系目前研究的很少。本研究探索lncRNA HOXA-AS2与血管内皮细胞功能紊乱及动脉粥样硬化的关系，为动脉粥样硬化的预防和治疗提供新的思路和研究方向。

目的：本研究旨在探讨lncRNA HOXA-AS2在TNF-α诱导的血管内皮细胞的粘附、增殖和凋亡中的作用及其分子机制。

结果：（1）q-PCR结果显示在TNF-α刺激下，血管内皮细胞中HOXA-AS2的表达量明显增加；（2）内皮细胞粘附实验表明敲低HOXA-AS2减少THP-1细胞的粘附，过表达HOXA-AS2增加THP-1细胞的粘附；MTT法细胞增殖实验表明敲低HOXA-AS2能促进血管内皮细胞的增殖，过表达HOXA-AS2能抑制内皮细胞的增殖；流式凋亡实验表明下调HOXA-AS2能抑制血管内皮细胞的凋亡；（3）q-PCR结果显示在TNF-α刺激下，HOXA-AS2敲低后ICAM-1粘附因子及IL-6、IL-1β和TNF-α炎症相关分子的表达明显减少；Western Blot结果表明HOXA-AS2敲低后ICAM-1，VCAM-1粘附蛋白及Caspase-3和Caspase-9凋亡蛋白表达降低；

结论：LncRNA HOXA-AS2在TNF-α诱导的血管内皮细胞中表达明显上调，敲低HOXA-AS2后可促进内皮细胞增殖，抑制内皮细胞粘附和凋亡，并且调控粘附分子、炎症相关分子和凋亡蛋白的表达水平。

关键词：血管内皮细胞，lncRNAs，HOXA-AS2，内皮功能紊乱

A-1-019 LPS induces mGluR5 expression of neutrophils in p65-dependent manner

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Lip polysaccharide (LPS) is an important pathogenic stimulator to activate neutrophils. Metabotropic glutamate receptor 5 (mGluR5) is highly expressed in central nervous system that modulates glutamate release and circuits activity. However, the roles and functions of mGluR5 in peripheral systems especially on neutrophils are poorly understood. Here we found that LPS induced both mRNA and protein expressions of mGluR5 in neutrophils. Activation of mGluR5 promotes p65 nuclear translocation in PKC-dependent manner. The ability of cytokines releasing and migration toward LPS treatment in mGluR5 KO neutrophils was dramatically impaired compared with WT neutrophils. Furthermore, LPS induced mGluR5 expression by enhancing p65 binding to mGluR5 upstream promoter element (-779 to -113), which was confirmed by Luciferace and CHIP experiments. These findings demonstrate that induction of mGluR5 by LPS is vital for neutrophils activation, which unveils the inducible functionality of mGluR5 in neutrophils.

Key words: LPS; neutrophil, p65; mGluR5

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A-1-020 The inhibition effect to bladder cancer metastasis and molecular mechanism of LINC00892

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LncRNA is a non-coding RNA (ncRNA) molecule of more than 200nt in length and rich in biological functions. It can directly interact with proteins, microRNAs, etc. LncRNA has been found to be associated with various types of cancer progression. By analyzing the information of bladder cancer patients in TCGA database, we found that LINC00892 was significantly down-regulated in cancer tissues, which was consistent with the expression of LINC00892 in our clinical specimens collected. The expression of LINC00892 was positively correlated with the survival of patients with bladder cancer. In vitro and in vivo function experiments have revealed that LINC00892 significantly inhibited the biological function of bladder cancer matastasis. Mechanism studies showed that LINC00892 downregulated RhoA and RhoC by decreasing the expression of nucleolin(NCL), which decreased RhoA and RhoC mRNA stability. Further, LNC00892 downgraded the expression of NCL by inhibiting the transcriptional activity of its promoter. Globally, LINC00892 inhibited the invasion-metastasis by NCL/ RhoA and RhoC cascade. The results have potential implications for us to understand the progress of bladder cancer and provide a theoretical basis for the development of anti-bladder cancer metastasis drugs or technologies targeting LINC00892 and related intermediate signaling molecules.

Keywords: bladder cancer metastasis; LINC00892; RhoA, RhoC; NCL; prognosis diagnosis

A-1-021 Oncogenic lncRNA *TURBOR* promotes Warburg effect by enhancing LDHA enzyme activity

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Aerobic glycolysis, also known as the Warburg effect, is a hallmark of cancer and essential for malignancies, but its regulation and modulation in cancer cells remain poorly understood. Here, by large-scale functional screening, we identified a tumor-associated and broadly expressed oncogenic long noncoding RNA *TURBOR*, which is highly expressed in multiple types of human cancers. Notably, knocking down *TURBOR* significantly inhibits the proliferation of multiple types of cancer cells and reduces tumor growth in vivo. Mechanistically, *TURBOR* directly binds to lactate dehydrogenase A (LDHA), an essential glycolysis-associated enzyme, and enhances its enzymatic activity, thereby promoting glycolysis. Clinically, high expression of *TURBOR* is significantly associated with poor prognosis in many types of human cancers. Together, our results identified a new long noncoding RNA (lncRNA) that modulated a cancer-specific regulation mechanism for the Warburg effect and suggested a potential target against one of cancer's vulnerabilities for developing broad-acting anti-cancer therapies.

Key Words: Long noncoding RNA, Warburg effect, Tumor proliferation

A-1-022 Dynamic imaging of RNA in living cells by CRISPR-Cas13 systems

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Visualizing the location and dynamics of RNAs, particularly long noncoding RNAs (lncRNAs), in live cells is key to understanding function, but currently available tools are limited. Here, we identify two endonuclease-deficient, single-component programmable RNA guided RNA-targeting Cas13 RNases (dCas13) that allow efficient lncRNA labeling in live cells, even using single guide RNAs with spacers of 20-27nt in length. Compared to traditional aptamer-based strategies (such as the MS2-MCP system), the optimized dCas13 RNA labeling system achieves a higher labeling efficiency and does not require genetic manipulation. Combination of two different CRISPR-dCas13 systems or CRISPR-dCas13 and MS2-MCP allows dual-color RNA tracking in single live cells and the study of the dynamics of the lncRNA NEAT1-associated paraspeckle nuclear bodies. Collectively, these results demonstrate that the CRISPR-dCas13 RNA labeling system is a user-friendly and efficient tool to visualize RNAs in live cells.

Keywords: CRISPR-Cas13; NEAT1; nuclear stress bodies; paraspeckle dynamics; RNA microscopy; RNA dynamics; SatIII

A-1-023 A combinatorial strategy for overcoming primary and acquired resistance of MEK inhibition in colorectal cancer

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[Abstract] Compared with traditional chemotherapeutic drugs, targeted therapeutic medicine has the advantages of high efficacy and less toxic side effects. However, in clinical practice for treatment of colorectal cancer, the primary and acquired resistance of these medicines limits the effectiveness of targeted therapy, which impedes development of precision medicine and personalized therapy. Currently, there are limited drugs for targeted therapy of colorectal cancer, mainly applying monoclonal antibodies against EGFR or VEGFR inhibitors. Trametinib, a MEK inhibitor approved by the US Food and Drug Administration, can significantly improve survival of melanoma patient. However, this agent has not been used for clinical treatment of colorectal cancer because of drug resistance. To identify the resistance mechanism of colorectal cancer cells to trametinib and overcome this situation, in present experiment, the Chou-Talalay method was performed to screen synergistic drug combinations. We obtained a synergistic combination of trametinib and GSK2126458, and the combination index values were less than 1 in the primary resistant cell lines SW480, CW-2 and the acquired drug-resistant cell line RKO-R. This combination could inhibit the colony formation of colorectal cancer cells and the growth of xenograft tumors in nude mice. Mechanistic analysis confirmed that trametinib can activate the alternative PI3K-AKT signaling pathway while inhibiting the MAPK pathway, which may be one of the molecular mechanisms of primary and acquired trametinib tolerance in colorectal cancer cells. This bypass activation can be blocked by combination with GSK2126458. These results suggest that combination of trametinib and GSK2126458 is an effective approach for treating colorectal cancer resistance to trametinib.

Keywords: Colorectal cancer; Trametinib; Targeted therapy; Drug resistance; Resistance mechanism; Drug combination

A-1-024 ALYREF links 3'-end processing to nuclear export of nonpolyadenylated mRNAs

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The RNA-binding protein ALYREF plays key roles in nuclear export and also 3'-end processing of polyadenylated mRNAs, but whether such regulation also extends to non-polyadenylated RNAs is unknown. Replication-dependent (RD)-histone mRNAs are not polyadenylated, but instead end in a stem-loop (SL) structure. Here, we demonstrate that ALYREF prevalently binds a region next to the stem loop on RD-histone mRNAs. SL-binding protein (SLBP) directly interacts with ALYREF and promotes its recruitment. ALYREF promotes histone pre-mRNA 3'-end processing by facilitating U7-snRNP recruitment through physical interaction with the U7-snRNP-specific component Lsm11. Furthermore, ALYREF, together with other components of the TREX complex, enhances histone mRNA export. Moreover, we show that 3'-end processing promotes ALYREF recruitment and histone mRNA export. Together, our results point to an important role of ALYREF in coordinating 3'-end processing and nuclear export of non-polyadenylated mRNAs.

Key words: ALYREF/mRNA export/RD-histone mRNA/SLBP/3'-end processing

A-1-025 Systematic analyses of m^{6}A methylomes revealed pervasive site-specific regulation of m^{6}A by RNA binding proteins

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N^6-methyladenosine (m^{6}A) is a reversible and dynamic RNA modification in eukaryotes. It plays important roles in a variety of physiological and pathological processes. However, it is still poorly understood how the cell-specific m^{6}A methylomes are established and whether there is prevalent site-specific *trans*-regulation of m^{6}A. In this study, based on the public available m^{6}A-seq data of 25 unique cell lines, we found the m^{6}A peaks near stop codons were stable, while the variable m^{6}A peaks were actually away from stop codons. We found the m^{6}A indexes of the co-methylation modules classified using the variable m^{6}A peaks exhibited remarkable topology-specificities and cell line-specificities. To systematically elucidate the *trans*-acting m^{6}A co-factors, we correlated these modules with the gene expressions, the binding targets, and the binding motifs of a large number of RNA binding proteins (RBPs). We found pervasive regulation of m^{6}A by RBPs and finally identified 17 high-confidence m^{6}A co-factors whose gene expressions were significantly correlated with the m^{6}A indexes of specific modules, which happened to enrich for the RBP binding targets or motifs. We further experimentally validated that the RNA binding proteins TRA2A and CAPRIN1 could selectively promote the methylations of the m^{6}A sites co-localized with their binding targets on RNAs through physical interactions with the m^{6}A writers. Our findings revealed that various RBPs worked as m^{6}A co-factors to specifically regulate m^{6}A *in trans*, providing novel insights into the mechanisms by which spatial and temporal dynamics of m^{6}A methylomes were established.

Key Words: *N*^{6}-methyladenosine, specific-methylation, RNA binding proteins

A-1-026 MiRNA-520c-3p 对血管内皮细胞功能的影及相关机制研究

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动脉粥样硬化（Atherosclerosis，AS）是发生于大中动脉常见且极具危害性的慢性炎症疾病，而内皮细胞的病理性凋亡会导致动脉粥样硬化斑帽的坏死落，诱发血栓的形成，因此，防止血管内皮细胞的病理性凋亡是预防 AS 疾病发生的关键因素。MicroRNA-520c-3p（miR-520c-3p）是近年来在肿瘤中新发现的一种单链小分子 RNA，属于 miR-520/373 家族，作为一种调控因子参与多种转录后基因的表达。血小板衍生生长因子（Platelet Derived Growth Factor，PDGF），是一种很强的促有丝分裂剂和体外化学趋化剂，可通过激活多条信号通路调节功能蛋白的磷酸化状态和相互作用关系，最终抑制或激活靶基因的表达。

方法：（1）CCK-8法检测 miR-520c-3p 的上调和下调对 HUVECs 细胞增殖能力的影响；（2）免疫荧光细胞粘附实验检测 miR-520c-3p 的上调和下调对细胞粘附功能的影响；（3）流式细胞术评价 miR-520c-3p 上调和下调对内皮细胞凋亡的影响；（4）通过 western blot 方法检测 miR-520c-3p 对 AKT/NF-κB 信号通路的影响。

结果：（1）miR-520c-3p通过下调粘附分子 ICAM-1 和 VCAM-1 及炎性因子 IL-6 和 TNF-α 的表达，减少

HUVECs 与 THP-1 细胞之间的粘附数量；（2）miR-520c-3p 通过下调促凋亡相关蛋白的表达，抑制血管内皮细胞的凋亡，促进血管内皮细胞的增殖及修复；（3）miR-520c-3p 抑制 AKT 和 RELA 蛋白的磷酸化，而 si-RELA 可以恢复 anti-miR-520c-3p 对丝氨酸苏氨酸蛋白激酶（Protein Kinase B，AKT）和 RELA蛋白磷酸化的促进作用，提示 miR-520c-3p 通过 AKT/NF-κB 信号通路来调节血管内皮细胞的增殖凋亡和粘附。

结论：（1）miR-520c-3p 靶向 RELA 抑制 HUVECs 细胞的粘附和凋亡，促进细胞增殖；（2）miR-520c-3p 通过 AKT/NF-κB 信号通路靶向作用 RELA 调控 HUVECs 细胞的增殖、凋亡及粘附功能。

关键词：动脉粥样硬化； HUVECs miR-520c-3p ；细胞粘附与凋亡； RELA

A-1-027 Subcellular transcriptome profiling by APEX2-mediated proximity-dependent RNA labeling

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The RNA localization is closely linked to its biological functions. Asymmetrically localized RNAs are vital to chromatin architecture, local translation, and RNA homeostasis. Current methods for studying RNA localization either lack throughput or suffer contaminations. In this study, we have developed a novel technique to profile local transcriptome. Our method, termed APEX2-mediated proximity-dependent RNA labeling (APEX-RNA labeling), utilizes reactive radicals generated by the reaction of the Btn-An probes with H_2O_2 at the catalysis of APEX2 to label proximal RNA. As a demonstration of the spatial specificity and depth of coverage in mammalian cells, we applied APEX-RNA labeling in the mitochondrial matrix, capturing all 13 mitochondrial mRNAs and no cytoplasmic RNAs. We then extended the methodology to open subcellular regions including nuclear lamina, nucleolus, and cell membrane. APEX-RNA labeling enriched 125 RNAs at nucleolus and 662 RNAs at nuclear lamina, with little overlapping between two sets of RNAs. The 119 mRNAs enriched by APEX-RNA labeling at cell membrane encode proteins that are involved in cell junction, motility, and anchoring. APEX-RNA labeling is thus a promising method for mapping the subcellular transcriptome, which could shed light on their functions in cell physiology.

Key Words: APEX2, proximity-dependent labeling, Biotin-aniline probe, subcellular transcriptome

A-1-028 RNA G-quadruplex Influences MicroRNA-26a Maturation and Function

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RNA G-quadruplexes (RG4s) are secondary structures known to influence RNA post-transcriptional mechanisms and have been explored as potential targets for treatment of human diseases. However, the in vivo evidence of RG4s, as well as their pathophysiological functions, remains extremely unknown. MicroRNAs (miRNAs), the best characterized class of small noncoding RNAs, are emerging as new regulators of gene expression and play critical roles in development and diseases. The biogenesis of miRNAs is under tight temporal and spatial control, and disruption of any step could influence miRNA abundance and outcome. RG4 presence within several precursor miRNAs (pre-miRNAs) has been reported to compete with hairpin formation, suggesting a role of RG4 in miRNA biogenesis and function. However, the biological implications between RG4 and miRNAs are so far limited. Here we report that RG4 regulates the expression and pathophysiological function of

miR-26a. Bioinformatic, biophysical and biochemical analyses collectively identify a guanine-rich sequence in pre-miR-26a-1 that can fold into RG4 structure. Chemical stabilization of this RG4 significantly reduces miR-26a expression *in vitro* and *in vivo*, primarily through impairing pre-miR-26a processing. Using miR-26a knockin or knockout mouse models, we show that this RG4 affects the physiological functions of miR-26a in insulin sensitivity and liver metabolism. Furthermore, we reveal that DHX36 can bind and unwind the RG4 structure in pre-miR-26a-1, thereby enhancing miR-26a maturation. In line with decreased miR-26a expression, DHX36 is prominently reduced in obese mouse livers. Therefore, our findings not only offer new insights into the structural and pathophysiological roles of RG4 in gene regulation, but also delineate a novel regulatory mechanism underlying the deregulation of miR-26a, a promising therapeutic target for various human diseases.

Key Words: G¬quadruplex, MicroRNA, DHX36, T2D, Insulin Sensitivity

A-1-029　miR-1290对IL-8诱导的血管内皮细胞黏附、凋亡的影响及其分子机制的研究

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动脉粥样硬化（Atheroserosis,AS）是发生在动脉血管壁以脂质代谢为特征的慢性炎症性疾病。内皮功能障碍是动脉粥样硬化的早期阶段，进而引发炎症反应。基于本课题组前期研究，我们发现MLCK与AS的发生发展相关联，通过对与MLCK相关的miRNA进行筛查发现了miR-1290等非编码RNA，同时发现miR-1290可能与细胞炎症相关。目前关于miR-1290在动脉粥样硬化领域未曾见相关报道。结果表明，高脂血症患者血清中miR-1290的表达量升高；在IL-8诱导下，血管内皮细胞中miR-1290的表达量升高，其靶基因GSK-3β在mRNA水平以及蛋白水平的表达量均降低；在IL-8刺激下，miR-1290mimic可以上调黏附相关因子ICAM-1、VCAM-1的表达量;同时，miR-1290inhibitor可以下调黏附相关因子ICAM-1、VCAM-1和增殖相关因子cyclinD1的表达量；miR-1290inhibitor可以减少血管内皮细胞的粘附数量，miR-1290mimic可以促进细胞间的粘附作用；miR-1290靶向调控GSK-3β。结论：miR-1290通过靶向GSK-3β对HUVEC的功能产生影响。

关键词：动脉粥样硬化；miR-1290； IL-8； 血管内皮细胞；细胞增殖；细胞黏附

A-1-030　LARP7-Mediated U6 snRNA Modification Ensures Splicing Fidelity and Spermatogenesis in Mice

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U6 snRNA, as an essential component of the catalytic core of the pre-mRNA processing spliceosome, is heavily modified post-transcriptionally with 2′-*O*-methylation being most common. The role of these modifications in pre-mRNA splicing, as well as their physiological function in mammals, has remained largely unclear. Here, we report that the La-related protein LARP7 functions as a critical cofactor for the 2′-*O*-methylation of U6 in mouse male germ cells. Mechanistically, LARP7 promotes U6 loading onto box C/D snoRNP, thereby facilitating U6 2′-*O*-methylation by box C/D snoRNP. Importantly, ablation of LARP7 in the male germline causes defective U6 2′-*O*-methylation, massive alterations in pre-mRNA splicing, and spermatogenic failure in mice, which can be rescued by ectopic expression of wildtype LARP7 but not an U6-loading

deficient mutant LARP7. Our data uncover a novel role for LARP7 in regulating U6 2′-*O*-methylation and demonstrate the functional requirement of such modification for splicing fidelity and spermatogenesis in mice.

Key Words: U6 snRNA, 2′-*O*-methylation, LARP7, box C/D snoRNP, pre-mRNA splicing, spermatogenesis

A-1-031 Intellectual disability associated protein FTSJ1 interacts with WDR6 to catalyze 2'-O-methylation on specific tRNAs

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Chemical modifications in tRNA impact its structure, ribosome binding affinity and codon deciphering, especially at positions of the anticodon stem loop (ASL). The defects of tRNA modifications are always associated with human diseases. $tRNA^{Phe}$(GAA) from nonsyndromic X-linked intellectual disability (NSXLID) patients with loss of *ftsj1* lacks Cm32 and Gm34, hinting that FTSJ1 is human putative tRNA 32 and 34 2'-O-methyltransferase. FTSJ1 is a homolog of yeast Trm7, which interacts separately with two auxiliary proteins, Trm732 and Trm734, for tRNA 32 and 34 2'-O-methylation. However, the catalytic mechanism and RNA substrates of FTSJ1 remain unknown. Our results showed that FTSJ1 directly binds to WDR6, the predicted yeast Trm734 homolog. We found that only the recombinant FTSJ1-WDR6 complex could 2'-O-methylate $tRNA^{Phe}$(GAA) with m^1G37 modification at G34 *in vivo* and *in vitro*, but not the transcript $tRNA^{Phe}$(GAA), suggesting that m^1G37 is the prerequisite for Gm34 formation. Conversely, loss of Gm34 influenced the conversion of m^1G37 to peroxywybutosine (o2yW) in $tRNA^{Phe}$(GAA). Moreover, the translation efficiency of TTT but not TTC codon decreased in CRISPR/Cas9-mediated *ftsj1* gene silencing cells, suggesting that Gm34 affect the interaction of wobble base pairing. These findings indicated the complicated network and crosstalk of tRNA modifications at ASL region.

Key words: tRNA, Trm7, FTSJ1, NSXLID, WDR6

A-1-032 长链非编码RNA SNHG5对人脐静脉内皮细胞功能的影响

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动脉粥样硬化(Atherosclerosis,AS)是一种与脂类代谢障碍有关的，以大中动脉内膜脂质沉积、粥样斑块形成、纤维组织增生、管壁硬化为特征，多种因素相互作用下发生的一种全身性慢性炎症性血管疾病。长链非编码RNA (long noncoding RNA, lncRNA)是一类存在于细胞核或细胞质内长度大于200nt，不编码蛋白质的线性RNAs，最新研究发现lncRNA通过参与血管内皮细胞的增殖、凋亡进而调节其功能，从而影响AS的进展。lncRNA–小核仁RNA宿主基因5 (small nucleolar RNA host gene 5,SNHG5)是全长为524 bp的细胞质lncRNA ，其在胃癌、结直肠癌等多种恶性肿瘤中异常表达，但在AS中的功能是完全未知的。本研究以人脐静脉内皮细胞（human umbilical vein endothelial cell,HUVECs）为研究对象，以IL–8为体外诱导剂建立HUVECs炎症损伤模型，探讨lncRNA SNHG5在IL–8诱导下对

HUVECs功能的影响。

结果显示：1.qRT-PCR结果显示，在IL-8刺激下，HUVECs中lncRNA SNHG5表达量减少；2.内皮细胞粘附实验结果显示，与对照组相比，lncRNA SNHG5过表达后粘附细胞数明显减少，而敲降后粘附细胞数明显增加，并且qPCR及Western blot结果显示lncRNA SNHG5过表达可以抑制ICAM-1 mRNA和蛋白水平的表达，而敲降后得到相反的结果；3. MTT实验结果显示，在IL-8刺激下，lncRNA SNHG5敲降可以明显抑制HUVECs的增殖，而过表达后可以明显促进其增殖；4.Western blot结果显示，lncRNA SNHG5敲降后可以明显抑制细胞增殖相关分子CyclinD1和PCNA的表达，而过表达后得到相反的结果。

结论：我们的研究表明了lncRNA SNHG5对HUVECs具有明显的促进增殖以及抑制细胞粘附的作用。

关键词：lncRNA SNHG5；人脐静脉内皮细胞；细胞增殖；细胞粘附；

A-1-033 LncRNA ZFAS1在动脉粥样硬化中作用的初步探讨

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动脉粥样硬化是一种多因素诱导的由动脉内皮细胞损伤或功能障碍启动的动脉壁的慢性炎症反应。近年来发现长链非编码RNA ZFAS1通过多种机制在各肿瘤进展中发挥促进作用，此外，lncRNA ZFAS1 也参与心肌细胞收缩的调控。但其在动脉粥样硬化中的作用还未明确。本研究拟初步探讨其在血管内皮细胞中的作用。

方法：(1)实时定量PCR（Q-PCR）检测不同浓度梯度的IL-8处理HUVECs后lncRNA ZFAS1的变化；（2）瞬时转染建立lncRNA ZFAS1的过表达和敲低模型；（3）MTT法和细胞粘附实验检测lncRNA ZFAS1过表达和敲低对HUVECs增殖及粘附的影响；（4）Q-PCR检测lncRNA ZFAS1过表达和敲低后炎症因子的变化；（5）Western Blot 检测lncRNA ZFAS1过表达和敲低后增殖、粘附及Wnt/β-catenin 信号通路相关蛋白的变化。

结果：（1）IL-8处理HUVEC后lncRNA ZFAS1的表达下调；（2）lncRNA ZFAS1过表达促进HUVECs增殖并抑制粘附，敲低后抑制HUVEC增殖并促进粘附；（3）lncRNA ZFAS1过表达抑制IL-1及ICAM-1表达，敲低后抑制IL-1、ICAM-1表达；（4）lncRNA ZFAS1过表达促进PCNA蛋白表达，抑制ICAM-1蛋白表达，敲低后与之相反；（5）lncRNA ZFAS1过表达促进β-catenin及cyclinD1蛋白的表达，抑制GSK-3β蛋白表达；敲低后与之相反。

结论：lncRNA ZFAS1影响HUVECs的粘附及增殖，并可能通过Wnt/β-catenin 信号通路影响HUVECs增殖。

关键词：动脉粥样硬化；lncRNA ZFAS1；HUVEC；

A-1-034 The Changes of MicroRNAs-Fkbps-GR Regulatory Circuits in Hippocampus of Aged Rats and the Adjustment Effect of Kidney-Tonifying and Yi Qi Recipe

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The 5 months male SD rats as the young control group, 23 months homologous strain rat are randomly divided into aged control group、aged Tonifying Kidney（Zuogui Pill） group, aged Yi Qi（Yiqi Smart Decoction）group, aged mixture (TK+YQ) group and aged antagonists(RU486) group. The main purpose of the project is to observe microRNAs-Fkbps-GR regulatory circuits affect GR nuclear translocation in aged rats and its effect on learning and memory related genes expression and the adjustment effect of Tonifying Kidney and Yi Qi recipe. The spatial memory ability of rats was observed by Morris water maze method. MicroRNAs-Fkbps-GR Regulatory Circuits(GC, Fkbp5/4, Dynein IC2, GR, miR-124a/511) and learning

memory related signal transduction molecules (Nptx2, Synapsin-1, Homer1) expression by biochemistry and molecular biology technology. The results showed that: Compared with the young control group, the spatial learning and memory ability of the aged rats was significantly decreased, and the serum corticosterone content was significantly increased. The expression of total GR protein and nuclear GR protein in hippocampus was significantly decreased, the expression in cytoplasmic GR protein was significantly increased, and the expression of GR mRNA was significantly decreased. The expression of Dynein IC2 was significantly decreased, the expression of Fkbp5 and Fkbp4 protein was significantly increased, and the expression of Fkbp5 mRNA was significantly increased. miR-511 expression was significantly decreased,miR-124a expression was significantly increased. The expression of learning and memory related genes Nptx2 and Synapsin-1 gene were significantly decreased, and the expression of Homer1 gene was not significantly changed. The medication groups（TK，YQ，TK+YQ and RU486）have different corrective effects on the abnormal changes of the above-mentioned indexes in the aged rats. Thus it can be seen, Tonifying Kidney(Zuogui Pill) and Yiqi Smart Decoction mainly reduce the serum GC content of aged rats, adjust the mRNA expression of miR-Fkbps-GR loop related genes, increase the nuclear translocation of GR, and increased the expression of learning and memory related genes. Resist the toxic effects of glucocorticoids on the hippocampus, thereby delaying the degradation of learning and memory in aged rats.

Key words: aged rat; glucocorticoid; learning and memory; Tonifying Kidney and Yiqi Recipe;

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A-1-035 MiR-196a-5p/NR6A1 regulates stemness and neural differentiation of Ntera-2 embryonal carcinoma cells via E-cadherin inhibition

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Cancer stem cells (CSCs) are a group of malignant tumors characterized by self-renewal, differentiation potential, high tumorigenicity and high drug resistance. Recent studies have shown that CSCs in solid tumors can produce functional neurons to participate in the construction of tumor microenvironment and support cancer growth. Therefore, the molecular targets with a capacity to promote or inhibit neural differentiation within CSCs, are of therapeutic importance. Using human Ntera-2 (NT-2) pluripotent embryonal carcinoma cells as a CSCs model in vitro, the present study investigated the function and regulatory mechanism of miR-196a-5p in CSCs and found that miR-196a-5p can inhibit the proliferation and metastasis as well as maintain the stemness of NT-2 cells through targeting the NR6A1 gene. E-cadherin (CDH1) is also identified as one of the important negative target molecules of NR6A1 in the process of RA-induced neurodifferentiation of CSCs. RNAi-mediated silencing of NR6A1 enhances the sphere formation capacity in NT-2 stem-like cells, and delayed the expression of neuron-specific genes during RA-induced differentiation. Mechanistically, NR6A1 promotes early neural differentiation by inhibiting E-cadherin through binding with DR0 sites in the CDH1 gene promoter. Our study revealed that the MiR-196a-5p/NR6A1/E-cadherin signaling axis is involved in the maintenance of NT-2 cell stemness and the inhibition of early neural differentiation.

Keywords: miR-196a-5p; NR6A1; E-cadherin; Ntera-2 cells, differentiation

A-1-036 KLF7 Promotes Transcription of *CDKN3* in Chicken Preadipocytes

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Krüppel like factor 7 (KLF7) inhibits preadipocyte differentiation and promotes proliferation in chicken. In our previous study, using ChIP-seq, a KLF7 binding peak which located in -96 nt upstream of the translation start codon of cyclin dependent kinase inhibitor 3 (*CDKN3*) was identified, implying that KLF7 may regulate transcription of *CDKN3*. In this study, in order to verified the transcriptional regulation of KLF7 on *CDKN3*, KLF7 was overexpressed in chicken preadipocytes, and the realtime RT-PCR showed that the transcription level of *CDKN3* was significantly increased ($P<0.05$). Then, we constructed a series of dual luciferase reporter vectors, pGL3-*CDKN3*-1912/-7, pGL3-*CDKN3*-758/-7, pGL3-*CDKN3*-450/-7 and pGL3-*CDKN3*-160/-7. Reporter gene analysis showed that -450/-7 had the highest promoter activity, whereas -160/-7 inhibit the lowest promoter activity. Furthermore, to determine the core promoter region of *CDKN3*, we construscted a dual luciferase reporter vector pGL3-*CDKN3*-450/-161. Reporter gene analysis showed that -450/-161 inhibit the highest promoter activity, suggesting that this region was the core promoter region of *CDKN3*. In order to determine the binding site of KLF7 on *CDKN3*, JASPAR was used to predict the binding site. And then, we carry out a series of putative binding site deletion mutation of *CDKN3*. Compared with the wild-type reporter, the -137/-128 deletion caused a 55% decline in basal promoter activity ($p < 0.05$), suggesting that "TGGGCGGGCT" (-137/-128) was the binding site of KLF7 on *CDKN3*. Moreover, the results of ChIP-qPCR showed that KLF7 could bind to the *CDKN3* promoter region. Taken together, KLF7 promotes transcription of *CDKN3* in chicken preadipocytes.

Key Words: Chicken, Cyclin dependent kinase inhibitor 3 (*CDKN3*), Krüppel like factor 7 (KLF7), Promoter

A-1-037 NRDE2 negatively regulates exosome functions by inhibiting MTR4 recruitment and exosome interaction

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The exosome functions in the degradation of diverse RNA species, yet how it is negatively regulated remains largely unknown. Here, we show that NRDE2 forms a 1:1 complex with MTR4, a nuclear exosome cofactor critical for exosome recruitment, via a conserved MTR4-interacting domain (MID). Unexpectedly, NRDE2 mainly localizes in nuclear speckles, where it inhibits MTR4 recruitment and RNA degradation, and thereby ensures efficient mRNA nuclear export. Structural and biochemical data revealed that NRDE2 interacts with MTR4's key residues, locks MTR4 in a closed conformation, and inhibits MTR4 interaction with the exosome as well as proteins important for MTR4 recruitment, such as the cap-binding complex (CBC) and ZFC3H1. Functionally, MID deletion results in the loss of self-renewal of mouse embryonic stem cells. Together, our data pinpoint NRDE2 as a nuclear exosome negative regulator that ensures mRNA stability and nuclear export.

Key words: NRDE2; the nuclear exosome; MTR4 recruitment; mRNA export

A-1-038 Structural and Functional Studies of *Arabidopsis thaliana* Guanosine Deaminase GSDA

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Nucleoside deaminase is an enzyme that catalyzes the hydrolysis of nucleosides with the elimination of the amino group in bases to release ammonia. Guanine deaminase (GDA) specifically deaminates of the guanine base and is found in many organisms including humans. The paralog of GDA in plants is named guanosine deaminase (GSDA), which belongs to the cytidine/deoxycytidylate deaminase enzyme family. Interestingly, GSDA is exclusively present in all known organisms within the Kingdom 'Plantae' so far (except for algae) and specifically catalyzes the deamination of guanosine and deoxyguanosine. In *A. thaliana*, xanthosine is generated first during the degradation process of guanosine, and is converted later to xanthine and uric acid respectively for nitrogen recycling, while the necessary nutrients for plants are retained. The distinct substrate specificity displayed by GDA and GSDA and the unique existence of the latter in plants are of interest. To gain structural insights into the substrate specificity and evolutionary origin of GSDA, we solved the enzyme structures in the free and in the ligand-bound forms. The structure shows that Leu119 in GSDA is replaced with a tryptophan residue in GDA, which is probably the main cause of the unique substrate guanosine at the active site of GSDA, instead of the normal substrate guanine for GDA. The comparison of GSDA to other structurally similar tRNA-deaminases was also conducted to reveal the potential structural basis leading to catalysis on different substrates. Our structural studies provide the first look of this important metabolic enzyme and shed lights on its catalytic pathway, which helps to unveil its physiological functions and indispensability in plants.

Key Words: GSDA, GDA, Crystal structures, Catalytic mechanism, Substrate specificity

A-1-039 ZNF281 Promotes Progression of Hepatocellular Carcinoma through Transcriptional Regulation of Annexin A10

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ZNF281 (zinc finger protein 281) is a transcription factor containing four zinc finger domains. It has been reported that ZNF281 is indispensable for pluripotency maintenance of embryonic stem cells dependent on its transcriptional activity. ZNF281 also contributes to the invasive growth of certain types of cancer cells through differential regulation of a panel of oncogenic or tumor suppressive genes like c-myc and p53, respectively. Nevertheless, the role of ZNF281 in the progression of hepatocellular carcinoma(HCC)remains elusive, which was investigated in the current study. The results showed that ZNF281 was up-regulated at both the mRNA and protein levels in HCC cells and tissues. Knockdown of ZNF281 decreased the proliferation, migration and invasion of the HCC cells. Mechanistically, RNA-seq indicates that depletion of ZNF281 with short hairpin RNAs results in alterations of a panel of genes related to tumor progression of HCC, including PTPN11, MDM2, and IGFBP4 etc. Interestingly, one tumor suppressor gene in HCC, gastric cancer, and colon cancer, Annexin A10 was the top up-regulated gene upon ZNF281 knockdown. Further analyses indicate that Annexin A10 was predominantly down-regulated in HCC cells and tissues, and low expression of Annexin A10 was associated withshorter overall survival and recurrence free survival. Furthermore, knockdown of Annexin A10 not only increased the proliferation, migration and invasion of ZNF281 intact HCC cells , but more importantly, restored the impaired proliferation, migration and invasion of HCC cells elicited by ZNF281, suggesting that ZNF281 promotes HCC progression probably through Annexin A10. Further

studies are needed to investigate the molecular detail of ZNF281 on the regulation of Annexin A10 expression in vitro and vivo, as well as the mechanisms on how Annexin A10 regulates the progression of HCC.

Key Words: ZNF281, ANXA10, Hepatocellular Carcinoma, Transcription, Invasion

A-1-040 香猪产仔数相关lncRNA的筛选

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长链非编码RNA（Long non-coding RNA，lncRNA）是一类转录本长度大于200nt、不编码蛋白的RNA分子。研究显示lncRNA在表观遗传调控、转录调控以及转路后调控等层面上参与调节胚胎发育、细胞增殖分化、器官发生等生物学过程。香猪作为贵州特有的小型地方猪种，繁殖性状特别是产仔数在群体间的变化较大，但总体上繁殖力低。为了探讨lncRNA在香猪繁殖性状特别是产仔数性状中的调控作用，本研究以高、低产香猪群体为研究对象，通过RNA-seq技术对香猪卵巢去rRNA的链特异性文库进行测序，运用生物信息学分析，从高、低产香猪群体卵巢组织中共筛选得到1615条lncRNA（已知lncRNA 245条，新鉴定的lncRNA 1370条）。对筛选得到的lncRNA进行差异分析，结果显示在高、低产香猪群体卵巢组织中差异表达的lncRNA共有 281条，其中低产香猪群体相对于高产香猪群体，40条lncRNA表达量显著上调、141条lncRNA表达量显著下调；差异表达的lncRNA中，低产香猪群体特有表达的lncRNA 8条、高产香猪群体特有表达的lncRNA 11条。对差异显著的lncRNA进行顺式作用靶基因预测，并进行GO、KEGG功能分析，发现候选的靶基因涉及繁殖、免疫及脂代谢等相关的生物功能和通路，如腺体形态发生（gland morphogenesis）、类固醇生物合成过程的调控（regulation of steroid biosynthetic process）、减数分裂细胞周期的负调控（negative regulation of meiotic cell cycle）、免疫反应的调节（positive regulation of immune response）和脂质代谢过程（lipid metabolic process）。综上所述，本研究通过初步解析高、低产香猪群体卵巢组织中lncRNA的表达谱特性,丰富了香猪卵巢组织lncRNA的数据，为进一步解析lncRNA在香猪繁殖性状特别是产仔数性状中的作用奠定了基础。

关键词：香猪；RNA-seq；产仔数；lncRNA

A-1-041 Functional proteomics identifies a PICS complex required for piRNA maturation and chromosome segregation

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piRNAs play significant roles in suppressing transposons and nonself nucleic acids, maintaining genome integrity, and defending against viral infections. In *C. elegans*, piRNA precursors are transcribed in the nucleus and are subjected to a number of processing and maturation steps. The biogenesis of piRNAs is not fully understood. We use functional proteomics in *C. elegans* and identify a piRNA processing and chromosome segregation (PICS) complex. The PICS complex contains TOFU-6, PID-1, PICS-1, TOST-1, and ERH-2, which exhibit dynamic localization among different subcellular compartments. In the germlines, the PICS complex contains TOFU-6/PICS-1/ERH-2/PID-1, is largely concentrated at the perinuclear granule

zone and engages in piRNA processing. During embryogenesis, the TOFU-6/PICS-1/ERH-2/TOST-1 complex accumulates in the nucleus and plays essential roles in chromosome segregation. The functions of these factors in mediating chromosome segregation are independent of piRNA production. We speculate that differential compositions of PICS factors may help cells coordinate distinct cellular processes.

Keywords: piRNA, TOFU-6, PID-1, TOST-1, PICS-1, ERH-2, chromosome segregation, perinuclear granule

A-1-042 Co-surveillance of ribosomal RNA by the exosome complex and small RNA in *C. elegans*

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RNA exosome is a 3' to 5' exonuclease complex, consisting of a 9-protein catalytically inactive core complex (EXO-9) and two catalytic subunits, Rrp6, and Dis3. EXO-9 forms a double-layered barrel-like structure. This complex functions as a robust surveillance system to degrade a variaty of aberrent RNAs. In a forward genetic screening, we have identified a risiRNA biogenesis suppressor SUIS-5. Through the SNP mapping method, this mutated gene was mapped to be the *dis-3* gene. DIS-3 is one of the two catalytic subunits of RNA exosome complex. Moreover, depletions of factors of RNA exosome induce the biogenesis of risiRNAs. We have constructed several *gfp* or *mCherry* tagged transgenes of subunits of RNA exosome complex. We will identify the erroneous ribosomal RNA upon depletion of RNA exosome complex in the future to investigate the functions of this complex in *C. elegans.*

Key Words: RNA exosome, risiRNA, *C. elegans*

A-1-043 人外周血miRNA作为辐射损伤评估指标的初步研究

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目的：随着核能和核技术的发展及广泛应用，人们受到电离辐射暴露的几率增加，对辐射损伤及时有效的评估是当前研究的热点之一。目前个体辐射损伤的评估往往依赖于个体的辐射时间和恶心、呕吐，淋巴细胞减少，细胞遗传学分析等的严重程度。但这些指标易受到如心理因素、炎症等影响，而且这些方法分析非常费时，常常难以满足大批量辐射损伤评估的需求，另外不利于辐射损伤的早发现。miRNA是一类广泛存在于真核生物中，在细胞增殖、分化、凋亡及基因调控中起重要作用，人类体液中miRNA耐酸、耐碱、物理性状非常稳定，很适合作为一种检测指标。本研究采用测序技术检测60co γ射线照射引起人外周血中差异表达的miRNA，为miRNA分子作为辐射损伤评估指标提供实验基础。**方法：**采取16名健康成年男性外周血，给予2.0 Gy的γ射线照射，通过染色畸变分析技术，筛选出对辐射敏感性较均一者进行miRNA差异分析。辐射敏感性较均一者接受0.2 Gy和2.0 Gy的γ射线照射，照射后6 h提取外周血miRNA进行高通量测序，照射组分别与假照组进行比较，分析差异的miRNA分子；采用miRDB和TargetScan2个软件对差异miRNA的靶基因进行预测，对靶基因进行GO和KEGG分析，进一步分析靶基因的生物学功能。**结果：**染色体畸变分析结果显示，在16名男性中有7名对电离辐射的敏感性较一致。进一步分析0.2 Gy和2.0 Gy照射后7名受试者外周血miRNA表达变化，结果显示与假照组相比，0.2 Gy组有10个差异表达（上调2个，下调8个），2.0 Gy有21个（上调9个，下调12个）。其中2个剂量组共同变化的miRNA有6个，且变化趋势一致（miR hsa-miR-23c，hsa-miR-1287-5表达在照射后均显著上调；hsa-miR-219a-2-3p，hsa-miR-6511b-3p等表达则均显著下

降）。通过对差异miRNA靶基因预测及功能分析，靶基因主要富集在Ras、MARK、P53等信号通路。**结论：**辐射敏感性较均一者受到不同剂量电离辐射作用后，外周血miRNA表达变化是不同的，这些差异的miRNA可能参与了电离辐射后细胞的增殖凋亡等过程，共同变化的miRNA分子可作为辐射损伤评估的潜在指标。

关键词：电离辐射；差异表达miRNA；生物信息学

A-1-044　辐射与酒精联合作用对子代肝细胞恶性转变的影响研究

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摘要：随着核能的快速发展，电离辐射对环境和人类健康的影响越来越受到重视。α粒子由于具有较高的传能线密度(LET)和较强的相对生物学效应，它对人体的健康影响一直是放射生物学研究领域中关注的热点。钚、钍及其衰变产物被国际癌症研究机构（IARC）列为I类致癌物质，主要危害由α粒子辐射所致，肝脏为其靶点之一。肝脏是人体代谢的主要场所，且几乎是酒精代谢的唯一场所，涉核工作人员的饮酒习惯是否会增加α粒子内照射对人体的健康风险是本研究的关注点。目的:本文通过体外细胞实验从细胞生物学和分子生物学水平探讨α粒子辐射、酒精单独作用和联合作用对肝细胞子代发生恶性转化趋势的影响，为职业人员健康监护和健康风险评估提供客观的体外实验数据。材料与方法: 人正常肝上皮细胞LO2经一定浓度酒精处理和一定剂量范围的241Amα粒子辐射源照射后建立α粒子辐射和酒精单因素染毒模型，在单因素作用模型的基础上，进一步开展α粒子辐射和酒精联合作用。实验分为5组:正常对照组（不进行任何处理，培养约30代）；辐射组（α粒子照射2Gy，每10代照射一次，累计2次照射，培养约30代）；酒精组（用含1%酒精浓度的培基培养，每代染毒24h，染毒20代，培养约30代）；辐射+酒精复合组（先α粒子以1Gy/次累计照射2次后，酒精染毒10代，培养约30代）；酒精+辐射复合组（先酒精染毒20代后，α粒子以1Gy/次累计照射2次，培养约30代）。采用RT-PCR方法检测细胞恶性转化相关指标（P53、CDH1和BCL2），通过Transwell方法测定细胞迁移侵袭能力。实验结果：与对照组比较，单因素作用和联合作用组，子代肝细胞的迁移和浸润能力均增加，CDH1和P53在mRNA表达水平均显著性下调。结论:单因素与复合因素均诱导子代肝细胞发生细胞迁移和浸润，且发生抑癌基因P53和E-钙黏蛋白相关基因CDH1下调，发生恶性转化趋势。在评价α粒子内照射致肝细胞恶性转变的过程中，酒精的作用不容忽视。

关键词：α粒子辐射 酒精 联合作用 细胞恶性转化

A-1-045　LncRNA CRNDE上调ATG4B的分子机制及其促HCC细胞自噬的作用研究

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肝细胞癌（hepatocellular carcinoma, HCC）严重威胁人类健康。索拉非尼是首个被批准用于治疗HCC的靶向药物，其虽有较好疗效，但容易产生耐药。细胞自噬是肿瘤耐药的重要机制之一，自噬相关蛋白ATG4B在其中发挥了关键作用，因此，靶向调节ATG4B及自噬对于提高HCC细胞对索拉非尼的敏感性具有重要意义。已有报道显示，参与调控ATG4B的分子包括酶类、转录因子及其结合蛋白、microRNA、siRNA及一些化合物等，而关于长非编码RNA（lncRNA）是否参与调控ATG4B，目前尚不清楚。近年研究表明，lncRNA CRNDE可通过调控多条信号通路促进HCC细胞的生长与转移，但其是否参与调控ATG4B，目前未见报道。在本研究中，我们发现，CRNDE可通过上调ATG4B而促进HCC细胞自噬，而CRNDE升高ATG4B是通过增加ATG4B mRNA的稳定性而实现的。进一步研究发现，miR-543可直接靶向下调ATG4B，而CRNDE则可通过抑制miR-543而升高ATG4B的水平，进而促进HCC细胞自噬。

药物敏感实验发现，抑制“CRNDE–ATG4B–自噬”通路可增强HCC细胞对索拉非尼的敏感性，提示该通路可能是增敏索拉非尼抗HCC的新靶点。

关键词：长非编码RNA；CRNDE；ATG4B；肝细胞癌；索拉非尼

A-1-046　原核生物翻译终止过程分子机理的研究

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摘要：蛋白质合成在生命活动中占有重要地位，分为起始、延伸、终止和核糖体回收四个阶段。多个GTPase参与翻译各过程进行且提供能量，包括翻译终止因子RF3 (release factor 3)。迄今为止，在原核生物中释放因子RF1 (release factor 1) 和RF3是如何协作推进翻译终止过程的发生，与核糖体又发生了怎样的相互作用，特别是核糖体与RF1构成的复合物是否作为RF3的鸟嘌呤核苷酸交换因子 (GEF, guanine nucleotide exchange factor)等科学问题尚未得到明确解释。本研究拟通过利用融合蛋白得到核糖体与终止因子复合物 (Ribosome・RF1・RF3)高分辨率晶体结构并结合体外翻译系统，对翻译终止过程的分子机理进行深入研究。

关键词：翻译，核糖体，RF1，RF3，GEF，GTPase

A-1-047　Biochemical characterization of cellular IRES using an in vitro reconstituted human cell-free translation system

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Internal ribosome entry site (IRES) elements are cis-acting RNA regions that promote internal initiation of protein synthesis with cap-independent mechanisms. IRES was first discovered in virus 31 years ago, and in recent years, increasing data have suggested that many cellular mRNAs may also make use of IRES to initiate their translation, and these IRESs are called cellular IRES. However, a consensus has not been reached over the true identity and function of cellular IRESs, let alone their working mechanisms. This is mainly due to the lack of an effective tool to characterize cellular IRESs. Here we set out to use a fully reconstituted in-vitro translation system to investigate cellular IRESs mechanistically. We have obtained nearly all the components for a functional translation system. Two biochemical assays are proposed to identify cellular IRESs and potential *trans*-acting factors.

Key Words: cellular IRES, translation initiation

A-1-048 In vitro assembly and functional study of Multi-aminoacyl-tRNA Synthetase Complex

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In mammals, nine aminoacyl-tRNA synthetases along with three auxiliary proteins assemble into a megacomplex called the multi-aminoacyl-tRNA synthetase complex (MSC). The physiological significance of MSC still remains elusive, although mounting data have suggested that MSC is involved in a variety of cell activities in addition to protein synthesis. Structural investigation of MSC has been a challenge due to its dynamic nature and difficulty in sample preparation. In this study, we biochemically characterized interactions among all recombinant MSC components, and successfully reconstituted the full MSC complex, providing a valuable framework to investigate its structure and function.

Key Words: MSC, Macromolecular, Protein synthesis, Assembly

专题二：代谢网络与稳态

A-2-001　人参皂苷Rg1对糖尿病大鼠周围神经氧化应激以及凋亡的影响

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摘要：目的：探讨人参皂苷Rg1（Ginsenoside Rg1，G-Rg1）对STZ糖尿病大鼠周围神经的氧化应激以及细胞凋亡的影响，阐明G-Rg1对糖尿病大鼠周围神经的修复作用及其机制。**方法：**取雄性健康SD大鼠，除正常对照组外，一次性腹腔注射STZ（ 60mg/kg）诱发糖尿病模型。糖尿病成模大鼠随机分为模型组、G-Rg1小剂量组、G-Rg1大剂量组。确定成模后立即开始给药，G-Rg1小剂量组和G-Rg1大剂量组分别每日灌胃一次40mg/kg和80mg/kg，连续给药8周。8周后检测坐骨神经的传导速度以及ROS和MDA水平，同时利用电子显微镜观察其超微结构变化。利用Western Blot检测坐骨神经中Bcl-2、Caspase-3、NF-κB等蛋白的表达。**结果：**模型组坐骨神经传导速度明显低于正常对照组，G-Rg1小剂量组和G-Rg1大剂量组坐骨神经传导速度与模型组比较，有明显改善（P<0.05）；模型组ROS、MDA水平明显低于正常对照组，G-Rg1小剂量组和G-Rg1大剂量组ROS、MDA水平与模型组比较，明显降低（P<0.05）；电镜观察G-Rg1小剂量组和G-Rg1大剂量组大鼠坐骨神经纤维与模型组比较超微结构有明显改善；模型组Bcl-2表达明显低于正常对照组，模型组Caspase-3、NF-κB表达明显高于正常对照组，G-Rg1小剂量组和G-Rg1大剂量组与模型组比较，坐骨神经Bcl-2表达升高，Caspase-3、NF-κB表达明显抑制（P<0.05）。**结论：**人参皂苷Rg1通过氧化应激、凋亡信号通路对坐骨神经有修复作用。

关键词：人参皂苷Rg1；糖尿病周围神经病变；细胞凋亡；氧化应激

A-2-002　Construction of a cDNA library and preliminary analysis of the expressed sequence tags of the earthworm Eisenia fetida (Savigny, 1826)

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Abstract: Earthworms are useful indicator organisms of soil health and Eisenia fetida have been extensively used as test organisms in ecotoxicological studies. In order to gain insight into the gene expression profiles associated with physiological functions of earthworms, a full length enriched cDNA library of the Eisenia fetida genome was successfully constructed using Switching Mechanism at 5'End of RNA Template technology. Construction of a cDNA library and analysis of Expressed Sequence Tags (ESTs) are efficient approaches for collecting genomic information and identifying genes impor¬tant for a given biological process. Furthermore, analysis of the expression abundance of ESTs was performed with the aim of providing genetic and transcriptomic information on the development and regenerative process of earthworms. Phrep and Crossmatch were used to process EST data and a total of 1,140 high quality EST sequences were determined by sequencing random cDNA clones from the library. Clustering analysis of sequences revealed a total of 593 unique sequences including 225 contiguous and 368 singleton sequences. Basic Local Alignment Search Tool analysis against the Kyoto Encyclopedia of Genes and Genomes database resulted in 593 significant hits (P value <1x10 8), of which 168 were annotated through Gene Ontology analysis. The STRING database was used to determine relationships among the 168 ESTs, identi¬fying associated genes involved in protein protein interactions and gene expression regulation. Based on nucleic acid and protein sequence homology, the mutual relationships between 287 genes could be obtained, which identified a portion of the ESTs as known genes. The present study reports on the construction of a high quality cDNA library representative of adult earthworms, on a preliminary analysis of ESTs and on a putative functional analysis of ESTs. The present study is expected to enhance our understanding of the molecular basis underlying the biological development of earthworms.

Key words: Earthworm Eisenia fetida (Savigny, 1826), cDNA library, expressed sequence tags, GO annotation, KEGG pathway

A-2-003 Isolation and purification of phytotoxic molecule from *Verticillium dahliae*

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Verticillium dahliae infects hundreds of crop plants, leading to yield losses heavily or even no crops. The phytotoxins of *V. dahliae* play an indispensable role in pathogenesis. However, the exact constituent remains unknown except for cinnamyl acetate. In this study, we purify phytotoxins produced by a highly virulent *V. dahliae* strain, L2-1. Both of lipophilic and hydrophilic toxins extracted by ethyl acetate cause wilt of cotton cotyledon, but their contamination differ significantly. The hydrophilic toxins account for 95% of total toxins. Using anion-exchange, reversed-phase chromatography and mass spectrometry, we obtained four components with strong wilting activity from hydrophilic toxins, and the m/z is 245.0630, 389.0692, 301.0976 and 389.0630, with speculated molecular formulas is C6H9N6O5, C11H13N6O10, C11H17N4O4S and C12H17N6O5S2, respectively. We also obtained three components from lipophilic toxins with strong wilting activity by reversed-phase chromatography, but their concentration is too low to identify and need to enrich further. This study expands the understanding of *V. dahliae* phytotoxins and may use for virulence detection of the pathogen.

Key Words: *Verticillium dahliae*, L2-1, Phytotoxins

A-2-004 Acetylshikonin induces apoptosis of human leukemia cell line K562 by involving the modulation of ROS accumulation and blocking NF-κB signaling

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ABSTRACT: Acetylshikonin, a natural naphthoquinone derivative compound, holds anti-bacterial, anti-inflammatory, and anti-tumor activities. However, the effect of acetylshikonin on human chronic myelocytic leukemia（CML）cell apoptosis and detailed regulation of NF-κB signaling pathway mechanisms remains uncertain. The purpose of the present study was to investigate whether acetylshikonin could induce K562 cells apoptotic and regulate the NF-κB signaling pathway, thereby leading to suppression of CML. K562 cells were treated with the acetylshikonin at different concentrations. The results showed that K562 cell viability was significantly inhibited by the acetylshikonin with an IC50 of 2.03μM at 24 h and 1.13μM at 48 h, respectively. The examination of cytotoxic effects on healthy cells showed that the acetylshikonin did not show any effect on healthy Vero cells. Selectivity indexes were greater than 18.23, suggesting that the acetylshikonin had selective toxicity against K562 cells. The increase of distribution in the S phase of the cell cycle suggested that acetylshikonin arrested the k562 cell cycle primarily at S phase. The results of annexin V-FITC/PI and AO/EB staining showed that acetylshikonin induced cell apoptosis in a dose-dependent manner. The apoptotic rate was increased in the treatment groups compared with that in the control group ($P<0.05$). K562 cells treated with acetylshikonin resulted in profound induction of apoptosis accompanied by rapid generation of reactive oxygen species (ROS). Scavenging of ROS completely blocked the induction of apoptosis following acetylshikonin treatment. The levels of the pro-apoptotic proteins Bax, cleaved PARP and

cleaved caspase-3 increased with increasing concentration of acetylshikonin, while the anti-apoptosis protein Bcl-2 was downregulated. Cyt c and AIF, which are characteristic proteins of the mitochondria-regulated intrinsic apoptosis pathway, also increased in the cytosol with increasing concentrations of the acetylshikonin. In addition, acetylshikonin could lead to a block of NF-κB signaling pathway via decreasing nuclear NF-κB p65 and increasing cytoplasm NF-κB p65. Moreover, acetylshikonin significantly inhibited the phosphorylation of IkBα and IKKα/β in K562 cells. These results showed that acetylshikonin significantly inhibits cell viability and induces cell apoptosis through the mitochondria-regulated intrinsic apoptotic pathway in K562 cell line. The mechanisms may involve inhibition of NF-κB. The NF-κB signal pathway inactivation caused by acetylshikonin treatment resulted in the k562 cells apoptosis. Together, our results indicate that acetylshikonin could serve as a promising therapeutic agent for future treatment of CML.

Key words: acetylshikonin; K562; apoptosis; ROS; blocking NF-κB signaling

A-2-005 Ponatinib Inhibits Proliferation and Induces Apoptosis of Liver Cancer Cells, but Its Efficacy Is Compromised by Its Activation on PDK1/Akt/mTOR Signaling

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Abstract: Ponatinib is a multi-target protein tyrosine kinase inhibitor, and its effects on hepatocellular carcinoma cells have not been previously explored. In the present study, we investigated its effects on hepatocellular carcinoma cell growth and the underlying mechanisms. Toward SK-Hep-1 and SNU-423 cells, ponatinib induces apoptosis by upregulation of cleaved caspase-3 and -7 and promotes cell cycle arrest in the G1 phase by inhibiting CDK4/6/CyclinD1 complex and phosphorylation of retinoblastoma protein. It inhibits the growth-stimulating mitogen-activated protein (MAP) kinase pathway, the phosphorylation of Src on both negative and positive regulation sites, and Jak2 and Stat3 phosphorylation. Surprisingly, it also activates the PDK1, the protein kinase B (Akt), and the mechanistic target of rapamycin (mTOR) signaling pathway. Blocking mTOR signaling strongly sensitizes cells to inhibition by ponatinib and makes ponatinib a much more potent inhibitor of hepatocellular carcinoma cell proliferation. These findings demonstrate that ponatinib exerts both positive and negative effects on hepatocellular cell proliferation, and eliminating its growth-stimulating effects by drug combination or potentially by chemical medication can significantly improve its efficacy as an anti-cancer drug.

Keywords: ponatinib; proliferation; apoptosis; liver cancer cells; PDK1/Akt/mTOR signaling

A-2-006 藏族青年肠道菌群的表型关联分析

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探究藏族青年肠道菌群与机体表型指标的关联性。采集92名藏族青年肠道菌群样本、血常规、BMI等信息，利用Illumina Miseq测序平台进行表型关联、宏基因组学和生物信息学数据分析。

研究发现与汉族青年相比，藏族青年肠道菌群的α多样性高，而β多样性低（P<0.05）。因藏族青年特殊的饮食习惯和地理环境，使得其肠道菌群以拟杆菌门和厚壁菌门为主。经FDR校正，筛选P<0.05，COR>0.3的表型关联指标，发现藏族青年肠道菌群中拟杆菌门、变形菌门、厚壁菌门主要与机体的红细胞、血红蛋白、血小板、白细胞、中间细胞、BMI等指标有所关联。在藏族青年口腔和肠道中特殊的菌群多样性和表型关联指标，可能是一种新的生物标志物，为寻找临床疾病的早期预防与诊断的标志物建立基础。

关键词：藏族；口腔菌群；肠道菌群；表型关联

A-2-007 Metformin attenuates Cd-induced neuronal apoptosis via blocking ROS-dependent AMPK/PP5-JNK signaling pathway

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Abstract: Cadmium (Cd), a toxic environmental contaminant, induces reactive oxygen species (ROS)-dependent neuronal apoptosis and consequential neurodegenerative disorders. Metformin has recently been received attention owing to its protection against neurodegenerative diseases. However, little is known regarding the effect of metformin on Cd-induced neurotoxicity. Here we show that metformin effectively prevented from Cd-evoked apoptotic cell death in neuronal cells. Metformin declined Cd-induced apoptosis by suppressing Cd activation of JNK, which was attributed to blocking Cd inactivation of PP5 and AMPK. Inhibition of JNK with SP600125, knockdown of c-Jun, or overexpression of PP5 potentiated metformin's prevention of Cd-induced phosphorylation of JNK/c-Jun and apoptosis. Activation of AMPK with AICAR or ectopic expression of constitutively active AMPKα strengthened the effects of metformin, whereas expression of dominant negative AMPKα prevented metformin from hindering Cd-induced dephosphorylation of AMPKα, phosphorylation of JNK/c-Jun, and apoptosis. Metformin repressed Cd-induced ROS, thereby diminishing cell death, as evidenced by the findings that N-acetyl-L-cysteine (NAC) enhanced the inhibitory effects of metformin on Cd-induced ROS and apoptosis. Moreover, using Mito-TEMPO, we further demonstrated that metformin attenuated Cd-induced cell death by suppressing induction of mitochondrial ROS. Taken together, these results indicate that metformin prevents mitochondrial ROS inactivation of PP5 and AMPK, thus attenuating Cd-induced JNK activation and apoptosis in neuronal cells. Our data highlight a beneficial role of metformin as a promising agent for prevention of Cd-induced oxidative stress and neurodegenerative diseases.

Keywords: Metformin; Cadmium; Apoptosis; ROS; PP5; AMPK

A-2-008 Acetyl shikonin inhibits proliferation and induces apoptosis by triggering endoplasmic reticulum stress in ESCC

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Acetyl shikonin (AS), one of active components of Lithospermum erythrorhizon Sieb. et Zucc, a well- known Chinese

traditional herb has been reported to exert antitumor activities in various cancer cell types. However, the effects of AS on esophageal squamous cell carcinoma (ESCC) and its underlying mechanisms remain unknown. Here, we observed that AS showed anti-proliferative action against 6 cell lines of ESCC, among which KYSE450 and KYSE180 are more sensitive to AS with IC_{50} values of 1.69 and 3.72μM respectively. AS induces endoplasmic reticulum stress (ER stress), as evidenced by increased GRP78, CHOP, ERO1-Lα, elevated phosphorylation of eIF2α at Ser51, splicing of XBP1. Further investigation discovered that AS-induced apoptosis was closely associated with ER stress. Moreover, administration of AS to xenograft mice reduced tumor growth, size and weight, and coordinately caused elevated expression of GRP78 and CHOP related to ER stress. Overall, our findings identified ER stress-dependent apoptosis with AS treatment in ESCC cells, as well as supported the continued research for the use of AS as a potential candidate to treat ESCC.

Keywords: Acetyl shikonin, esophageal squamous cell carcinoma, Endoplasmic reticulum stress, Apoptosis

A-2-009 UCP2参与人参皂苷PPD对肺腺癌A549细胞的抑制作用

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人参皂苷PPD是由人参中提取的天然抗癌活性成分，但其抗癌作用机制尚未阐明。本研究观察了人参皂苷PPD对于肺腺癌细胞A549的作用并对其可能的机制进行了初步探讨。研究发现，人参皂苷PPD（10–30ug/mL）作用A549细胞24小时显著抑制了细胞增殖、阻滞细胞周期于G1期，并诱导其凋亡。为阐明PPD抑制肺癌细胞增殖并最终诱导其凋亡作用的相关分子机制，通过线粒体通透性转换孔道（mPTP）变化检测发现，PPD具有诱导A549细胞线粒体损伤的能力；采用Real–time PCR和Western–blot 检测发现，人参皂苷PPD（30ug/mL）抑制了A549细胞中解偶联蛋白2（UCP2）的表达水平。以上结果表明，PPD能抑制肺癌A549细胞增殖并促其凋亡，其机制与抑制UCP2表达，促进线粒体凋亡损伤有关，同时提示抑制UCP2是治疗肺癌手段之一。

关键词：人参皂苷PPD；解偶联蛋白2（UCP2）；A549细胞

A-2-010 肌肽对高糖诱导H9c2心肌细胞线粒体损伤的保护作用

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肌肽是一种天然的抗氧化剂，对高糖诱导的氧化应激损伤具有保护作用，但能否对线粒体损伤起到保护作用及相关机制尚未明确。本研究探讨肌肽对高糖诱导H9c2心肌细胞线粒体损伤的影响及可能的作用机制。研究发现，20 mmol/L肌肽可以显著减少线粒体通透性转换孔道（mPTP）开放并恢复线粒体膜电位（ΔΨm），表明肌肽对高糖诱导的线粒体损伤具有保护作用。为进一步探讨其相关的作用机制，通过实时PCR技术和免疫荧光实验检测发现，肌肽可逆转高糖诱导的UCP2、Grx1和Trx1的表达水平降低。以上结果表明，肌肽对高糖诱导的线粒体损伤具有保护作用，其机制与调节内源性抗氧化体系功能相关。

关键词：线粒体损伤；高糖；肌肽；H9c2心肌细胞

A-2-011 血小板修复衰老卵巢功能

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高龄女性的生育需求和生殖衰老之间的矛盾日以冲突。生殖系统衰老关乎着女性的生育能力和身体状况，卵巢的排卵功能是女性生育的前提但如今二胎放开，越来越多的高龄备孕女性面临着卵巢功能退化，卵子质量差甚至没有卵子发育。干细胞移植虽可改善了老化卵巢功能，但潜在风险不容忽视。我们发现血小板可以促进体外培养颗粒细胞的增殖。本研究应用衰老小鼠模型，静脉回输血小板后观察治疗卵巢功能衰退效果，研究不同的血小板给与方式,给与周期对卵巢功能的作用，分析卵巢的排卵功能和内分泌功能变化以及卵巢功能改变后对大脑的负反馈和子宫内膜的调整。并利用蛋白质谱、Realtime PCR技术和免疫荧光等技术研究血小板回输对卵泡发育闭锁相关分子及通路变化，分析血小板因子在衰老小鼠卵泡生长发育中的作用及其机制。血小板作为较安全的血液回输成分，该研究将为寻找卵巢功能衰退治疗提供依据，为临床高龄女性的卵巢衰老提供治疗思路。

关键词：血小板；卵巢早衰；卵子质量；不孕不育

A-2-012 Taurine-mediated browning of white adipose tissue is involved in its protective role against obesity in mice

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Taurine, a nonprotein amino acid, is widely distributed in almost all animal tissues. Ingestion of taurine helps to improve obesity and its related metabolic disorders. However, the molecular mechanism underlying the protective role of taurine against obesity is not completely understood. In this study, it was found that intraperitoneal treatment of the mice with taurine alleviated high-fat diet (HFD)-induced obesity, improved insulin sensitivity, and increased energy expenditure and adaptive thermogenesis of the mice. Meanwhile, administration of the mice with taurine markedly induced the browning of inguinal white adipose tissue (iWAT), with significantly elevated expression of PGC1α, UCP1 and other thermogenic genes in iWAT. In vitro studies indicated that taurine also induced the development of brown-like adipocytes in C3H10T1/2 white adipocytes. Knockdown of PGC1α blunted the role of taurine in promoting the brown-like adipocytes phenotype in C3H10T1/2 cells. Moreover, taurine treatment enhanced AMPK phosphorylation in vitro and in vivo, and knockdown of AMPKα1 prevented taurine-mediated induction of PGC1α in C3H10T1/2 cells. Consistently, specific knockdown of PGC1α in iWAT of the HFD-fed mice inhibited taurine-induced browning of iWAT, with the role of taurine in the enhancement of adaptive thermogenesis, the prevention of obesity and the improvement of insulin sensitivity being partially impaired. These results reveal a functional role of taurine in facilitating the browning of white adipose tissue, which is dependent on the induction of PGC1α. Our studies also suggest a potential mechanism for the protective role of taurine against obesity which involves taurine-mediated browning of white adipose tissue.

Key Words: Taurine, White adipose tissue (WAT) browning, Obesity, Energy expenditure, Adaptive thermogenesis, PGC1α, AMPK

A-2-013 Inhibition of CCR2 protein reduces macrophages in atheromata

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Circulating monocytes, the precursors of macrophages, display heterogeneity in mice and humans. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. Monocytes recruitment to sites of inflammation is regulated by members of the chemokine family of chemotactic cytokines. Ly-6Chi monocytes emigration from bone marrow requires signals mediated by Chemokine receptor CCR2 .CC chemokine receptor 2 (CCR2) governs Ly6Chi monocyte emigration from bone marrow to circulating blood, which is a key step for the Macrophage accumulation in the development and exacerbation of atherosclerosis. However, the mechanisms that regulate the migration of monocytes from bone marrow to blood are not well understood. We report here that AMPK activation can inhibit CCR2 expression and CCR2 mediated Ly-6Chi monocytes emigration from bone marrow to circulating blood. In vitro, CCR2 protein expression of THP-1 cells treated with AMPK activators including AICAR, A769662 and Metformin were decreased when compared with control group and Chemotaxis analysis revealed that AMPK activation can inhibit monocytes migration. In vivo, AMPK activation can reduce peripheral blood monocytosis induced by atherogenic diet via inhibition of CCR2 protein expression. AMPK activation has no effect on cell number of annexin V+ cells and cell circle within Ly-6Chi monocytes in the bone marrow. In addition, AMPK activation can promote the conversion from Ly-6Chi monocytes to Ly-6Clo monocytes. In conclusion, activation of AMPK reduced the formation of atherosclerosis plaque via inhibition of monocytes CCR2 expression and CCR2 mediated Ly6Chi monocytes egress from bone marrow.

Key Words: CCR2, atherosclerosis, Ly6Chi monocyte migration

A-2-014 Comprehensive DNA methylation analysis of Chinese patients with first-episode schizophrenia

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Schizophrenia (MIM 181500) is a chronic, severe mental disorder that 64-80% of the cases are inherited with a prevalence of ~1%. Recently, accumulated evidences suggested that epigenetics played an important role in the etiology of schizophrenia. In the current study, we investigated the methylation differences between schizophrenia patients and healthy controls in the *Han* Chinese of Northwest China using Illumina Human Methylation 450K Beadchip chip. Finally, 4,494 locus differences in DNA methylation were found, including 1,592 methylation degree modulation points and 2,902 methylation degree down-regulation points. The gene corresponding to the differential methylation site was analyzed by Gene Ontology to describe the function of the gene, and significantly related genes were involved in protein kinase regulation, cell morphological differentiation, actin cytoskeletal organization, etc. Based on the results of the Chip and GO analysis, we selected TNIK for further pyrosequencing verification. The results showed that the difference in methylation site of TNIK between cases and controls was statistically significant ($P<0.01$). Overall, our study have revealed the differential methylation sites between schizophrenia cases and healthy controls in the *Han* Chinese. Meanwhile, the aberrant methylation of the TNIK gene in schizophrenia patients was demonstrated for the first time, providing a new clue for the development of epigenetic mechanisms in schizophrenia. Then, the methylation of TNIK should be explored further at the cellular and animal levels for its molecular mechanisms in schizophrenia.

Keywords: Schizophrenia, DNA Methylation, TNIK, *Han* Chinese, Chip

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A-2-015 Molecular mechanisms of the αβ and αγ heterodimers of human NAD-dependent isocitrate dehydrogenase

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Abstract: Human NAD-dependent isocitrate dehydrogenase, existing as the α2βγ heterotetramer, catalyzes the decarboxylation of isocitrate into a-ketoglutarate in the Krebs cycle, which can be allosterically regulated by citrate, ADP, ATP and NADH. We systematically studied the enzymatic properties of the α2βγ heterotetramer and the αβ and αγ heterodimers, and performed the structural and functional studies of the αβ and αγ heterodimers. Our results show that like the α2βγ heterotetramer, the αγ heterodimer can be activated by CIT or/and ADP and the γ subunit contains the allosteric site consisting of the CIT and ADP binding sites, whereas the αβ heterodimer cannot be activated and the β subunit contains a pseudo allosteric site unable to bind the activators. There are structural communications between the allosteric site and the active sites of both heterodimers in the α2βγ heterotetramer via the interfaces, and the binding of activators to the allosteric site can regulate both α subunits. Our structural and biochemical data data together reveal the molecular mechanisms of the functional roles and the allosteric regulations of the αβ and αγ heterodimers of human and possibly other eukaryotic NAD-IDHs.

Keywords: Isocitrate dehydrogenase, IDH, NAD-IDH, allosteric regulation, conformational change, TCA cycle.

A-2-016 生酮微环境对胶质瘤干细胞的代谢重编程及其增殖抑制作用

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胶质瘤是常见的颅内肿瘤，尤以胶质母细胞瘤（GBM）恶性程度最高,患者预后不良。近些年来由于代谢组学的发展，前期研究发现胶质瘤会导致机体代谢紊乱以及微环境失调，进一步加重胶质瘤的发展进程，目前仍无有效方法。生酮饮食作为高脂、低糖、低碳水化合物饮食，其广泛用于小儿难治性癫痫治疗。目前临床研究显示，生酮饮食对可明显延长部分胶质母细胞瘤瘤患者生存期，而其作用机制尚不明确。本研究首先在体外模拟生酮微环境（BHB-G^{low}），发现BHB-G^{low}可显著控制胶质瘤干细胞（GSCs）的代谢重编程过程：抑制GSCs的ATP产生比率；下调糖酵解关键酶HK2、PKM2、LDHA的蛋白表达量；降低GSCs对葡萄糖的摄入。通过透射电镜、细胞糖酵解代谢率OCR、ROS生成及抗氧化应激酶检测发现，BHB-G^{low}培养后，GSCs线粒体密度增加、细胞间连接消失；OCR代谢率降低，并且细胞氧化应激平衡失调。通过Edu染色、干细胞克隆球形成实验、CCK8细胞活性检测发现，GSCs

在BHB-G^{low}中，细胞增殖明显抑制。随后，清除ROS后，BHB-G^{low}中的GSCs线粒体形态、功能恢复，GSCs细胞增殖抑制作用解除。提示ROS在BHB-G^{low}对GSCs增殖抑制作用中发挥重要作用。此研究为临床治疗提供了新的诊疗思路，具有广泛的应用前景。

关键词：胶质瘤干细胞；生酮微环境；线粒体异常；氧化应激

A-2-017 Gut Flora-Dependent Metabolite Trimethylamine-N-oxide Accelerates Vascular Aging through Oxidative Stress

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Trimethylamine-N-oxide (TMAO), gut microbiota-dependent metabolites, has been shown to be associated with cardiovascular diseases. However, little is known about the relationship between TMAO and vascular aging. Here, we observed a change in TMAO during the aging process and the effects of TMAO on vascular aging and endothelial cell (EC) senescence. We analyzed age-related plasma levels of TMAO in young adults (18-44 years old), older adults (≥65 years old), and 1-month-old, 3-month-old, 6-month-old and 10-month-old senescence-accelerated mouse prone 8 (SAMP8) and age-matched senescence-accelerated mouse resistance 1 (SAMR1) models. We found that circulating TMAO increased with age both in humans and mice. Next, we observed that a TMAO treatment for 16 weeks induced vascular aging in SAMR1 mice and accelerated the process in SAMP8 mice, as measured by an upregulation of senescence markers including senescence-associated β-galactosidase (SA-β-gal), p53, and p21, vascular dysfunction and remodeling. In vitro, we demonstrated that prolonged TMAO treatment induced senescence in human umbilical vein endothelial cells (HUVECs), characterized by reduced cell proliferation, increased expressions of senescence markers, stagnate G0/G1, and impaired cell migration. Furthermore, TMAO suppressed sirtuin 1 (SIRT1) expression and increased oxidative stress both in vivo and in vitro and then activated the p53/p21/Rb pathway resulting in increased p53, acetylation of p53, p21, and decreased CDK2, cyclinE1, and phosphorylation of Rb. In summary, these data suggest that elevated circulating TMAO during the aging process may deteriorate EC senescence and vascular aging, which is probably associated with repression of SIRT1 expression and increased oxidative stress, and, thus, the activation of the p53/p21/Rb pathway.

Key Words: Trimethylamine-N-oxide (TMAO), vascular aging, endothelial cell senescence, SIRT1, oxidative stress

A-2-018 PCBPs Regulate Circadian Rhythms

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Circadian rhythms are endogenous, entrainable, rough 24h oscillations that coordinate daily changes in physiology and behavior. These oscillations exist widely in most organisms and are controlled by the internal time-keeping systems called circadian clocks. The core mechanism of circadian clocks at the molecular level has been considered to be a transcription/translation feedback loop(TTFL) model which consists of positive and negative arms. In addition to the core TTFL loop, there are several additional loops. Period, amplitude and phase are three key properties of a circadian oscillator. From the data

of a previous genome-wide RNAi screen, we identified poly(rC) binding proteins(PCBPs) as candidates for clock regulator. Our current study shows that PCBP1, PCBP4 can regulates the period of circadian clock, hnRNPK can induce the circadian clock of arrhythmic. So next,we plan to further investigate the detailed mechanism(s) on how PCBPs regulate the circadian rhythms.

Key Words: circadian, rhythms, PCBP

A-2-019　辐射致染色体损伤的遗传易感位点的筛选

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摘要：目的 辐射致染色体损伤的遗传易感位点。材料和方法 采集20–30岁健康成年男性外周血，给予0、2 Gy ^{60}Co γ射线照射，剂量率为0.46 Gy/min。2 Gy γ射线照射后的样本利用染色体畸变分析技术分析，将该人群分为辐射致染色体损伤易感组和不易感组。分别提取0 Gy照射后易感组和不易感组的基因组DNA。利用全外显子测序技术及生物信息学分析筛选差异单核苷酸多态性（SNP）位点。结果 利用染色体畸变分析2 Gy γ射线照射后的样本，得到辐射致染色体损伤易感组和不易感组，易感组平均染色体畸变率为19.1 %，不易感组平均染色体畸变率为10.5 %。经全外显子测序及生物信息学对0 Gy照射后样本进行分析，初步获得易感组和不易感组差异SNP位点共2077个，进一步筛选与损伤修复、辐射应答相关SNP位点共60个。结论 初步筛选出辐射致染色体损伤遗传易感位点，后续将利用飞行时间质谱法进行大样本量的验证，进而获得辐射致染色体损伤的遗传易感位点，这有望成为辐射损伤的标志物，为制定个性化放射治疗方案提供参考，为医用放射线的风险评估提供支持。

关键词：辐射损伤，遗传易感，染色体畸变，SNP

A-2-020　Dichloroacetate enhances the anti-tumor effect of Pirarubicin via ROS-JNK pathway in liver cancer cells

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Abstract: Liver cancer is one of the most common malignancies with high recurrence rate. Besides radiotherapy and surgery, chemotherapy also plays an important role in the treatment of liver cancer. However, the existing strategies are not satisfactory in clinic. Therefore, it is urgent to investigate the methods to enhance the effectiveness of chemotherapy for liver cancer. In present study, our data showed that dichloroacetate (DCA) can significantly enhance the anti-tumor effect of pirarubicin (THP) on liver cancer cells, including changed the morphology and adherence ability, reduced the viability and increased the mortality of liver cancer cells. The results of flow cytometry showed that the combination of THP and DCA dramatically promoted the apoptosis in liver cancer cells. Moreover, compared to that of THP treatment alone, DCA combination significantly increased THP-triggered ROS generation in liver cancer cells. Antioxidant NAC can reverse the synergetic effect of DCA and THP on ROS generation, cell death and apoptosis. Furthermore, we found that the phosphorylation of JNK was obviously increased in DCA combined THP group. And the effects of DCA and THP on cell viability and cell death could also be inhibited by JNK inhibitor SP600125. The data indicate that DCA enhances the anti-tumor effect of THP via ROS-JNK pathway in liver cancer cells.

Key words: Dichloroacetate, Pirarubicin, ROS, JNK, liver cancer cells

专题三：蛋白质功能与修饰

A-3-001 Dual inhibition of ACK1 and KIT in Gastrointestinal Stromal Tumor

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Gastrointestinal stromal tumor (GIST) is the most common malignant tumor of gastrointestinal tract.

Mutated and activated Oncogene *KIT* and *PDGFRA* mutation and abnormal activation are the main factors for the occurrence and progression of GIST.GISTs have KIT (85%) or PDGFRA (10%) gene mutations. At present, KIT has become an important tumor biomarker for the diagnosis of GIST. Imatinib (IM), a specific KIT/PDGFRA inhibitor, become a first-line drug for the treatment of GIST. Clinical studies have demonstrated that IM has a remarkable benefit for GIST patients. However, most of the patients eventually acquired resistance to IM after treatment with drugs for two years. The second-line drug sunitinib has a good effect on IM resistant GISTs, but it has badly clinical side effects. Therefore, it is urgent to solve the problem of GIST IM resistance.

ACK1 is a member of the non-receptor tyrosine kinase family, studies have reported that ACK1 has abnormal expression and activation in many kinds of tumors, such as prostate cancer, hepatocellular carcinoma, breast cancer, stomach cancer, pancreatic cancer and so on. Therefore, in the present project, we focused on studying the expression, activation and biological function of ACK1 and the additive effects of dual targeting ACK1 and KIT on GIST signaling pathway, proliferation and metastasis.

The results showed that ACK1 overexpressed in GIST cell lines and tumor samples. In addition, it was observed that ACK1 strongly phosphorylated in IM sensitive GIST cell lines (GIST-T1) and IM resistant GIST cell line (GIST 430) by Co-immunoprecipitation (Co-IP). However, there is no interaction between ACK1 and KIT proteins. Inhibition of KIT (IM and Sunitinib) did not reduce ACK1 phosphorylation, and inhibition of ACK1 (AIM-100) little affected KIT activation. Treatment with AIM-100 inhibited ACK1 activation in a dose-dependent manner, which further verified that there is no interaction between ACK1 and KIT. MTT assay showed that ACK1 inactivation only partially inhibited the growth of GIST cells. However, AIM-100 can significantly inhibit the wound healing in GIST cells, indicating that ACK1 regulates the migration of GIST cells. Dual targeting ACK1 (AIM-100) and KIT (IM) resulted in that the inactivation of ACK1, KIT and downstream intermediates including AKT, MAPK and S6 (mTOR), in IM sensitive and IM resistant GIST cell lines. As compared to the inhibition effect of KIT (IM) in GIST cell lines, the combination of AIM-100 and IM had additive inhibition effects on the cell growth, migration, invasion in GIST430, but not in GIST-T1 cells. These findings demonstrate that co-targeting ACK1 and KIT may be novel clinical drug management and treatment of IM resistant GIST. This topic clarifies the mechanism and function of ACK1 in GIST, indicating that ACK1 and KIT synergistic targeted inhibition may provide new therapeutic strategies and methods for IM drug-resistant GIST clinical drug and treatment.

Key words: GIST; ACK1; KIT; AIM-100; IM resistance

A-3-002 IL-17B/IL-17RB signaling regulates lysine 63-linked Beclin-1 ubiquitination to strengthen self-renewal and tumorigenesis in gastric cancer

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Cancer stem cells (CSCs) display many malignant biological traits including tumorigenesis, metastasis, drug resistance and angiogenesis. Discovering new markers specific for CSCs and elucidating their regulatory mechanisms is a significant goal. Herein, we found that IL-17B /IL-17RB signaling promoted self-renewal and tumorigenesis of gastric cancer (GC) cells by activating autophagy. We identified this pathway by first determining that IL-17RB expression is significantly up-regulated in spheroid cells, which was closely associated with the degree of differentiation of patient-derived GC tissues. Exogenous recombinant IL-17B (rIL-17B) promoted the self-renewal capacity of GC cells in vitro and enhanced tumor growth and metastasis in vivo. Moreover, we found that rIL-17B induced autophagosome formation and cleavage-mediated transformation of LC3 in GC and 293T cells. Interestingly, inhibition of autophagy by ATG7 knockdown reversed rIL-17B induced self-renewal of GC cells. Further study revealed that rIL-17B promoted K63-linked ubiquitination of beclin-1 by mediating the binding of TRAF6 to beclin-1. Interfering with IL-17RB expression abolished all the effects of rIL-17B on ubiquitination of beclin-1 and autophagic activation of GC cells. Lastly, we discovered that IL-17B expression in the serum of patients was positively correlated with IL-17RB expression in GC tissues. In addition, rIL-17B increased IL-17RB expression in GC cells. Direct overexpression of IL-17RB in 293T cells mimics stimulated rIL-17B, which promoted K63-linked ubiquitination of beclin-1 and binding of TRAF6 to beclin-1. Together, these results revealed the novel action of IL-17B/IL-17RB signaling on CSCs and might provide new therapeutic targets against gastric cancer.

Key Words: IL-17B; IL-17RB; autophagy; K63-linked ubiquitination of Beclin-1; gastric cancer; cancer stem cell

A-3-003　铁储藏蛋白自组装设计及结构研究

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铁储藏蛋白（Ferritin）是生物机体内调控铁离子代谢的重要蛋白。人源铁储藏蛋白通过形成稳定的球状24聚体实现铁离子高效贮存和转运，从而完成维持生物体系铁代谢平衡的重要功能。作者通过设计和调控特定区域的氨基酸序列实现了铁蛋白从球形24聚体到8聚体纳米环状结构的重新装配，利用上海光源解析了其原子分辨率的结构，在晶体结构中其呈现纳米管状堆积和长程有序排列。该组装体在生物活性小分子的可调控装载和转运以及量子纳米生物材料等方面具有重要的应用价值。

关键词：铁储藏蛋白；自组装；可调控；蛋白质纳米管

A-3-004　泛素化介导的p53非蛋白酶体依赖途径研究进展

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肿瘤抑癌基因p53被称为“细胞的看门人”或“基因组卫士”。在多种细胞损伤应激反应中，p53基因通过引起细胞周期阻滞和细胞凋亡维持基因组稳定性。p53的翻译后修饰是调控p53活性的重要机制之一。泛素化、磷酸化、乙酰化是主要的修饰方式，其中泛素化对p53的调控发挥中心作用。p53的泛素–蛋白酶体途径对其蛋白水平调节起至关重要的作用，除此之外，p53的泛素化还介导其他非蛋白酶体依赖途径，这些途径并不引起p53蛋白的降解。研究发现p53在一些调控因子的作用下被泛素化后，并不被26S蛋白酶体识别，而是促进p53的细胞质定位，进而介导细胞的凋亡、自噬等途径。与泛素化蛋白酶体途径中p53的多泛素化标志相比，该途径的p53泛素化往往呈现单一位

点泛素化修饰。

关键词：p53；泛素化；非蛋白酶体依赖途径

A-3-005 A *Salmonella* Effector Reveals the V-ATPase-ATG16L1 Axis that Initiates Bacterial Autophagy

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Antibacterial autophagy (xenophagy) is an important host defense, but how it is initiated is unclear. Here we performed a bacterial transposon screen and identified a T3SS effector SopF that potently blocked *Salmonella* autophagy. SopF was a general xenophagy inhibitor without affecting canonical autophagy. S. Typhimurium Δ*sopF* resembled *S. flexneri* Δ*virA*Δ*icsB* with the majority of intracellular bacteria targeted by autophagy, which permitted a CRISPR screen to identify host V-ATPase as an essential factor. Upon bacteria-triggered vacuolar damage, the V-ATPase recruited ATG16L1 onto bacteria-containing vacuole, which was blocked by SopF. Mammalian ATG16L1 bears a WD40 domain that was required for interacting with the V-ATPase. Inhibiting autophagy by SopF promoted *S*. Typhimurium proliferation *in vivo*. SopF targeted Gln124 of ATP6V0C in the V-ATPase for ADP-ribosylation. Mutation of Gln124 also blocked xenophagy but not canonical autophagy. Thus, the discovery of SopF reveals the V-ATPase-ATG16L1 axis that critically mediates autophagic recognition of intracellular pathogen.

Key Words: *Salmonella* effector, bacterial autophagy, V-ATPase, ADP-ribosylation

A-3-006 干细胞标志物CD133蛋白在肿瘤干细胞中的功能和机制

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肿瘤干细胞标志物CD133在肿瘤启动、耐药、侵袭中发挥着重要的功能，有望成为肿瘤治疗的新靶点。然而CD133 直接介导的信号通路不明，成为其在肿瘤干细胞分选、功能研究和临床应用的瓶颈之一。本团队长期从事干细胞标志物CD133蛋白的糖链结构、功能和信号通路研究，如发现CD133具有唾液酸化修饰、CD133和Src、P85相互等促进肿瘤干细胞自我更新等（相关研究发表于PNAS、J Biol Chem等杂志）。发现CD133通过其C端磷酸化修饰与p85相互作用后激活Akt信号通路，从而促进胶质瘤干细胞自我更新和成瘤；发现和Scr相互作用后促进肿瘤细胞迁移。近期本团队近期利用糖链结构分析平台，解析了正常、肿瘤干细胞中 CD133 的糖链结构特征，寻找合成该结构的关键糖基转移酶；利用干细胞、小鼠动物模型研究 CD133 与胞外分子相互作用的机制、 调控因素及其在肿瘤干细胞微环境中的作用与机制。 从而为阐释干细胞标志物CD133蛋白调控干细胞命运的机制。本研究获得国家自然基金（31770856、81773164和81472724）资助。

关键词：肿瘤干细胞; CD133蛋白; N–糖基化

A-3-007 Akt通路在CD133$^+$ 甲状腺癌干细胞中的特点及意义

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甲状腺癌是常见的内分泌系统肿瘤。据统计,在过去30年中,甲状腺癌在全球的发病率不断上升,在所有恶性实体肿瘤中增长速度最快,严重影响病人生活质量并带来巨大的经济及心理负担。其中甲状腺未分化癌是人类甲状腺癌相关死亡的主要原因,而且目前的医治方法大多收效甚微。研究证明在人甲状腺癌中也存在肿瘤干细胞,是甲状腺癌复发、转移、碘131抵抗的种子细胞。本团队长期从事甲状腺癌干细胞的分子机制研究。研究发现CD133$^+$ 甲状腺癌细胞高表达干细胞基因、具有自我更新和高成瘤能力。Akt磷酸化修饰是调控肿瘤、肿瘤干细胞特性的关键信号通路，其能促进肿瘤干细胞的自我更新和成瘤。我们研究发现CD133$^+$ 甲状腺癌细胞中的ROS高于CD133$^-$甲状腺癌细胞。ROS主要是由NADPH氧化酶家族成员NOX1–5产生ROS产生氧化酶NADPH氧化酶1（NOX1）在CD133$^+$ 甲状腺癌细胞中高表达。NOX1的敲低降低了PI3K/ Akt途径的活性。 Akt激活形式的过表达部分回补NOX1下调抑制CD133 $^+$甲状腺癌细胞的自我更新能力作用。可见，一定程度的NOX1高表达可以通过激活Akt通路促进甲状腺癌干细胞的自我更新和成瘤。除此之外，我们研究发现CD133通路可以促进Akt的磷酸化修饰。CD133可以通过激活Akt通路促进甲状腺癌干细胞自我更新。综上所述，Akt通路是维持甲状腺癌干细胞自我更新的关键信号通路，对其激活机制的研究有助于阐释肿瘤干细胞自我更新的维持机制。

关键词：甲状腺癌干细胞; CD133蛋白; NOX1; Akt

A-3-008 Capsid structure of a freshwater cyanophage *Siphoviridae* Mic1

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Cyanobacteria are the most abundant photosynthetic microorganisms, the global distribution of which are mainly regulated by the corresponding cyanophages. A systematic screening of water samples in the Lake Chaohu enabled us to isolate a freshwater siphocyanophage that infects *Microcystis wesenbergii,* thus termed Mic1. Using the cryo-electron microscopy, we solved the 3.5 Å structure of Mic1 capsid. The major capsid protein gp40 of an HK97-like fold forms two types of capsomers: hexons and pentons. The capsomers interact with each other via the interweaved N-terminal arms of gp40 in addition to a tail-in-mouth joint along the three-fold symmetric axis, resulting in the assembly of capsid in a mortise-and-tenon pattern. The novel-fold cement protein gp47 sticks at the two-fold symmetric axis and further fixes the capsid. These findings provide structural insights into the assembly of cyanophages, and set up a platform to explore the mechanism of specific interactions and co-evolution with cyanobacteria.

Key Words: cyanophage, capsid assembly, cryo-EM structure, cement protein

A-3-009 YdiU通过催化蛋白UMP修饰调控细菌压力信号

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YdiU/SelO是三界中广泛存在且高度保守的蛋白家族，被预测为假激酶。其真正生理功能未知，多次被评为Top10“最具研究价值的未知功能蛋白家族”。我们研究发现细菌感受外界压力后启动YdiU的表达，并发现YdiU在细菌的热应激中发挥重要保护作用。进一步揭示YdiU催化蛋白的酪氨酸和组氨酸上发生尿嘧啶单核苷酸修饰（UMPylation），鉴定出YdiU的多种蛋白底物。有意思的是，YdiU可通过对C末端保守丝氨酸的自我AMP化来调节其对底物的UMP化活性。通过解析Apo-YdiU，YdiU-ATP 和YdiU-AMP的晶体结构及YdiU-UTP的分子动力学模拟模型，我们从分子水平上阐明YdiU催化UMP化及底物选择性的机制。分子伴侣蛋白是YdiU的UMP化底物之一。生化实验证明YdiU介导的UMP化修饰阻断分子伴侣与下游底物或辅助蛋白的相互作用，并促进分子伴侣的降解。体内数据表明，热损伤修复过程中，YdiU通过UMP化修饰动态调控分子伴侣通路的活性，有效防止ATP耗竭引起的细菌死亡。

关键词：YdiU蛋白家族；假激酶；蛋白UMP化修饰，分子伴侣翻译后修饰

A-3-010 Base-flipping dynamics from an intrahelical to an extrahelical state exerted by thymine DNA glycosylase during DNA repair process

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Thymine DNA glycosylase (TDG) is a DNA repair enzyme that excises a variety of mismatched or damaged nucleotides (nt), e.g., dU, dT, 5fC, and 5caC. TDG is shown to play essential roles in maintaining genome integrity and correctly programming epigenetic modifications through DNA demethylation. After locating the lesion sites, TDG employs a base-flipping strategy to recognize the damaged nucleobases, thereby the interrogated nt is extruded from the DNA helical stack and binds into the TDG active site. The dynamic mechanism of the base-flipping process at an atomistic level, however, remains elusive and challenging to be probed by current experimental techniques. Here, we employed the Markov State Model (MSM) constructed from extensive all-atom molecular dynamics (MD) simulations to reveal the base-flipping mechanism in the presence of TDG at tens of microsecond timescales. We capture several critical intermediate states of the mispaired dT nt during its extrusion process and identify several key TDG residues that are directly involved in the inter-state transitions. Moreover, our studies reveal the atomistic-level details of how TDG participates in sculpturing the DNA backbone and penetrates into the DNA minor groove. Notably, our comparative simulations of the naked and TDG-bound DNA systems indicate that the key intercalated residue Arg275 is critical to stabilize the partially flipped nt by forming a cation-π interaction, most likely via an active recognition scenario. The subsequent base eversion process is shown to be actively promoted by TDG. Finally, we extended our studies to three additional TDG substrates (dU, 5fC, and 5caC) to evaluate the substituent effects of various chemical modifications of the pyrimidine rings on the base-flipping dynamics.

Key words: DNA repair, molecular dynamics simulations, Markov state model.

A-3-011 Structural insights into trans-histone regulation of H3K4 methylation by unique histone H4 binding of MLL3/4

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Abstract: MLL3 and MLL4 are two closely related members of the SET1/MLL family of histone H3K4 methyltransferases and are responsible for monomethylating histone H3K4 on enhancers, which are essential in regulating cell-type-specific gene expression. Mutations of MLL3 or MLL4 have been reported in different types of cancer. Recently, the PHD domains of MLL3/4 have been reported to recruit the MLL3/4 complexes to their target genes by binding to histone H4 during the NT2/D1 stem cell differentiation. Here we show that an extended PHD domain (ePHD6) involving the sixth PHD domain and its preceding zinc finger in MLL3 and MLL4 specifically recognizes an H4H18-containing histone H4 fragment, and modifications of residues surrounding H4H18 modulate H4 binding to MLL3/4. Our In vitro methyltransferase assays and cellular experiments further reveal that the interaction between ePHD6 of MLL3/4 and histone H4 is required for their nucleosomal methylation activity and MLL4-mediated neuronal differentiation of NT2/D1 cells.

Key words: MLL3, PHD domain, histone H4

A-3-012 转录因子的磷酸化修饰对大豆耐盐响应机制的研究

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转录因子的激活通常依赖蛋白质的翻译后修饰来实现，在众多的蛋白质翻译后修饰当中，磷酸化/去除磷酸化是变化最迅速、对环境变化最敏感的类型之一。为应对高盐胁迫，大豆根部通过磷酸化修饰激活GmMYB173转录因子，提升B环含双羟基的类黄酮花青素3–阿拉伯糖苷的积累；通过磷酸化修是抑制GmMYB183转录因子活性，降低B环含单个羟基的芒柄花苷的含量，从而缓解其遭受到的盐胁迫危害。由于B环上含有双羟基的类黄酮清除ROS的能力通常强于含单个羟基的类型，这两类类黄酮物质组成的合理调配可能是大豆应对盐胁迫引起的ROS胁迫所采用的重要策略之一。类黄酮的不同亚类、不同修饰在植物耐盐响应后期清除活性氧化物的过程中可能起正、负调控两种作用，探究类黄酮的合成与修饰机制将为我们全面理解植物抵御ROS次级危害提供依据。

关键词：大豆；盐胁迫；转录因子；磷酸化修饰；类黄酮

A-3-013 Annexin A11 inhibition decreases malignant behaviours and induces megakaryocytic differentiation of CML cells through FAK/STAT5 and MEK/ERK2/Elk1

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Annexin A11 (ANXA11) is a member of the group A family of annexins in vertebrates. The dysexpression of ANXA11 is already linked to a variety of cancers, while, with unclear function in leukemia diseases. This work explored the role of ANXA11 in chronic myeloid leukemia (CML) using K562 cell line that expresses BCR-Abl. To investigate the level change of ANXA11 on the malignant properties of K562 cells, we first constructed and screened K562-shANXA11 cell line with stable knockdown of ANXA11 by short hairpin RNA (shRNA)-mediated interference against G418 screening using limited dilution method. ANXA11 knockdown decreased the proliferation, colony forming, migration and invasion capacities and

potently induced megakaryocytic differentiation of K562 cells compared with the empty vector transfected K-562 cells. ANXA11 knockdown resulted in the decreased expression levels of FAK, p-FAK, STAT5b, p-STAT5, BCL-2, BCL-XL and MMP-2, and increased expressions of p-MEK, p-ERK2, ElK1, CD61 and TIMP-1. Current work indicated that ANXA11 upregulation probably increases CML cell proliferation, colony forming, migration and invasion abilities through FAK and STAT5 signaling pathway, which contributes to malignant progression of CML. Its knockdown induced the megakaryocytic differentiation of K562 cells *via* MEK/ERK2/ElK1 signaling pathway, which implicates the ameliorated malignancy of tumor cells.

Key Words: CML, Annexin A11, FAK, STAT5, MEK/ERK2

A-3-014　靶向Akt精准治疗及光免疫治疗2.0时代

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PI3K/Akt在多种类型的肿瘤发生发展中发挥重要作用。我们首次在单个活细胞内实时动态监测Akt的全过程。我们发现，Akt的异常激活是许多传统化疗药物（如5-氟尿嘧啶）产生耐药的主要原因。因此，靶向Akt的小分子抑制剂能很好解决抗药性问题，达到更好的肿瘤治疗效果。我们在结直肠癌模型中筛选出两个重要的Akt下游PUMA及SIRT6。结果显示：靶向Akt小分子抑制剂可通过Akt/FoxO3a, NF-yb或p53途径上调促凋亡蛋白PUMA的表达，从而抑制肿瘤生长。首次发现并验证了SIRT6作为的FoxO3a调控的下游基因在结直肠癌细胞凋亡中的发挥重要作用。 低功率激光照射（Low power laser irradiation, LPLI）是一种无损的物理治疗手段，近年肿瘤的光疗法得到迅速发展。我们课题组研究光对生物体作用已有近20年之久，我们发现低功率激光照射（LPLI）激活Akt蛋白激酶，而高通量低功率激光照射（High-fluence LPLI, HF-LPLI）则显著抑制Akt活性，抑制癌细胞及肿瘤生长。我们近期的研究更是发现HF-LPLI还激活免疫系统，促进抗肿瘤免疫反应，但机制不清楚。因此，靶向Akt的肿瘤光免疫疗法的机制阐明为无损的光疗法应用于临床，并推动肿瘤的精准及免疫治疗提供有力证据。

关键词：PI3K/Akt, 低功率激光照射（LPLI），肿瘤靶向治疗，精准治疗

A-3-015　蛋白质二硫键异构酶对Tau蛋白相分离及细胞毒性的调控

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Tau蛋白是一种神经元特异的微管结合蛋白，过度磷酸化修饰的Tau在细胞内形成的神经纤维缠结（NFT）是阿尔茨海默症的病理标志之一。蛋白质二硫键异构酶（PDI）是一种二硫键异构酶，同时又作为分子伴侣发挥功能。多项研究表明，PDI在神经退行性疾病中发挥着重要的作用，而PDI在该过程中如何发挥作用仍未可知。在体外水平，我们发现PDI显著抑制了Tau蛋白病理突变体K280的液-液相分离，通过不同的荧光染料标记，我们还发现PDI能够被Tau蛋白吸入和招募到液滴中，并减缓液滴内部的液-固相变，而当PDI被亚硝基化修饰后，这种抑制作用和吸入效应也随之丧失。在细胞中，我们发现PDI和Tau蛋白之间的相互作用主要发生在内质网中，尽管Tau蛋白是如何进入内质网这一途径仍未可知。我们进一步使用激光共聚焦、免疫印迹等方法发现PDI在细胞中显著降低了Tau蛋白的异常磷酸化和聚集，而且PDI的这种抑制作用并不依赖于其位于硫氧还蛋白样催化结构域中CGHC基序的半胱氨酸残基活性，而主要是依靠其分子伴侣活性。此外，我们还发现PDI可以显著降低由Tau蛋白寡聚体诱导的细胞毒性和线粒体损伤。我们的发现揭示了PDI与Tau之间相互作用和调控的分子机制，为研究PDI在阿尔茨海默症中的作用提

供了理论依据，并为治疗阿尔茨海默病提供了一种新的策略。

关键词：Tau蛋白；阿尔茨海默病；蛋白质二硫键异构酶；分子伴侣；液–液相分离；细胞毒性

A-3-016　杨梅素抑制人Tau蛋白修饰、积聚及毒性的机制研究

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自噬在细胞内蛋白质积聚和功能损害的细胞器清除过程中扮演着重要的角色。Tau蛋白的异常磷酸化与Tau蛋白毒性直接相关，也是阿尔茨海默病另一个重要的病理特征。我们在体外纯化了人Tau蛋白及其病理突变体Tau–ΔK280，通过硫黄素T结合实验和透射电子显微镜观察到低浓度杨梅素显著抑制了人Tau蛋白在体外的积聚，通过等温滴定量热法检测到杨梅素与Tau蛋白具有中等强度的结合。我们发现杨梅素明显降低了SH–SY5Y和293T细胞中人Tau蛋白及其病理突变体396位丝氨酸的磷酸化修饰水平，同时观察到杨梅素显著促进细胞自噬。我们通过超速离心取沉淀做免疫印迹观察到杨梅素显著抑制Tau蛋白的胞内聚集；同时观察到LC3B在细胞内募集到溶酶体周围以及胞内Tau蛋白聚集体的减少。上述实验表明，杨梅素通过细胞自噬抑制胞内Tau蛋白异常磷酸化。我们通过MTT和细胞早期凋亡检测发现杨梅素能够显著降低由刚果红诱发的细胞毒性以及由Tau蛋白片段引起的细胞早期凋亡水平。综上所述，体外杨梅素直接结合Tau蛋白片段抑制其积聚，细胞内杨梅素通过细胞自噬途径抑制人Tau蛋白的异常磷酸化以及胞内积聚从而提高细胞活性。上述研究有益于阐释杨梅素抑制人Tau蛋白的病理修饰和积聚的分子机制，为基于杨梅素的小分子抑制剂的设计和研发奠定基础。

关键词：Tau蛋白；阿尔茨海默病；磷酸化修饰；蛋白质积聚；自噬；细胞凋亡

A-3-017　小分子化合物抑制人朊蛋白错误折叠的机制研究

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朊病毒疾病，又被称为传染性海绵状脑病 (TSE)，是一种致死型的神经退行性疾病，其潜伏期很长，发病周期很短，一旦发病，病人往往在一年之内死亡。其主要特征为朊蛋白由细胞型的PrP^{C}转变为致病型的PrP^{Sc}。目前还没有确定有效的治疗药物能够治愈朊病毒疾病，也没有好的疫苗用来预防朊病毒的感染。我们通过硫黄素T结合实验和Sarcosyl可溶SDS–PAGE筛选，发现杨梅素显著抑制了人朊蛋白错误折叠；运用等温滴定量热、透射电子显微镜和圆二色光谱实验，我们发现杨梅素可以和人朊蛋白发生相互作用，改变朊蛋白聚集体的二级结构。我们用低浓度的杨梅素处理人朊蛋白稳转的RK13细胞系以及SH–SY5Y细胞系，通过PK梯度酶解、超离以及流式细胞术等实验发现杨梅素显著抑制了细胞中朊蛋白的错误折叠，同时也显著抑制了由毒性片段PrP106–126引发的细胞毒性。我们运用AutoDock软件将杨梅素和人朊蛋白进行分子对接，发现杨梅素和朊蛋白的His–155、Thr–183、Gln–186和His–187形成氢键网络和稳定的复合体结构，抑制朊蛋白的积聚。我们的实验证明了杨梅素可以有效抑制人朊蛋白错误折叠，并且主要抑制朊蛋白积聚的成核过程。因此杨梅素可以作为潜在的药物用于prion疾病预防和延缓潜伏期的朊病毒疾病患者疾病的发生，对朊病毒疾病的药物研发具有重要意义。

关键词：小分子化合物；人朊蛋白；朊病毒疾病；杨梅素；蛋白质错误折叠；细胞毒性

A-3-018　次磺酸化修饰在渐冻症致病机制中的作用

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肌萎缩脊髓侧索硬化症（ALS）是一种致命的神经退行性疾病，其重要病理特征之一就是由铜锌超氧化物歧化酶（SOD1）以及TAR DNA结合蛋白43（TDP–43）在中枢神经系统的运动神经元以及星形胶质细胞中形成纤维样聚集体。散发型ALS发病的根本原因仍不清楚。我们使用基于双甲酮的次磺酸化检测抗体，检测到无论在体外还是在活的神经细胞中，病理浓度过氧化氢都可以使得野生型SOD1发生次磺酸化修饰，而且这种可逆的氧化修饰对于SOD1生长纤维和发挥病理功能至关重要。我们运用免疫共沉淀和酶联免疫的方法检测了ALS病人的脑脊液样本和相同年龄段的对照组病人（未患ALS的病人）脑脊液样本，发现散发型ALS病人脑脊液样本中的次磺酸化修饰SOD1水平显著高于对照组脑脊液样本，而且修饰位点为111位的半胱氨酸，但散发型ALS病人脑脊液样本中的总SOD1水平并没有显著高于对照组脑脊液样本，因此次磺酸化修饰SOD1是散发性ALS早期诊断生化指标。脑脊液中次磺酸化修饰的SOD1与散发型ALS的相关性这一发现将为早期ALS的诊断和治疗提供一条全新的途径。进一步的实验表明，次磺酸化修饰的SOD1寡聚体具有Prion–like的特性，能够诱导神经细胞中正常的野生型SOD1在胞质中发生异常积聚，同时诱导野生型TDP–43错误定位和在胞质中发生异常积聚，最终导致神经细胞发生凋亡。这些发现为研究病理过氧化氢以及次磺酸化修饰在散发型ALS发病机制中的作用提供了有用的线索，也为散发性ALS疾病的起因和发生以及早期诊断提供了有用的线索。

关键词：SOD1；次磺酸化修饰；ALS；蛋白质聚集；错误折叠；SOD1毒性

A-3-019　Genome annotation of a model diatom *Phaeodactylum tricornutum* using an integrated proteogenomic pipeline

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Diatoms comprise a diverse and ecologically important group of eukaryotic phytoplankton that significantly contributes to marine primary production and global carbon cycling. *Phaeodactylum tricornutum* is commonly used as a model organism for studying diatom biology. Although its genome was sequenced in 2008, a high quality genome annotation has still not been obtained for this diatom. Here we develop a systematic approach for conducting an integrated proteogenomic analysis of *P. tricornutum* using mass spectrometry (MS) based proteomics data. Our proteogenomic analysis unambiguously identifies close to 8300 genes and reveals 606 novel proteins, 506 revised genes, 94 splice variants, 58 single amino acid variants, and a holistic view of posttranslational modifications in *P. tricornutum*. We experimentally confirm a subset of novel events and provide MS-evidence for more than 200 micropeptides in *P. tricornutum*. These findings expand the genomic landscapes of *P. tricornutum* and provide a rich resource for the study of diatom biology. The proteogenomic pipeline we developed in this study is applicable to any sequenced eukaryotes and so represents a significant contribution to the toolset for eukaryotic proteogenomic analysis. The pipeline and its source code are freely available at https://sourceforge.net/projects/gapeproteogenomic.

Key words: *Phaeodactylum tricornutum*; proteogenomics; mass spectrometry (MS); genome annotation

A-3-020 蛋白质二硫键异构酶对Tau蛋白相分离及细胞毒性的调控

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Tau蛋白是一种神经元特异的微管结合蛋白，过度磷酸化修饰的Tau在细胞内形成的神经纤维缠结（NFT）是阿尔茨海默症的病理标志之一。蛋白质二硫键异构酶（PDI）是一种二硫键异构酶，同时又作为分子伴侣发挥功能。多项研究表明，PDI在神经退行性疾病中发挥着重要的作用，而PDI在该过程中如何发挥作用仍未可知。在体外水平，我们发现PDI显著抑制了Tau蛋白病理突变体□K280的液–液相分离，通过不同的荧光染料标记，我们还发现PDI能够被Tau蛋白吸入和招募到液滴中，并减缓液滴内部的液–固相变，而当PDI被亚硝基化修饰后，这种抑制作用和吸入效应也随之丧失。在细胞中，我们发现PDI和Tau蛋白之间的相互作用主要发生在内质网中，尽管Tau蛋白是如何进入内质网这一途径仍未可知。我们进一步使用激光共聚焦、免疫印迹等方法发现PDI在细胞中显著降低了Tau蛋白的异常磷酸化和聚集，而且PDI的这种抑制作用并不依赖于其位于硫氧还蛋白样催化结构域中CGHC基序的半胱氨酸残基活性，而主要是依靠其分子伴侣活性。此外，我们还发现PDI可以显著降低由Tau蛋白寡聚体诱导的细胞毒性和线粒体损伤。我们的发现揭示了PDI与Tau之间相互作用和调控的分子机制，为研究PDI在阿尔茨海默症中的作用提供了理论依据，并为治疗阿尔茨海默病提供了一种新的策略。

关键词：Tau蛋白；阿尔茨海默病；蛋白质二硫键异构酶；分子伴侣；液–液相分离；细胞毒性

A-3-021 Zinc Enhances the Formation of Liquid and Hydrogel Phases of Human Tau and thereby Increases Tau Toxicity in Neuronal Cells

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The homeostasis of zinc is fundamentally important in the CNS to ensure many physiological processes, but dyshomeostasis and accumulation of zinc are observed in the brain of patients with Alzheimer's disease. The transition between soluble intrinsically disordered Tau protein and Tau filaments from Alzheimer's disease is unknown. Liquid-liquid phase separation by low-complexity domains of proteins is the first step of protein aggregation, which can generate membrane-less organelles and drive the formation of pathological filaments. In this paper, we studied the influences of zinc on phase transition, filament formation, and neurotoxicity of a pathological mutant ΔK280 of full-length human Tau to elucidate the molecular mechanism underlying Alzheimer's disease. We report that zinc enhances the intrinsic ability of ΔK280 to complete the required phase transition to form pathological filaments. Substitution of Cys-291 and Cys-322 with Ala, however, essentially eliminates such enhancing effects of Zn^{2+} on the fibrillization and phase transition of ΔK280. Zinc dramatically accelerates hyperphosphorylation and abnormal aggregation of ΔK280 both in vitro and in SH-SY5Y neuroblastoma cells. Furthermore, zinc enhances the formation of liquid and hydrogel phases and elevated ROS production. These findings elucidate how zinc regulates Tau phase separation, aggregation associated with Alzheimer's disease.

Key Words: Tau protein, Zinc, Alzheimer's disease, Liquid-liquid phase separation, Protein aggregation, Tau toxicity, Phase transition

A-3-022 Neutralizing Mutations Significantly Inhibit Phase Separation of Human Prion Protein and Decrease Its Cytotoxicity

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Prion disease, such as Creutzfeldt-Jakob disease and bovine spongiform encephalopathy, is a kind of fatal neurodegenerative disease that affects many mammals including humans, and is caused by the misfolding of prion protein (PrP). A naturally occurring protective polymorphism G127V in human PrP has recently been found to greatly attenuate prion disease, but the mechanism has remained elusive. We herein report that the hydrophobic chain introduced in G127V significantly inhibits phase separation, transition and fibril formation of the human PrP, highlighting the protective effect of the G127V polymorphism. We further introduce an amino acid with a different hydrophobic chain (Ile) at the same position and find G127I having similar protective effects of G127V. Moreover, we show that two neutralizing mutations G127V and G127I significantly decrease human PrP cytotoxicity that results from its fibril formation, mitochondrial damage, and elevated ROS production enhanced by a strong prion-prone peptide PrP 106-126. These findings elucidate the molecular basis for a natural protective polymorphism in PrP and will enable the development of novel therapeutic strategies against prion disease.

Key Words: Prion protein, Neutralizing mutation, Protein phase separation, Protein aggregation, Prion disease

A-3-023 维生素c介导P4HA1 N259位翻译后糖基生成促进胶原的分泌

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胶原是人体内最大量的蛋白。由胶原分泌失调产生的病理占所有人类疾病的60%以上。维生素c是胶原蛋白成熟的重要辅因子而P4HA1是胶原成熟的主要催化酶，催化胶原脯氨酸的羟化促进其成熟。N–链接糖基化是一种进化上非常保守的蛋白质修饰方式，可以区分为共翻译修饰和翻译后修饰。共翻译修饰主要由STT3A复合物完成而翻译后修饰主要由STT3B/MAGT1复合体完成。在P4HA1上存在两个糖基花位点，此前报道表明这两个糖基化修饰和酶活性无关，但是N259位糖基位于酶结合底物的活性区。我们的研究发现P4HA1的N113 糖基修饰发生不依赖于维生素c处理而N259位糖基化依赖于维生素c处理并且有STT3B/MAGT1复合体参与的。敲降STT3B/MAGT1影响N259位N–链接糖基、减少一型胶原可以结合的P4HA1的酶量从而降低一型胶原特定位点的羟化而增加另外一些位点的羟化。我们的研究阐明了维生素c的新生理功能并为理解蛋白质N–链接糖基的产生机制开辟了新方向。

关键词：胶原成熟分泌；维生素C；脯氨酸羟化酶；N–链接糖基化修饰；STT3B； MAGT1

A-3-024 Thioredoxin-1 regulates learning and memory deficits in MPTP-induced Parkinson's disease model in mice

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Abstract: Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), characteristic motor symptoms and cognitive impairment. Thioredoxin-1 (Trx-1) is a redox protein and protects neurons from various injuries. Our study has shown that Trx-1 overexpression improves learning and memory impairments induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). However, whether Trx-1 downregulation aggravates learning and memory deficits is still unknown. In present study we found that downregulation of Trx-1 in SNpc aggravated dysfunction induced by MPTP. The decreased expressions of tyrosine hydroxylase (TH), dopamine D1 receptor (D1R), N methyl D aspartate receptor 2B subunit (NR2B), extracellular signal-regulated kinase (ERK1/2) and cAMP-response element binding protein (CREB) in the hippocampus were further down-regulated. These results suggest that Trx-1 regulates learning and memory deficits in MPTP-induced PD model via modulating the D1R and the NMDAR-ERK1/2-CREB pathway. Trx-1 may be a therapy target for learning and memory deficits in PD.

Key Words: Thioredoxin-1, Parkinson's disease, Hippocampus, Learning and memory

A-3-025 Glucagon-like peptide 1 improves vascular remodeling by down-regulating matrix metalloproteinase 1 expression through ERK1/2/NF-κB signaling pathway

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Abstract: As an incretin used to treat type 2 diabetes, glucagon-like peptide 1 (GLP-1) improves cardiovascular diseases by regulating the heart rate and blood pressure and ameliorating endothelial function and vascular remodeling. Vascular remodeling is known as the pathological basis of vascular diseases, including hypertension and atherosclerosis. The reversal of vascular remodeling is one of the possible targets for the clinical treatment of hypertension. However, it is untill unclear that the molecular mechanisms of the GLP-1 in vascular remodeling. Cell proliferation and viability was evaluated using MTT assays and Flow cytometry. Cell migration was determined by scratch wound assays. Immunofluorescence technique was used to check subcellular localization.Western blotting weas used to determine protein expression. GLP-1 treatment attenuated the proliferation and migration of rat aorta smooth muscle cells induced by angiotensin II (AngII). In addition, treatment with GLP-1 agonist Liraglutide decreased systolic blood pressure and partially restrained the remodeling of blood vessels in spontaneously hypertensive rats as indicated by the reduced vascular remodeling. ERK1/2 and NF-κB signaling pathways participated in these processes. Furthermore, the abated expression of matrix metalloproteinase 1 (MMP1) eliminated the effect GLP1 on proliferation and migration induced by AngII. In summary,GLP-1 inhibited vascular remodeling by down-regulating MMP1 expression, which was mediated by ERK1/2 and NF-κB signaling pathway.

Key Words: GLP-1, vascular remodeling, ERK1/2, NF-κB, MMP1

A-3-026 HIF employs CHD4 to stimulate RNA polymerase II recruitment in response to hypoxia

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Metastatic breast cancer has high rates of relapse and mortality. The transcription factor hypoxia-inducible factor (HIF) plays a crucial role in breast cancer metastasis by inducing hundreds of the downstream target genes. RNA polymerase II is essential for HIF-mediated transactivation. However, the mechanism by which RNA polymerase II is loaded to HIF target genes remains unknown.

Based on mass spectrometry analysis, we identified epigenetic remodeler CHD4 as a novel HIF regulator. CHD4 is highly expressed in invasive breast tumors and this overexpression is significantly correlated with poor clinical outcomes in patients with breast cancer. CHD4 depletion dramatically attenuates the tumorigenic potential of breast cancer cells in colony formation, migration and invasion in vitro, and suppresses breast tumor growth and metastasis in mice. To elucidate the underlying mechanism, we performed Co-IP, RNA-seq, ChIP-qPCR and RT-qPCR assays, and found that CHD4 interacts with HIF complex at the hypoxia inducible elements (HREs) to provoke the expression of HIF target genes *LOX, ANGPTL4, NDNF* and *VEGFA* in a HIF-dependent manner. CHD4 interacts with and recruits RNA Polymerase II to the HREs, thereby promoting the formation of transcription preinitiation complex. Together, CHD4-HIF-RNA polymerase II axis is critical for breast cancer progression and metastasis.

In summary, CHD4 augments HIF-mediated breast cancer progression and metastasis, and provides a potential prognostic biomarker and therapeutic target for breast cancer.

Key words: breast cancer; HIF; CHD4; RNA polymerase II

A-3-027 Acetylation regulates ribonucleotide reductase activity and cancer cell growth

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Abstract: Ribonucleotide reductase (RNR), composed of RRM1 and RRM2 subunits, catalyzes the rate-limiting step in the de novo synthesis of deoxyribonucleotides triphosphate (dNTP) needed for DNA synthesis. Although allosteric regulation has been defined to control its activity to manipulate the dNTP pool balance and genomic integrity, insight into fine regulation is still limited. Here, we report that acetylation and deacetylation of the RRM2 subunit of RNR acts as a molecular switch that impacts RNR activity, dNTP synthesis, and DNA replication fork progression. Acetylation of RRM2 at K95 abrogates RNR activity by disrupting its homodimer assembly. RRM2 is directly acetylated by KAT7, and deacetylated by Sirt2, respectively. Sirt2, which level peak in S phase, sustains RNR activity at or above a threshold level required for dNTPs synthesis. We also find that radiation or camptothecin-induced DNA damage promotes RRM2 deacetylation by enhancing Sirt2-RRM2 interaction. Acetylation of RRM2 at K95 results in the reduction of the dNTP pool, DNA replication fork stalling, and the suppression of tumor cell growth *in vitro* and *in vivo*. This study therefore identifies acetylation as a regulatory mechanism governing RNR activity.

Keywords: Ribonucleotide reductase; Acetylation; Cancer nucleotide metabolism

A-3-028 CRISPR/Cas9-mediated gene knockout for DNA methyltransferase Dnmt3a in CHO cells displays enhanced transgenic expression and long-term stability

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Aims: DNA methyltransferase Dnmt3a was knocked out by CRISPR/Cas9 technology to construct Dnmt3a-deficient CHO cell expression system. The molecular mechanism of DNA methylation affecting the expression stability of recombinant protein in CHO cells was also revealed. Methods: The sgRNA target of Dnmt3a KO was designed to construct plasmid. Selection and identification of monoclonal Dnmt3a KO CHO cells by PCR, Western blotting and sequencing. Cell proliferation was detected by CCK-8, and apoptosis was detected by Annexin V-FITC /PI double staining. Transgene expression vectors driven by two commonly used CMV and EF1a promoters were constructed and transfected into CHO cells. Polycolonies of the 3a-30 and control CHO-K1 cells stably transfected with CMV or EF1a were passaged under selection pressure in the presence (G418+) or absence (G418) of G418 for 60 passages. The MFIs of eGFP in the recombinant CHO cells were detected to evaluate the intensity values of the expressed eGFP at 10, 20, 30, 40, 50 and 60 passages. The total DNA methylation level in CHO cells was analyzed by flow cytometry. Detection of CpG island methylation in different promoter regions of recombinant CHO cells by MALDITOF mass array. Results: Six Dnmt3a-deficient CHO cell monoclonal clones were identified by PCR and sequencing. The results of cell proliferation and apoptosis showed that the biological characteristics of Dnmt3a defective CHO cell line were not significantly different from those of normal CHO cells. The Dnmt3a-deficient 3a-30 cell line transfected with CMV promoter displays the most stable and the highest expression levels regardless of the presence or absence of G418, suggesting that the Dnmt3a KO in CHO cells can enhance the long-term stability of recombinant protein expression by using CMV promoter for at least 50 passages. The results of DNA methylation analysis revealed that the rate of DNA methylation in the analysed 10 CpG sites of CMV promoter at high passage (P50) was lower in 3a-30 cell line than in natural CHO cell line. By contrast, Dnmt3a KO exerted no influence on the reduction in the DNA methylation of EF1a promoter in the transfected CHO cells. Conclusion: The Dnmt3a gene in CHO-K1 cells was knocked out by CRISPR/Cas9 technology, screened and obtained the Dnmt3a defective CHO-K1 cell line. Dnmt3a KO positively affected the maintenance of recombinant protein production in stably transfected CHO cells. The stability of transgene expression during long-term cultivation could be distinctly enhanced in Dnmt3a-deficient CHO cells transfected with CMV promoter, presumably because of the distinct reduction in the DNA methylation of CMV promoter in stably transfected Dnmt3a-deficient CHO cells.

Key Words: Chinese hamster ovary cell, DNA methylation, DNA methyltransferase Dnmt3a, gene knockout, transgene expression

A-3-029 生物质谱技术在蛋白质研究中的应用

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生物质谱技术是自然科学领域一种广泛应用的检测研究手段。随着“组学”（蛋白质组学，代谢组学等）概念的提出，生物质谱技术更是蓬勃发展，在生物学研究领域有着广泛的应用。生物质谱技术在生物学中的应用使得蛋白质研究的通量、灵敏度、精确度得到了显著地提升，并越来越多的被应用到化学生物学、分子生物学、细胞生物学、医学、药学等多个学科领域。

随着新概念与新方法、新技术不断被引进和多个革新，蛋白质相互作用信号通路分析更加便利化，更有效的推

动药物靶蛋白的筛选和疾病相关信号通路等的研究工作；蛋白质后修饰位点及定位分析大大促进了表观遗传学及生物化学等方向的研究；定量蛋白质组学分析方法的进步则使得生物标志物的筛选成为可能；样品类型兼容性得到了提高，检测技术方法也随之日新月异地发展。

与此同时，生物质谱技术在蛋白质结构解析研究工作中的作用也崭露头角。虽然，目前基于生物质谱对蛋白质结构的解析研究还无法与传统的X-Ray，NMR以及cryo-EM相提并论。但是，基于生物质谱技术的蛋白质交联质谱联用检测技术、氢氘交换质谱联用检测技术等从自身的解析角度出发，从蛋白质的空间位置、组成、动态分析等多个方面给予蛋白质的结构解析和功能研究提供巨大的帮助。

蛋白质设施质谱分析系统经过几年的积累，已经形成了蛋白质研究中多项能力：通过对蛋白质的高分辨、高准确性鉴定确定功能系统中起重要作用的蛋白质，从而提示进行结构分析和分子影像分析发现蛋白质的功能；通过质谱分析定位发生在蛋白质上的修饰位点进一步指导蛋白质结构的测定和功能分析；通过对重要功能蛋白质的精确定量分析可追踪在不同时间和处理条件下的蛋白质表达变化，从而为解释细胞活动的分子机制及筛选疾病生物标志物和药物靶点提供分子基础。

同时，系统经过技术开发与建立，也开展了一系列生物质谱新技术新方法的开发，特别是在蛋白质表征，如结构解析、蛋白质糖基化等方面的众多工作。交联质谱联用技术和氢氘交换质谱联用技术就是其中两个重要的方面。化学交联结合质谱技术（chemical cross-linking of proteins coupled with mass spectrometry），简称交联质谱技术、CXMS或XL-MS，是近年发展起来的新方法。它利用化学交联剂 （chemical cross-linker）处理蛋白质样品，将空间距离足够接近、可以与交联剂反应的两个氨基酸以共价键连接起来，然后利用基于高精度质谱的蛋白质组学手段分析交联产物，可以反映了蛋白的溶液构象。氢氘交换质谱联用技术（HDX MS, hydrogen deuterium exchange mass spectrometry）是一种结合了氢氘交换实验特点与质谱检测能力，用以研究蛋白质空间构象的新型技术。该项技术在蛋白质结构及动态变化研究、蛋白质相互作用位点发现、蛋白表位及活性位点鉴定方面有着广泛的应用。随着氢氘交换质谱技术的不断发展，它正在成为结构生物学家及生物药物研发的重要手段。

通过不同类型技术的整合，可以为蛋白质功能和表征、结构解析等研究提供更好的推动力。

关键词：定量蛋白质组学、蛋白质结构解析、蛋白质修饰组、蛋白质糖基化

A-3-030 hCINAP regulates the DNA damage response and mediates the resistance of acute myelocytic leukemia cells to therapy

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Maintenance of genomic stability is critical for the proper function of organisms. When DNA damage occurs and is detected, a series of repair proteins sequentially accumulate at the damaged site and induce signal transduction pathways to initiate the subsequent repair of double-stranded DNA breaks (DSB). Acute myeloid leukemia (AML) is a genetically heterogeneous malignant disorder of the hematopoietic system, characterized by the accumulation of DNA-damaged immature myeloid precursors. In this study, we find that hCINAP is involved in the repair of DSB and that its expression correlates with AML prognosis. hCINAP is a highly conserved adenylate kinase that plays essential roles in the growth of eukaryotic cells. Following DSB, hCINAP is recruited to damage sites where it promotes SENP3-dependent deSUMOylation of NPM1, which in turn results in the dissociation of RAP80 from the damage site and CTIP-dependent DNA resection and homologous recombination. NPM1 SUMOylation is required for recruitment of DNA repair proteins at the early stage of DSB, and SUMOylated NPM1 impacts the assembly of the BRCA1 complex.

Moreover, we find that knockdown of hCINAP also sensitizes a patient-derived xenograft (PDX) mouse model to chemotherapy. In clinical AML samples, low hCINAP expression is associated with a higher overall survival rate in patients. These results provide mechanistic insight into the function of hCINAP during the DNA damage response and its role in AML resistance to therapy. Meanwhile, the discovery of sumoylation/desumoylation pathways that function to control DNA

damage repair highlights the possibility of modulating these PTM activities in order to protect healthy cells from the effects of genotoxic anticancer therapies, while still eliminating the cancer cells. As the analogous ubiquitination system can easily be targeted by drugs, such pleiotropic mechanisms can be of substantial use in cancer treatments, offering a number of possibilities for future applications.

Key Words: hCINAP, NPM1, double-stranded DNA breaks (DSB), SUMOylation, acute myeloid leukemia (AML)

Reference

Ruidan Xu, Shuyu Yu, Dan Zhu, Xinping Huang, Yuqi Xu, Yimin Lao, Yonglu Tian, Jinfang Zhang, Zefang Tang, Zemin Zhang, Jing Yi, Hong-Hu Zhu, and Xiaofeng Zheng*. hCINAP regulates the DNA damage response and mediates the resistance of acute myelocytic leukemia cells to therapy. Nature Communications. 2019. Accepted.

A-3-031 A multi-lock inhibitory mechanism for fine-tuning enzyme activities of the HECT family E3 ligases

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The HECT type E3 ligases are key regulators of cell growth and proliferation, and malfunctions of these enzymes are associated with a broad range of human tumors and other diseases. The ligase activities of Nedd4 family E3s, the largest and best characterized subfamily of HECT E3s, are tightly controlled via auto-inhibition. However, the molecular mechanisms underlying Nedd4 E3s auto-inhibition and activation are poorly understood. We show that multiple WW domains play an inhibitory role by sequestering HECT using a multi-lock mechanism in most Nedd4 E3s, though each with a distinct mode. The structure of fully inhibited WWP1 reveals that many WWP1 mutations identified in cancer patients result in a partially active state with increased E3 ligase activity, and the WWP1 mutants likely promote cell migration by enhancement of ΔNp63*α* degradation. We further demonstrate that WWP2 and Itch utilize a highly similar multi-lock autoinhibition mechanism as that utilized by WWP1, whereas Nedd4/4L and Smurf2 utilize a slightly variant version. Binding of the Ndfip1 adaptor or JNK1-mediated phosphorylation relieves the auto-inhibition of Itch in a WW domain-dependent manner. Aberrant activation of Itch leads to migration defects of cortical neurons during development. Overall, these results reveal versatile autoinhibitory mechanisms that fine-tune the ligase activities of the HECT family enzymes.

Key Words: Nedd4 family E3 ligases; HECT; ubiquitination; allosteric regulation; cell migration; WWP1; ΔNp63*α*; Itch; Nedd4/4L; Smurf2

A-3-032 Design and Preliminary Activity Determination of anti-tumor peptides targeting integrin αvβ3

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Abstract: Objective Design a couple of RGD loop-containing peptides on the basis of the structure of echistatin and evaluate the preliminary activity. **Methods** The peptides were designed based on the spatial structure of echistatin with combination of other reports. The preliminary activity of the peptides was analyzed using MTT. The expression of integrin αvβ3 in five

colorectal cancer cells was analyzed by Western blot. **Results** Six peptides (cEchi-L-1, cEchi-L-2, Ω-Echi, Echi-L, cEchi-M and cEchi) were designed and synthesized. Among them, cEchi-L-1 and cEchi-L-2 exhibited antitumor activity, and their IC50 were 59.92 μmol/L and 18.33 μmol/L, respectively. **Conclusion** Two peptides with a certain anti-tumor activity were found, the results indicate that the quantity of amino acids between the RGD loops and the C-terminal of echistatin may play a role on the anti-tumor activity of the peptidemimetics.

Keywords: Integrin αvβ3; RGD loop; Echistatin; Colorectal cancer

A-3-033 Role of phosphorylation at Serine 1439 in subcellular localization and function of L-periaxin in Schwann cells

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L-periaxin is an important scaffold protein in nerve cells of mammals. During the development and maturation of Schwann cells, L-periaxin redistributes its subcellular localization from the nucleus to the cytoplasm, where it is concentrated at the abaxonal membrane. In order to determine its effect on the localization of L-periaxin, the reversible Akt-dependent phosphorylation of L-periaxin has been studied. First, PI3K/Akt signaling pathways participated in L-periaxin's cell migration. Scan-site predictions and analysis of conserved amino acid sequence among vertebrate species indicated that Ser1439 located in the C-terminal acidic structure-domain was a target site of phosphorylation by Akt. Subsequently, the mutated Ser1439 into Ala (1439S/A) or Glu (1439S/D) found that pEGFP-*L-PRX*$^{1439S/A}$ mainly located to the cell nucleus, while pEGFP-*L-PRX*$^{1439S/A}$ mainly located at the cell membrane. According to the results of SRLCA (Split-Renilla luciferase complementary assay), the interaction between the N-terminal and C-terminal domains of L-periaxin can be affected by the phosphorylation of Ser1439. Furthermore, the phosphorylation and dephosphorylation of L-periaxin can dynamically regulate RSC96 cell proliferation and cell migration. In summary, the unique cytology positioning and protein expression of L-periaxin is associated with the Ser1439 phosphorylation state. This finding can further provide the reference for studying L-periaxin's role in development, maturation and other processes related to the myelin sheath of mammals.

Key Words: L-periaxin, Phosphorylation, Acidic structure-domain, Subcellular locational; Akt

A-3-034 Growth phase-dependent changes in the size and infectivity of SDS-resistant Sup35p assemblies associated with the [*PSI*$^+$] prion in yeast

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The translation termination factor Sup35p can form self-replicating fibrillar aggregates responsible for the [*PSI*$^+$] prion state. Sup35p aggregation yields detergent-resistant assemblies detectable on agarose gels under semi-denaturant conditions and fluorescent puncta within the yeast cytosol when the protein is fused to GFP. It is still unclear whether any of theses manifestations of [*PSI*$^+$] truly correspond to the Sup35p assemblies that faithfully transmit the [*PSI*$^+$] prion from mother to

daughter cells. The infectious titer of prions in cells can be indirectly assessed by the ability of [*PSI*$^+$] cells lysates to induce the prion state when introduced into naïve cells. Here, we report that the dramatic changes in the size and amounts of SDS-resistant Sup35p that occur during growth do not correlate with the infectious titer. Our results suggest that fluorescent Sup35p-GFP puncta and detergent-resistant Sup35p assemblies are good indicators of Sup35p conversion to the prion state but not of infectious particles number.

Key words: SUP35, yeast, prion, amyloid, [*PSI*$^+$]

A-3-035 Ezrin interacts with L-periaxin by the "head to head and tail to tail" mode and influences the location of L-periaxin in Schwann cell RSC96

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In the peripheral nervous system (PNS), Schwann cells (SCs) are required for the myelination of axons. Periaxin (PRX), one of the myelination proteins expressed in SCs, is critical for the normal development and maintenance of PNS. As a member of the ERM (ezrin-radxin-moesin) protein family, ezrin holds our attention since their link to the formation of the nodes of Ranvier. Furthermore, PRX and ezrin are co-expressed in cytoskeletal complexes with periplakin and desmoyokin in lens fiber cells. In the present study, we observed that L-periaxin and ezrin interacted in a "head to head and tail to tail" mode in SC RSC96 through NLS3 region of L-periaxin with F3 subdomain of ezrin interaction, and the region of L-periaxin (1368-1461aa) with ezrin (475-557aa) interaction. Phosphorylated ezrin resulted in L-periaxin accumulation on SC RSC96 membrane. Ezrin could inhibit the self-association of L-periaxin, and ezrin overexpression in sciatic nerve injury rats could facilitate the repair of impaired myelin sheath. Therefore, the interaction between L-periaxin and ezrin may adopt a close form to complete protein accumulation and to participate in myelin sheath maintenance.

Key Words: Schwann cells, L-periaxin, Ezrin, interaction, myelination

A-3-036 Prohibitin1对动脉粥样硬化发生发展及分子机制的探究

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动脉粥样硬化(atherosclerosis, AS)是一种复杂的慢性炎症性疾病，是多种心脑血管疾病和周围血管病的重要病理基础，严重威胁人类健康及生命。白细胞介素8（IL-8）是一种趋化因子，来源于单核/巨噬细胞、血管内皮细胞、血管平滑肌细胞，可引起中性粒细胞在炎症部位聚集，诱导血管内皮细胞，平滑肌细胞增殖和迁移。PHB由两个高度相似的亚基组成，即：PHBl和PHB2， Prohibitin1（PHB1）定位于染色体17q21，是由275个氨基酸残基组成分子量为32kd的蛋白，在细胞膜，细胞核，细胞质均有定位。过往的研究表明其在细胞核中与转录因子p53，Rb，E2F，AIF等均存在共定位。这些肿瘤抑制因子或原癌基因与细胞的增殖或凋亡的调控作用密切相关，暗示PHB1功能的多样性。

目的：PHB1与炎症及细胞的增殖、凋亡等有关，但与心血管疾病关系的研究资料较少。本研究旨在探讨PHB1对动脉粥样硬化发生发展及分子机制的探究。

材料和方法：（1）构建小鼠AS模型，采用组织免疫荧光和免疫组化检测AS斑块中PHB1的表达量，qPCR检测IL-8刺激下人血管平滑肌细胞（AOSMC）、大鼠血管平滑肌细胞（A7r5）、血管内皮细胞（HUVEC）中PHB1的表达情况;（2）MTT检测PHB1对AOSMC和A7r5增殖的影响；（3）transwell检测PHB1对AOSMC和A7r5迁移的影响；（4）流式细胞术方法检测AOSMC、A7r5细胞周期；（5）Elisa检测健康人群和高脂血症人群血清中PHB1的含量。

实验结果：（1）PHB1在AS斑块中和IL-8的刺激下，在AOSMC、A7r5和HUVEC表达量明显升高；（2）外源性siPHB1可以明显抑制AOSMC、A7r5的迁移和增殖，而过表达PHB1可以明显促进其增殖。（3）Elisa结果显示，与健康人群相比，高脂血症人群血清中PHB1含量明显增高。

结论：（1）IL-8可以诱导AOSMC、A7r5和HUVEC中PHB1的表达，促进AOSMC、A7r5的增殖和迁移，促进HUVEC的增殖（2）外源性siPHB1可抑制AOSMC、A7r5的增殖和迁移，而过表达PHB1可以明显促进其增殖。

关键词：血管平滑肌细胞 血管内皮细胞 抑制素1 细胞增殖 细胞迁移

A-3-037 Non-classical estrogen signaling in ovarian cancer improves chemo-sensitivity and patients' outcome

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Deficiency in homologous recombination repair (HRR) is frequently associated with hormone-responsive cancers, especially the epithelial ovarian cancer (EOC) which shows defects of HRR in up to half of cases. However, whether there are molecular connections between estrogen signaling and HRR deficiency in EOC remains unknown. We analyzed the estrogen receptor α (ERα) binding profile in EOC cell lines and investigated its association with genome instability, HRR deficiency and sensitivity to chemotherapy using extensive public datasets and in vitro/in vivo experiments. We found an inverse correlation between estrogen signaling and HRR activity in EOC, and the genome-wide collaboration between ERα and the co-repressor CtBP. Though the non-classical AP-1-mediated ERα signaling, their targets were highly enriched by HRR genes. We found that depleting ERα in EOC cells up-regulates HRR activity and HRR gene expression. Consequently, estrogen signaling enhances the sensitivity of ovarian cancer cells to chemotherapy agents in vitro and in vivo. Large-scale analyses further indicate that estrogen replacement and ESR1 expression are associated with chemo-sensitivity and the favorable survival of EOC patients. These findings characterize a novel role of ERα in mediating the molecular connection between hormone and HRR in EOC and encourage hormone replacement therapy for EOC patients.

Key Words: Ovarian cancer; Estrogen signaling; Deficiency of homologous-recombination; Chemotherapy; Hormone replacement

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A-3-038 Evolution analysis of aerolysin-like proteins in metazoan

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Aerolysin-like proteins (ALPs) are aerolysin domain containing family of pore-forming proteins. They widely distributed in bacteria, fungi, plants and animals. Previous investigation indicated that a rich diversity of ALPs existed in vertebrates. Meanwhile, evolutionary analysis illustrated that ALPs evolved from prokaryotic to eukaryotes through horizontal genes transfer. In present investigaion, to solve the problem of low primary structure similarities of ALPs, we used hidden Markov model (HMM) as the core algorithm to retrieve ALPs sequences. Different from previous studies, HMMs employed for search was constructed by the sequences in unidentified protein databases from different organism groups and subfamilies in proportion and iterated. The low complexity sequences (short fragment repeat regions/full sequence>40%) were removed after searching and the candidate sequences were further confirmed by PDB and Pfam public databases. This method can attenuate the limitations in HMM caused by lacking of characteristics and over-fitting of some strength features. After collecting the sequences by present modified method, more sequences were retrieved from public databanks, including 15 sequences from platypus genome. Taking ep37 (an ALP composed of crystallin and aerolysin domains) as an example, we found that their molecular evolution is basically consistent with phylogenetic development, while the evolution of these two domains are different. Considering that ep37-like 2 domain ALPs are not widely distributed in invertebrates, domain fusion or replacement events might be pappened in the evolution process of crystallin and aerolysin domain containing ALPs.

Key Words: ALPs, aerolysin, evolution, HMM

A-3-039 Purification, characterization and functional analysis of peptidyl-aminoacyl-L/D-isomerase from *Bombina maxima*

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Although most natural proteins are composed exclusively of L-configuration amino acids, more and more emerging evidences showed that D-amino acids play crucial roles in sustaining the functions of proteins or peptides, as well as in the development of diseases. Peptidyl-aminoacyl-L/D-isomerase is a key enzyme to catalyze the transition from L-amino acids to D-amino acids. However, the molecular characterization and functional analysis of the enzyme in amphibian species of *Bombina maxima* were unclear. In this study, the enzyme was purified from skin secretions of *B. maxima.* The apparent molecular weight of the purified enzyme was determined to be 35 kDa. Interestingly, no internal or interchain disulfide bonds was found for this enzyme. Functional study revealed that the isomerase has a strong ability to catalyzes the isomerization of an L-Ile in position 2 of a model peptide (IIGPVLGC) to D-allo-Ile. Mutation study demonstrated that the lysine in position[122] of the enzyme was essential for its function. Interestingly, a D-amino acids containing peptide named maximin H was identified from *B. maxima* skin secretions and displayed a strong antimicrobial activity. However, the activity of maximin H was significant decreased once the its D-Ile changed to L-Ile. These findings suggested that peptidyl-aminoacyl-L/D-isomerase was vital for sustaining the functions of frog-derived bio-active peptides and the survival of frogs.

Key Words: peptidyl-aminoacyl-L/D-isomerase, D-amino acid, *Bombina maxima*

A-3-040 Comparison of γ-bungarotoxin variations in 2 regions of china and systematic determination of LD50 via different routines

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Studies have revealed that snake venoms from identical species in different regions differ in composition, toxicity and activity. This geographical venom variation might be caused by ontogeny, geographical location or seasonal climate differences, and might also be related to existing diets. However, the main factor causing this geographical venom variation is geographical location. Present investigation focus on γ-bungarotoxin (the most poisonous component of post-synapse contained in *Bungarus multicinctus* venom) of 2 geographical regions in China. The crude venoms used in this study were collected from Zhejiang and Hunan province of China, respectively. Gel filtration, ion-exchange chromatography, high performance liquid chromatography were used to separate and purify γ-bungarotoxin from different regions. Although the primary structures of γ-bungarotoxins isolated from different locations are identical as revealed by mass spectrometry analysis, the quantity of γ-bungarotoxin contained in crude venom from different regions differs greatly. LD50 values of γ-bungarotoxin were accrurately determined in Kunming mice by intraperitoneal injection, intramuscular injection and tail vein injection, respectively.

Key Words: γ-bungarotoxin, geographical variation, toxicity, LD50

A-3-041 Sprouty4 regulated vascular permeability by regulating the tyrosine phosphorylation and degradation of VE-cadherin

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Regulation of endothelial cell adherent junctions is important to pathophysiological processes, in which vascular integrity is disrupted such as during tumor angiogenesis and chronic inflammatory conditions. The effects of Sprouty4 (Spry4) on endothelial barrier function was investigated using vascular endothelial cell growth factor (VEGF)-induced vascular permeability model. In the present study we showed that overexpression of Spry4 inhibited vascular permeability in primary human endothelial cells in vitro and in Spry4-expressing transgenic mice in vivo. Conversely, knockdown of Spry4 in vitro and conditional endothelial cell-specific knockout of Spry4 in endothelial cells in vivo enhanced VEGF-induced vascular permeability. In addition, overexpression of Spry4 inhibited VEGF-induced vascular endothelial cadherin (VE-cadherin) tyrosine phosphorylation, in part by inhibiting c-Src activation, and resistance to VEGF-induced vascular permeability. Inhibition of VEGF-induced VE-cadherin tyrosine phosphorylation and decreased vascular permeability by Spry4 overexpression could be rescued by expression of constitutively active c-Src. Conversely, knockdown of Spry4 in endothelial cells increased tyrosine phosphorylation of VE-cadherin and vascular permeability, which was repressed by dominant negative Src (DNSrc). Consistent with these results, DNSrc repressed the increase in vascular permeability that resulted due to knockdown of Spry4. Taken together, these results suggested that Spry4 regulated vascular permeability via regulating the activation of c-Src by VEGF signaling and consequently the tyrosine phosphorylation of VE-cadherin.

Key Words: Vascular integrity, Endothelial permeability, Vascular endothelial growth factor receptor 2, Vascular endothelial cadherin, Sprouty

A-3-042 嗜热四膜虫组蛋白分子伴侣的鉴定与功能分析

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核小体是真核生物中染色质的基本结构和功能单位，由DNA与组蛋白八聚体构成。组蛋白的储存、转运及翻译后修饰由组蛋白分子伴侣协助完成的。四膜虫细胞具有核的二态性，包括生殖系的小核和体细胞的大核。功能不同的大小核为研究组蛋白靶向转运提供了良好的模式体系。本研究鉴定了嗜热四膜虫不同的组蛋白分子伴侣，组蛋白H3分子伴侣Nrp1，组蛋白H2A–H2B的分子伴侣Pob3，染色质组装因子Caf1复合物。不同的组蛋白分子伴侣在大核和小核均有定位，蛋白亲和纯化和质谱分析鉴定了不同的相互作用因子。基因敲减分析发现突变体细胞生长期出现小核不均等分裂，大核无丝分裂异常，逐出体增多；在有性生殖时期，出现小核拉伸异常和配子核无法选择，突变体细胞无法完成正常的有性生殖进程。表明不同组蛋白分子伴侣的正常表达对于四膜虫的有性生殖过程中细胞核的减数分裂是必需的。

关键词：嗜热四膜虫；核定位；组蛋白分子伴侣；核分裂

A-3-043 自噬相关基因*ATG5*在嗜热四膜虫细胞核程序性降解中的功能分析

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自噬是真核生物中一种必不可少且高度保守的细胞内组分降解过程，通过溶酶体机制响应营养饥饿或其他恶劣环境以维持细胞稳态。根据自噬对底物是否具有选择性，可分为非选择自噬和选择性自噬。嗜热四膜虫有性生殖过程中亲本大核程序性降解（programmed nuclear death，PND）是一种独特的细胞核选择性自噬过程。自噬相关蛋白Atg8，Vps34是PND的重要调控因子。然而，其他自噬相关蛋白如何参与调控PND的分子机制还未知。通过同源序列比对，嗜热四膜虫中存在自噬相关蛋白Atg5，基因表达谱分析*ATG5*在有性生殖时期10 h有表达峰值，蛋白相互作用预测Atg5与Atg8–2存在相互作用，暗示*ATG5*可能参与调控PND过程。间接免疫荧光定位分析表明 Atg5在anlagen之前定位于细胞质中，anlagen时期Atg5定位于亲本大核上。经Cd2+诱导高效表达后，不影响四膜虫核发育形态以及亲本大核的正常酸化，但延缓了亲本大核的凝缩降解。anlagen时期，敲减*ATG5* 阻碍了亲本大核向细胞底部的迁移，同时亲本大核无法正常凝缩降解，无法正常酸化，有性生殖36 h时仍存在未降解的亲本大核。上述结果表明Atg5参与调控亲本大核PND过程，与亲本大核向细胞底端的移动以及酸化相关。

关键词：自噬；四膜虫；亲本大核程序性降解；*ATG5*

A-3-044 Ionic protein–lipid interactions at the plasma membrane regulate the structure and function of immunoreceptors

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The Adaptive lymphocytes express a panel of activating and inhibitory immunoreceptors on their cell surface. Phospholipids are the major components of cell membranes, but they have functional roles beyond forming lipid bilayers. In particular, acidic phospholipids form microdomains in the plasma membrane and can ionically interact with proteins via polybasic sequences, which may have functional consequences for the protein. We have shown in our previous studies that negatively charged acidic phospholipids can interact with positively charged juxtamembrane polybasic regions of immunoreceptors, such as TCR CD3ε, CD28 and IgG-BCR, to regulate protein structure and function. Furthermore, we pay our attention to protein transmembrane domains (TMDs). TMDs are generally hydrophobic but our bioinformatics analysis targeting TMDs shows that many TMDs contain basic residues at terminal regions. Taking the integrin as an example, we showed that a membrane-snorkeling Lys residue in integrin αLβ2 (also known as LFA-1) regulates transmembrane heterodimer formation and integrin adhesion through ionic interplay with acidic phospholipids and calcium ions (Ca^{2+}) in T cells, thus providing a new mechanism of integrin activation. Here, we will review our recent progress showcasing the importance of both intramembrane and juxtamembrane ionic protein–lipid interactions.

Key Words: ionic protein–lipid interaction, immunoreceptors, Ca^{2+}

A-3-045 Structural insights into the Ragulator- and EGO-TC-mediated membrane tethering of the mTORC1-regulatory Rag GTPases

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The Rag/Gtr GTPases serve as a central module in the nutrient-sensing signaling network upstream of TORC1. In human, the nutrient-activated Rag heterodimer binds to mTORC1 to promote its recruitment to lysosomal membranes, where it is in a position to interact with the small GTPase Rheb that promotes its kinase activity. The Rag GTPases are recruited to lysosomes via the scaffold Ragulator/LAMTOR complex, consisting of p18, MP1, p14, HBXIP and C7orf59. In yeast, Gtr1 and Gtr2 are orthologs of mammalian Rag GTPases, which also form a functional obligate heterodimer. The anchoring of Gtr1-Gtr2 to membranes depends on the Ego1-Ego2-Ego3 ternary complex (EGO-TC), resulting in an EGO-TC-Gtr1-Gtr2 complex (EGOC). Intriguingly, EGO-TC and human Ragulator share no obvious sequence similarities and also differ in their composition with respect to the number of known subunits. Here, we report the structures of Ragulator and EGOC. The functional roles of key residues involved in the assembly are validated by in vivo assays. Our structural and functional data combined demonstrate that Ragulator-Rag and EGOC are structurally conserved and that Ragulator and EGO-TC are essential to recruit Rag GTPase to membranes to ensure appropriate TORC1 activation.

Key Words: mTORC1, crystal structure, protein complex

A-3-046 Osteopontin splicing isoform-c promotes the survival from 5-Fu dosing in colorectal cancer cells with a dependence to MeCP2 phosphorylation

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Abstract: The resistance to nutrient deprivation or chemotherapy in tumor cells involved cell communication and microenvironmental factors. The cytokine-like protein osteopontin (OPN) is suggested as a secretory signal molecule to promote colorectal cancer (CRC) progression. This study aimed to investigate the regulation and the effects of OPN splicing isoforms in CRC cells of differed chemo-sensitivities. Results showed that OPNc, one of the three major splicing isoforms, was significantly increased in CRC cells treated with 5-fluorouracil (5-Fu) as demonstrated in the percentage of OPNc to OPNt (total OPN). Overexpression of OPNc promoted the survivals with the increased resistance to 5-Fu in HCT-8 and HT115 cells. A similar response was also observed in cells cultured in OPNc condition medium. Treatments of 5-Fu or OPNc condition medium also increased the levels of nucleus Ca^{2+} concentration indicated by a genetically engineered fluorescent calcium indicator. The subsequent activation of CaMKII kinase lead to MeCP2 phosphorylation at the S421 residue, thus reduced its binding to *opn* gene, and eventually increased the skipping of OPN exon 5 to form OPNc. By the supplement of OPN neutralizing antibody in cultural medium, the 5-Fu induced nuclear Ca^{2+} was dramatically attenuated, suggesting the cellular functions of OPN might related to Ca^{2+} dependent nuclear signaling. Collectively, our data implied that OPNc could be a fast-responsive and horizontal transmissible factor to facilitate tumor survival and adaptation to stress conditions by influencing intercellular communication.

Key Words: colorectal cancer, OPN splicing isoform, 5-Fu, nuclear Ca^{2+}, MeCP2

A-3-047 Mydgf在心肌再生过程中的作用及机制研究

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心肌再生是目前治疗心力衰竭并提高预后的有效途径之一。新生小鼠具有强大的心肌再生能力，为研究哺乳动物心肌再生提供理想模型。已有研究表明，巨噬细胞在新生小鼠心肌再生过程中发挥重要作用。Myeloid-derived growth factor（Mydgf）为骨髓来源的单核巨噬细胞产生的旁分泌蛋白。然而，巨噬细胞是否通过分泌Mydgf参与调控心脏再生尚无报道。首先，我们检测了C57BL/6小鼠出生后不同时间点巨噬细胞中Mydgf的表达，结果发现，随着小鼠年龄增长Mydgf表达下降，表明Mydgf可能与心肌细胞增殖能力丢失相关。随后我们对新生1天小鼠进行心尖切除手术（AR），结果发现，与对照组相比，AR后显著激活巨噬细胞中Mydgf的表达，表明Mydgf可能参与新生小鼠心肌再生过程。接着我们引进Mydgf-KO小鼠，并在出生后1天进行AR，术后7天检测心肌细胞增殖情况，结果发现Mydgf-KO鼠心肌细胞增殖能力（$pH3^+$、$Ki67^+$的心肌细胞）显著下降；术后21天心动超声显示心功能显著降低，并取心脏进行MASSON染色显示心尖不能再生；此外，我们还构建成年小鼠心肌梗死手术（MI），给予Mydgf重组蛋白处理，术后21天通过心动超声和MASSON染色检测心功能及心肌纤维化情况，结果表明Mydgf处理可显著减小成年MI后的心肌纤维化程度，并改善心功能，说明Mydgf可以调控心肌再生及损伤修复。体外分离新生小鼠原代心肌细胞给予Mydgf处理16小时，免疫荧光染色显示心肌细胞增殖能力显著增强；将细胞RNA送去RNA-seq，对RNA-seq结果分析得出共有1005个差异基因表达，其中有707个基因表达上调，298个基因表达下调。对上调基因进行KEGG分析，显著富集于Cell cycle及PI3K-Akt信号通路。Western blot结果显示Cyclin D1、CDK2、CDK6、p-Akt蛋白水平明显上调。综上结果，我们发现，新生小鼠心肌损伤后，巨噬细胞通过分泌Mydgf，激活心肌细胞中PI3K-Akt信号通路促进心肌细胞增殖，进而调控新生小鼠心肌再生。

关键词：心肌再生；心尖切除；Mydgf；心肌细胞增殖

A-3-048 MLLT11 Upregulation Promotes Cancer Cell Growth and Metastasis in Bladder Cancer

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MLLT11, also named ALL1-fused from chromosome 1q (AF1q), was first identified from acute myeloid leukemia (AML) and located on chromosome band 1q21. Our recent study has demonstrated that MLLT11 played a significant role in a cancer progress that miR-411 downregulation enhanced tumor growth in human bladder cancer (BC). However, so far it is not clear precisely that the depth biological function of MLLT11 in BC. Here, we found that MLLT11 was dramatically upregulated in major of bladder cancer patients, which was significantly poorer overall survival (OS) that based on the TCGA database. Moreover, MLLT11 overexpression was observed in n-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN)-induced BCs. The same is true for evaluation of the expression of MLLT11 in human BC tissues and cell lines. In addition, knock out and knock down MLLT11 markedly inhibited BC cell proliferation and metastasis *in vitro* and *in vivo*. Mechanistically, knock out MLLT11 on the one hand upregulated transcription factor c-Jun expression in turn promoted p21 expression and resulted in cycle arrest at the G2/M phase, further hindered BC tumor growth, on the other hand inhibited BC cell invasion by downregulation of MMP2 expression and inhibited BC cell migration through accelerating RhoC protein degradation respectively. In conclusion, our studies make people have a better understanding of the role of MLLT11 in human BC development and suggest MLLT11 acts as a novel target for treatment and prognosis of BC patients.

Key words: MLLT11, bladder cancer, cell growth, metastasis

A-3-049 RYBP-PRC1复合物建立与维持H2AK119ub1的表观遗传机制

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在细胞的自我更新过程中，维持细胞自身性状的稳定需要表观遗传信息的精准继承。多梳抑制复合物参与了兼性异染色质区域的建立与维持，对于细胞的命运决定发挥了重要的作用。多梳抑制复合物主要分为PRC1和PRC2两类复合物。PRC2以正反馈的方式介导H3K27me3的建立和维持。然而，PRC1是如何在细胞内建立和维持H2AK119ub1的目前还不清楚。我们研究发现RYBP–PRC1的RYBP亚基能够识别H2AK119ub1并建立正反馈回路，通过该机制使细胞内的H2AK119ub1维持在正常水平。有趣的是，我们还发现H1通过调节相临核小体的距离促进了RYBP–PRC1介导的H2AK119ub1在染色质上的蔓延。另一方面，我们利用TetO–TetR targeting系统发现，来源于亲代的H2AK119ub1可以作为表观遗传种子，指导子代细胞染色质上H2AK119ub1的建立。利用体外分化模型，我们发现H1和RYBP–PRC1依赖的H2AK119ub1对于神经分化至关重用。总之，我们的研究首次阐明了H2AK119ub1在细胞内建立、维持以及遗传的具体机制，该研究为深入理解表观遗传的分子机理奠定了理论基础。

关键字：RYBP–PRC1；组蛋白H1；H2AK119ub1；表观遗传，染色质结构

A-3-050 Structural insights into the activation of ubiquitin-specific protease 46 by WDR48 and WDR20

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Ubiquitination is an important and reversible post-translational modification that regulates the stability, localization and function of proteins in many cellular processes. Deubiquitinases are responsible for the removal of ubiquitin chains from proteins, and ubiquitin-specific proteases (USPs) are the largest family of deubiquitinases, some of which are regulated by WD40-repeat proteins. The deubiquitinating activity of USP46 can be activated by WDR48 and WDR20; however, the molecular mechanism remains elusive. We determined the crystal structure of the USP46-WDR48-WDR20 complex at 3.1 Å resolution and validated the functional roles of key residues involved in the assembly of the complex with both *in vitro* and *in vivo* functional assays. The structural and functional data demonstrate that the binding of WDR48 and WDR20 can activate the activity of USP46 independently and synergistically and plays an indispensible role in USP46-mediated deubiquitination of PHLPP1 in the Akt signaling pathway. Detailed comparison of all available USP46 and USP12 structures reveals that the WDR48 binding not only stabilizes the Fingers subdomain but also increases conformational flexibility of several structural elements surrounding the catalytic center; and the combined effects render a moderate activation of the USPs. The further binding of WDR20 largely restores the WDR48-binding induced conformational changes and stabilizes the conformations of those structural elements surrounding the catalytic center, and thus potentiates the activity of the USPs. These results provide new insights into the molecular mechanism of the activation of USPs by WD40-repeat proteins.

Key words: Ubiquitination, deubiquitinases, ubiquitin-specific proteases, USP46, WD40-repeat protein, allosteric regulation

A-3-051 Structural insight into human N6amt1-Trm112 complex functioning as a protein methyltransferase

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Keywords: N6amt1, Trm112, 6mA modification, DNA methyltransferase, protein methyltransferase, substrate specificity.

DNA methylation is an important epigenetic modification in many organisms and can occur on cytosine or adenine. N6-methyladenine (6mA) exists widespreadly in bacterial genomes, which plays a vital role in the bacterial restriction-modification system. Recently, 6mA has also been reported to exist in the genomes of a variety of eukaryotes from unicellular organisms to metazoans. There were controversial reports on whether human N6amt1, which was originally reported as a glutamine MTase for eRF1, is a putative 6mA DNA MTase. We report here the crystal structure of human N6amt1-Trm112 in complex with cofactor SAM. Structural analysis shows that Trm112 binds to a hydrophobic surface of N6amt1 to stabilize its structure but does not directly contribute to substrate binding and catalysis. The active site and potential substrate-binding site of N6amt1 are dominantly negatively charged and thus are unsuitable for DNA binding. The biochemical data confirm that the complex cannot bind DNA and has no MTase activity for DNA, but exhibits activity for the methylation of Gln185 of eRF1. Our structural and biochemical data together demonstrate that N6amt1 is a bona fide protein MTase rather than a DNA MTase.

A-3-052 A novel regulator of hepatic insulin signaling: extracellular thioredoxin

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Type-2 diabetes is a metabolic disease. This study has revealed that the activity of serum thioredoxin (Trx) appears as a good biomarker for type-2 diabetes. To address the underlying mechanism, we considered the effect of extracellular Trx on hepatic insulin signaling. Here, we report that extracellular Trx acts as a double-edged sword for liver insulin signaling, depending on the level ratio of extracellular and intracellular Trx. Under the conditions with type-2 diabetes/insulin resistance, the presence of increased serum Trx and decreased liver Trx causes the negative effect on insulin signaling, which results from an increase in extracellular Trx entry via insulin receptor, the latter induces upregulation of PTP1B. We have demonstrated that extracellular Trx-induced upregulation of PTP1B expression is mediated by insulin receptor and not by Trx itself. In contrast, under the healthy conditions with normal levels of serum and liver Trx, the amount of extracellular Trx entering the cells is less, and the role of extracellular Trx is involved in promoting insulin binding to its receptor, leading to an increase in phosphorylation of insulin receptor β subunit/AKT and glucose uptake. Our findings provide a new mechanism for regulation of hepatic insulin signaling by extracellular Trx.

Key words: glucose, insulin signaling, PTP1B, thioredoxin, type-2 diabetes

A-3-053 The saponin D39 blocks dissociation of nonmuscular myosin heavy chain IIA from TNF receptor 2, suppressing tissue factor expression and venous thrombosis

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Non-muscular myosin heavy chain IIA (NMMHC IIA) plays a key role in tissue factor expression and venous thrombosis. Natural products might inhibit thrombosis through effects on NMMHC IIA. Here, we have shown that a natural saponin, D39, from Liriope muscari exerted anti-thrombotic activity in vivo, by targeting NMMHC IIA. Expression and activity of tissue factor in endothelial cells were analysed in vitro by Western blot and simplified chromogenic assays. Interactions between D39 and NMMHC IIA were assessed by serial affinity chromatography and molecular docking analysis. D39-dependent interactions between NMMHC IIA and TNF receptor 2 (TNFR2) were measured by immunofluorescence, co-immunoprecipitation and proximity ligation assays. Anti-thrombotic activity of D39 in vivo was evaluated with a model of inferior vena cava ligation injury in mice. D39 inhibited tissue factor expression and procoagulant activities in HUVECs and decreased thrombus weight in inferior vena cava-ligated mice dose-dependently. Serial affinity chromatography and molecular docking analysis suggested that D39 bound to NMMHC IIA. In HEK293T cells, D39 inhibited tissue factor expression evoked by NMMHC IIA overexpression. This effect was blocked by NMMHC IIA knockdown in HUVECs. D39 inhibited dissociation of NMMHC IIA from TNFR2, which subsequently modulated the Akt/GSK3β–NF-κB signalling pathways. D39 inhibited tissue factor expression and thrombus formation by modulating the Akt/GSK3β and NF-κB signalling pathways through NMMHC IIA. We identified a new natural product that targeted NMMHC IIA, as a potential treatment for thrombotic disorders and other vasculopathies.

Key Words: nonmuscular myosin heavy chain IIA, TNF receptor 2, saponin D39, tissue factor, venous thrombosis, signalling pathways

A-3-054 The N-end rule ubiquitin ligase UBR2 mediates NLRP1B inflammasome activation by anthrax lethal toxin

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Anthrax lethal toxin (LT) is known to induce NLRP1B inflammasome activation and pyroptotic cell death in macrophages from certain mouse strains in its metalloprotease activity-dependent manner, but the underlying mechanism is unknown. Here we establish a simple but robust cell system bearing dual fluorescence reporters for LT-induced ASC specks formation and pyroptotic lysis. A genome-wide siRNA screen and a CRISPR-Cas9 knockout screen were applied to this system for identifying genes involved in LT-induced inflammasome activation. UBR2, an E3 ubiquitin ligase of the N-end rule degradation pathway, was found to be required for LT-induced NLRP1B inflammasome activation. LT is known to cleave NLRP1B after Lys-44. The cleaved NLRP1B, bearing an N-terminal leucine, was targeted by UBR2-mediated ubiquitination and degradation. UBR2 partnered with an E2 ubiquitin-conjugating enzyme UBE2O in this process. NLRP1B underwent constitutive autocleavage before the C-terminal CARD domain. UBR2-mediated degradation of LT-cleaved NLRP1B thus triggered release of the noncovalent-bound CARD domain for subsequent capase-1 activation. Our study illustrates a unique mode of inflammasome activation in cytosolic defense against bacterial insults.

Key Words: Anthrax lethal toxin, N-end rule pathway, NLRP1B inflammasome, UBR2

A-3-055 An insight into the biological function of a highly conserved C-terminal domain of Indian hedgehog N-fragment

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Indian hedgehog (Ihh) is a highly conserved secreted signaling protein involved in regulating cartilage formation and development. Under physiological conditions, Ihh is synthesized as a precursor that undergoes auto-processing into the highly conserved *N*-terminal domain (IhhN), which is responsible for the signaling activities by forming multimers, and the C-terminal domain (IhhC), catalyzing auto-processing reaction. However, there has been little study for the functions of the C-terminus of IhhN although it is near the self cleavage site and highly conserved at various homologs. In this study, we mutated certain amino acids of the C-terminus and found that four amino acids including K191, S192, E193 and H194, acting as a domain, are essential for Ihh self cleavage. In addition, S195, A196 and A197 are important for the formation of the stable multimers. All results suggest that the C-terminus of IhhN plays an important role in the physiological function of Ihh, and these findings contribute to a better understanding of the biochemical properties of the Ihh and Hedgehog protein families.

Key Words: Indian hedgehog signaling pathway, C-terminus of IhhN, Biological function, Self cleavage, Formation of multimer

A-3-056 TCAB1 induces Epithelial-to-mesenchymal transition by recruiting USP11 deubiquitized TGFβR2 to activate TGFβ pathway

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TCAB1 (Telomere Cajal Body protein 1) is an important component of Cajal body. It participates in telomere elongation, repairs DNA double strands break and promotes tumor development. Its high expression is related to cell proliferation and poor prognosis of patients. However, little is known about the mechanism through which it regulates cancer progression. Our team found that overexpression of TCAB1 can promote the proliferation and migration of A549 cells. Conversely, downregulation of TCAB1 inhibited cell proliferation and migration of A549 cells. GST-Pulldown and Co-IP experiments showed that TCAB1 interacted with USP11 in cells. USP11（Ubiquitin-specific peptidase11）is a deubiquitinase, which can activate the TGFβ pathway and promote EMT by stabilizing TGFβR Ⅱ. Moreover, we found that overexpression of TCAB1 increased the protein level of intracellular TGFβR Ⅱ. Our research group will further examine the effect of TCAB1 on the activity of USP11 deubiquitinase through in vitro deubiquitination experiments, and detect the mechanism of TCAB1 on EMT. Our study provides evidence that TCAB1 promotes tumor migration by activating TGFβ pathway and suggests that downregulating TCAB1 may be a promising therapeutic approach for cancer treatment.

Key Words: TCAB1, USP11, TGFβR Ⅱ, EMT

A-3-057 SIRT6 Inhibitor, OSS_128167 Restricts HBV Transcription and Replication through Targeting Transcription Factor PPARα

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Hepatitis B virus (HBV) is a major public health threat and leading to end-stage liver diseases, such as hepatitis and hepatocellular carcinoma. Current FDA approved therapeutic for chronic HBV infection (CHB) are limited to nucleos(t)ides (NAs) and interferon alfa (IFNα). NAs decrease HBV DNA level markedly by inhibiting viral reverse transcription. However, long-term use of these NAs often results in generation of drug-resistant virus. IFNα is showed to be useful in part of individuals, but has serious side effects. Therefore, identification of new agents will contribute to the development of curative therapies for CHB. To identify the effective molecular, we screened 3000 compounds from a small molecular compound library and focused on OSS-128167, a selective inhibitor of Sirtuin 6 (SIRT6). In this study, we revealed that OSS_128167 could decrease the level of HBV core DNA and 3.5-Kb RNA in vitro. Furthermore, OSS_128167 administration to HBV transgenic mice also markedly suppressed hepatic HBV DNA and 3.5-Kb RNA level. Then, we explored the underlining mechanism. We found that depletion of SIRT6 inhibited HBV transcription and replication in HepG2.2.15 and HBV-infected HepG2-NTCP cells, whereas overexpression of SIRT6 enhanced HBV transcription and replication. In addition, HBV core promoter was significantly activated by SIRT6 through upregulating peroxisome proliferator-activated receptors α (PPARα) expression. Consistently, silence of PPARα relieved the enhancement of HBV transcription induced by SIRT6. Notably, our data suggest that OSS_128167 may serve as a potent therapeutic for treatment of HBV.

Key Words: OSS_128167, SIRT6, HBV, antiviral, core promoter, PPARα

A-3-058 Molecular Mechanisms and Therapy Strategies of AGR2 induced Chemoresistance

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Malignant tumors are serious diseases that seriously threaten human health. The treatment is mainly based on surgical treatment, radiation therapy, chemotherapy and immunotherapy in recent years. The low selectivity of drug resistance and precision medication is a difficult problem to control. Anterior gradient 2 (AGR2), an endoplasmic reticulum (ER)-resident protein-disulfide isomerase (PDI), is associated to cancer development and malignant progression. There are few studies on AGR2 and tumor resistance. Screening AGR2 expression and sensitivity to cytotoxic chemotherapy drugs and targeted drugs of tumor cells, we show that low level of AGR2 promotes the Multi-drug resistant phenotype of prostate cancer and lung cancer. We found that both intracellular and secreted AGR2 participated in drug resistance through different mechanisms. Firstly, extracellular AGR2 directly interacts with VEGFA to promote angiogenesis and resistance to bevacizumab in prostate cancer. Importantly, GSH and cabozantinib effectively blocked the pro-angiogenic effect of rhAGR2 in vitro and in vivo, providing evidence that secreted AGR2 acts as a predictive biomarker for selection of angiogenesis-targeting therapeutic drugs based on its levels in the circular system. We also provided evidence identifying intracellular AGR2 negatively regulates MRP2 expression and mediates multidrug resistance of tumor chemotherapy. Molecular mechanism indicates AGR2 promote MRP2 endocytosis and degradation through promote its ubiquitination. In addition, AGR2 negatively regulates the proteasome activity of tumor cells through regulates the proteasome RP-CP association, and mediates tumor cell sensitive to proteasome inhibitor Bortezomib. Finally, we provide a Docetaxel and Bortezomib sequential therapy strategy to overcomes acquired resistance in Chemotherapy. Those founds highlighting the importance of AGR2 as a predictive marker for selection of subgroup patients in chemotherapy.

Key Words: AGR2, disulfide isomerase, multidrug resistance, angiogenesis, proteasome activity

A-3-059 REGγ Ablation Impedes Dedifferentiation of Anaplastic Thyroid Carcinoma and Accentuates Radio-therapeutic Response by regulating Smad7-TGF-β pathway

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Anaplastic thyroid cancer (ATC) is the most aggressive human thyroid malignancy, characterized by dedifferentiation and resistance to radioiodine therapy. The underlying mechanisms regulating ATC dedifferentiation are largely unknown. Here, we show that REGγ, a non-canonical proteasome activator highly expressed in ATC, is an important regulator of differentiation in ATC cells. Ablation of REGγ significantly restored expression of thyroid specific genes, enhanced iodine uptake, and improved the efficacy of ^{131}I therapy in ATC xenograft models. Mechanistically, REGγ directly binds to the TGF-β signaling antagonist Smad7 and promotes its degradation, leading to the activation of TGF-β signal pathway. With gain- and loss-of-function studies, we demonstrate that Smad7 is an important mediator for the REGγ function in ATC cell dedifferentiation and metastasis, which is supported by expression profiles in human ATC tissues. It seems that REGγ impinges on repression of thyroid-specific genes and promotion of tumor malignancy in ATC cells by activating TGF-β signal pathway via degradation

of Smad7. Thus, REGγ may serve as a novel therapeutic target for allowing radioiodine therapy in anaplastic thyroid cancer patients with poor prognosis.

Keywords: REGγ, anaplastic thyroid cancer, NIS, radioiodine therapy, TGF-β, Smad7

A-3-060 LUTI：从亚麻籽中分离得到的一种双功能抑制剂

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餐后的急性葡萄糖波动会增大患2型糖尿病（T2DM）的风险。由于α-葡萄糖苷酶抑制剂可阻碍碳水化合物降解为葡萄糖，因此被广泛用于控制高血糖症。常见的α-葡萄糖苷酶抑制剂有阿卡波糖、伏格列波糖、米格列醇和二甲双胍等。然而，它们经常导致胃肠道副作用，例如胃肠胀气和腹泻。因此，迫切需要更安全和更有效的α-葡萄糖苷酶抑制剂。

在本研究中，使用Q-Sepharose4B柱一步纯化，然后使用Sephacryl S-200排阻色谱法分离得到来自亚麻（*Linum usitatissimum*）种子的具有α-葡萄糖苷酶抑制活性的蛋白质。它被鉴定为胰蛋白酶抑制剂，命名为LUTI（*Linum usitatissimum* trypsin inhibitor）。经抑制酶活测定分析，LUTI对α-葡萄糖苷酶的半抑制浓度（IC_{50}）为113.92μM，对胰蛋白酶的IC50为6.17μM。 Lineweaver-Burk动力学实验表明，该蛋白质表现出两种不同的抑制模式，即α-葡萄糖苷酶的竞争性抑制剂类型和胰蛋白酶的非竞争性抑制剂类型。并通过凝胶过滤色谱和动态光散射（DLS）检测LUTI和α-葡萄糖苷酶之间的相互作用。用LUTI处理Caco-2和HepG2细胞后也观察到LUTI促进细胞中乳酸的生成以及葡萄糖的消耗。LUTI不仅抑制胰蛋白酶的活性而且抑制α-葡萄糖苷酶的活性。因此，LUTI有望成为潜在的T2DM的口服降血糖多肽药物。

关键词：LUTI； α-葡萄糖苷酶抑制剂；双功能抑制剂

A-3-061 Reductive stress induced by Ero1α S-nitrosation in endoplasmic reticulum accelerates cell senescence

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Redox homeostasis in cells is crucial for the function of biomacromolecules. Oxidative stress is known to mediate many cellular signal transduction in physiological and pathological processes, however, the biological effects and mechanisms of reductive stress (RS, an abnormal increase in electron pressure or reducing equivalents (GSH/GSSG; $NADH/NAD^+$; $NADPH/NADP^+$) are still poorly understood. In this study, we found that the ER exhibited reductive stress in senescent cells, and reductive stress accelerated senescence. Further study showed that the decrease of Ero1α activity is an important cause of ER reductive stress in the process of aging, and delaying aging was successfully achieved by increasing the oxidative power in the ER. For mechanism study, we found the Ero1α S-nitrosation increased in senescence cells. Ero1α C166 and C131 were identified as the site of S-nitrosation and result in decreasing of Ero1α activity. Moreover, we found that ER reductive stress led to protein homeostatic imbalance and decreased ER folding ability which accelerated aging. Our results suggested a new mechanism of aging caused by insufficient oxidation in the ER.

Key Words: Reductive Stress, ER, Ero1α, S-nitrosation, Cell senescence

A-3-062 Protein lysine de-2-hyroxyisobutyrylation by CobB in prokaryotes

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Abstract: The novel post-translational modification, lysine 2-hydroxyisobutyrylation (Khib), has recently been thought to be an evolutionarily conserved histone mark. Here we report a comprehensive identification of Khib in Proteus mirabilis (*P. mirabilis*). 4735 2-hydroxyisobutyrylation sites that were identified on 1051 proteins in *P. mirabilis* were characterized in abundance, distribution and functions.1 Furthermore, we demonstrate that CobB serves as a lysine de-2-hydroxyisobutyrylation enzyme that regulates glycolysis and cell growth in prokaryotes, for the first time. We identified the specific binding of CobB to Khib using a novel self-assembled multivalent photo-crosslinking peptide probe,2 demonstrated that CobB can catalyze lysine de-2-hyroxyisobutyrylation both in vivo and in vitro. The R58 of CobB is a critical site for its lysine de-2-hyroxyisobutyrylase activity. Using quantitative proteomics approach, we identified 99 endogenous substrates that are targeted by CobB for de-2-hyroxyisobutyrylation. We further revealed that CobB can regulate catalytic activities of ENO by removing the K343hib and K326ac of ENO simultaneously, which account for changes of bacterial growth. In brief, we systematically identified Khib in prokaryotes and dissected a Khib-mediated molecular mechanism that is catalyzed by CobB for the regulation of the activity of metabolic enzymes as well as the cell growth of bacteria.

Key Words: Post-translational modification, Lysine 2-hydroxyisobutyrylation, Enzyme, CobB, Biochemistry

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A-3-063 利用CRISPR-Cas9全基因组文库筛选奥西替尼耐药基因FDX1

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摘要：目的 利用非小细胞肺癌细胞研究对奥西替尼潜在耐药基因。**方法** 首先利用CRISPR-Cas9全基因组文库在人非小细胞肺癌细胞NCI-H1975中筛选出奥西替尼潜在耐药铁氧还蛋白1（FDX1）基因，建立敲除FDX1和对照AAVS1表达的sg-FDX1及sg-AAVS1稳定细胞系。提取 sg-FDX1 细胞基因组并且 PCR 扩增出sgRNA序列两侧的 DNA 序列，连接到 T载体后进行测序；Western-blot检测sg-FDX1和sg-AAVS1细胞中FDX1蛋白表达水平；将构建的sg-FDX1和sg-AAVS1细胞分别用不同浓度奥西替尼培养30 h后，Western印迹法检测细胞中c-PARP蛋白表达水平以及EGFR和ERK磷酸化水平。采用平板克隆和CCK8实验检测肿瘤细胞的存活率。**结果** 测序结果表明，sg-FDX1细胞中的FDX1基因发生缺失，同时FDX1蛋白表达水平被有效抑制；和sg-AAVS1细胞相比，50 nmol•L-1奥西替尼处理后的sg-FDX1 细胞中c-PARP 表达水平显著降低（P<0.01）；奥西替尼在sg-FDX1 细胞中对 p-EGFR 和 p-ERK 蛋白表达抑制作用相对于 sg-AAVS1 细胞显著降低（P<0.01）。克隆形成和CCK8实验表明，相对于sg-AAVS1细胞，奥西替尼

对sg–FDX1细胞的克隆形成率和增殖抑制作用显著降低（P<0.05）。结论 NCI–H1975细胞敲除 FDX1，减弱了奥西替尼对细胞凋亡的诱导功能，可能是FDX1参与NCI–H1975细胞对奥西替尼耐药机制之一。

关键词：CRISPR–Cas9文库；奥西替尼；非小细胞肺癌；FDX1

A-3-064 Arginine methylation of SIRT7 couples glucose sensing with mitochondria biogenesis

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Sirtuins (SIRTs) are a class of lysine deacylases that regulate cellular metabolism and energy homeostasis. Although sirtuins have been proposed to function in nutrient sensing and signaling, the underlying mechanism remains elusive. SIRT7, a histone H3K18-specific deacetylase, epigenetically controls mitochondria biogenesis, ribosomal biosynthesis, and DNA repair. Here, we report that SIRT7 is methylated at arginine 388 (R388), which inhibits its H3K18 deacetylase activity. Protein arginine methyltransferase 6 (PRMT6) directly interacts with and methylates SIRT7 at R388 *in vitro* and *in vivo*. R388 methylation suppresses the H3K18 deacetylase activity of SIRT7 without modulating its subcellular localization. PRMT6-induced H3K18 hyperacetylation at SIRT7-target gene promoter epigenetically promotes mitochondria biogenesis and maintains mitochondria respiration. Moreover, high glucose enhances R388 methylation in mouse fibroblasts and liver tissue. PRMT6 signals glucose availability to SIRT7 in an AMPK-dependent manner. AMPK induces R388 hypomethylation by disrupting the association between PRMT6 and SIRT7. Together, PRMT6-induced arginine methylation of SIRT7 coordinates glucose availability with mitochondria biogenesis to maintain energy homeostasis. Our study uncovers the regulatory role of SIRT7 arginine methylation in glucose sensing and mitochondria biogenesis.

Key Words: arginine methylation, glucose sensing, mitochondria biogenesis, PRMT6, SIRT7

A-3-065 Structural Insights into Repression of the *Pneumococcal* Fatty Acid Synthesis Pathway by Repressor FabT and Co-repressor Acyl-ACP

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The *Streptococcus pneumoniae* fatty acid synthesis pathway is globally controlled at the transcriptional level by repressor FabT and its co-repressor acyl cattier protein (acyl-ACP), the intermediate of phospholipid synthesis. Here, we report the crystal structure of FabT complexed with a 23-bp dsDNA, which indicates that FabT is a weak repressor of low DNA-binding affinity in the absence of acyl-ACP. Modification of ACP with a long-chain fatty acid is necessary for the formation of a stable complex with FabT, mimicking *in vitro* by cross-linking, which significantly elevates the DNA-binding affinity of FabT. Altogether, we propose a putative working model of gene repression under the double control of FabT and acyl-ACP,

elucidating a distinct repression network for *Pneumococcus* to precisely coordinate fatty acid synthesis.

Key Words: co-repressor, fatty acid synthesis, repression, transcription repressor

A-3-066 Structure and genome investigations of two freshwater podoviridae cyanophages from Lake ChaoHu

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The Caudovirales, known as tailed bacteriophages, comprises the vast majority (>95%) of bacteriophages. They all have in common a proteinaceous head, which encloses a single linear double-stranded DNA, and a tail. Three families are distinguished by the morphology of their tail: Myoviridae (long contractile tail), Podoviridae (short noncontractile tail), and Siphoviridae (long flexible noncontractile tail). Cyanophages are bacteriophages that specifically infect their host cyanobacteria, which contribute to the carbon cycle of the water biosphere by infecting and degrading their hosts, and thus play an active role in water ecology. Cyanophages have evolved together with their host for 3 billion years. Studies of cyanophages would improve our understanding on the co-evolution between cyanobacteria and cyanophages. However, compared to the well-studied marine cyanophages, structural and genomic information of freshwater cyanophages is remain largely unknown. Here, we separated and identified two Podoviridae cyanophages with similar morphology, who are termed Pam1-1 and Pam1-2, respectively. We solved the capsid structure of Pam1-1 at the resolution of 3.6 angstrom, and analyzed the genome sequences of Pam1-1 and Pam1-2. Moreover, we will solve the structures of the tail and baseplate, and further figure out their co-evolution with host and the molecular mechanisms on self-assembly and host-phage recognition specificity.

Key Words: Podoviridae, cyanophage, genome, cryo-EM structure

A-3-067 Coordinated regulation on transcription of *nirA* operon via NtcA and NtcB in *Anabaena* sp.PCC7120

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Cyanobacteria are prokaryotes, also known as “blue-green algae”, which can execute photosynthesis and thus play an important role in the early formation of the Earth′s atmosphere. Cyanobacteria can use a variety of nitrogen sources, but they prefer to use ammonium. The nitrate assimilation pathway (termed *nirA* operon) begins when the environment is lack of ammonium.

NtcB, a LysR-type transcriptional regulator (LTTR), functions as a homo-tetramer, each subunit of which consists of two domains: an N-terminal DNA-binding domain (DBD) and a C-terminal effector-binding domain (EBD). NtcB regulates the genes involved in nitrate assimilation in *Anabaena* sp. PCC7120.

NtcA is a cAMP receptor protein (CRP), which is also known as catabolite activator protein (CAP). NtcA is a global transcription factor in *Anabaena* sp. PCC7120 and responds to nitrogen starvation signal 2-oxogluatarate (2-OG). NtcA binds to -41.5 upstream of the transcription start site of *nirA* operon, which belongs to the "class II" CRP-dependent promoter. In fact, the *nirA* operon is regulated by two transcription activators (NtcA and NtcB) and the cognate co-activators (2-OG and nitrite)

Key Words: coordinated regulation, NtcA, NtcB, transcription activation

A-3-068 抑制Sirt2-Atg4B（Acetylation）-autophagy通路增敏索拉非尼抗肝癌细胞的效应及机制研究

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索拉非尼作为FDA批准的首个肝癌分子靶向药物，其有效性和安全性得到广泛认可，但是也有相当一部分接受索拉非尼治疗的患者，不但没有确切的疗效，反而增加由此带来的副作用和沉重的经济负担。如何抑制肝癌细胞索拉非尼耐药的发生，提高其治疗效果，逐渐成为近年来人们关注的热点。在本研究中，我们发现索拉非尼诱导的保护性自噬是肝癌细胞对索拉非尼的抵抗的重要机制，我们首次证明了自噬相关蛋白ATG4B存在乙酰化修饰并参与调节ATG4B的功能活性，而这一现象参与并影响索拉非尼诱导的保护性自噬进程。索拉非尼处理过程中，ATG4B的乙酰化明显降低，我们通过药物筛选，RNA干扰筛选，定点突变技术筛选出SIRT2在ATG4B的第39、244和259位赖氨酸进行乙酰化修饰，进而影响ATG4B的功能活性。通过抑制Sirt2–Atg4B（Acetylation）–autophagy通路，能够抑制索拉非尼介导的以ATG4B乙酰化降低为特征的保护性自噬，从而增敏索拉非尼对肝癌细胞的杀伤性。在本研究中，我们首次确定了Sirt2–Atg4B（Acetylation）–autophagy信号通路在索拉非尼诱导肝癌细胞保护性自噬中的重要作用，进一步阐述了肝癌细胞对索拉非尼的药物机制，以ATG4B乙酰化修饰变化为靶点，为索拉非尼更好地应用肝癌的临床治疗提供了新的思路和依据。

关键词：肝癌；ATG4B；SIRT2；索拉非尼；乙酰化

A-3-069 33-kDa annexin A3 isoform promotes HCC and its knockdown suppresses tumorigenesis and malignancy via activating intrinsic apoptosis and suppressing ERK/I3K/Akt pathway

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Hepatocellular carcinoma (HCC) is the third leading cause of most common cancer-related deaths due to its higher metastasis, recurrence and chemoresistance. The applicable targets for its progression, drug-tolerance and prognosis are required. Annexin A3 (ANXA3) is a member of the group A family of annexins in vertebrates. It contains two isoforms with the mole masses of 36-kDa and 33-kDa. The majority of researches have been focused on the function and underlying mechanism of the overall ANXA3 especially the 36-kDa ANXA3 in carcinogenesis and drug resistance, the role of 33-kDa ANXA3 in is undistinguished. This work indicated that 33-kDa ANXA3 was significantly upregulated in tumorous tissues from HCC patients. The knockdown of 33-kDa ANXA3 decreased the *in vivo* tumor growing velocity and malignancy of HepG2 cells transplanted in nude mice. The in vitro experimental characterizations indicated the stable suppression of 33-kDa ANXA3 decreased the proliferation, colony forming, migration, invasion and angiogenesis abilities of HepG2 cells through decreasing

the expressions of Rap1b, Rac1, CRKL, pMEK1/2, pERK2, c-Myc in ERK pathway, deactivating pAkt and HIF-1α in PI3K/Akt pathway, and enhanced the apoptosis, resistances to cisplatin and 5-fluorouracil of HepG2 cells through intrinsic apoptosis signaling pathway. 33-kDa ANXA3 plays a vital role in HCC malignancy and chemoresistance. It is of potential use in diagnosis and treatment for HCC.

Key Words: HCC, 33-kDa ANXA3, tumorigenesis, chemo-resistance, apoptosis

A-3-070 CRKL suppression decreases the proliferation, invasion and imatinib resistance of CML cells

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BCR-ABL kinase activation triggers the downstream signaling molecules including phosphoinositide 3-kinase (PI3K)/Akt, FAK/Src, STAT5 and c-MYC. The constitutive phosphorylation of CRKL attributed by BCR-ABL in chronic myeloid leukemia cell line, K562, suggesting its important role of CRKL in BCR-ABL signaling transduction. In current work, we obtained the monoclonal stable knockdown of CRKL by over 90% in K562 cells using shRNA interference and screening against G418 through limited dilution method. CRKL knockdown decreased the proliferation, colony forming, migration and invasion capacities of K562 cells, which was interpreted by the decreased expressions of p-Akt, FAK, p-Src, MMP2, MMP9, p-STAT5 and c-Myc, and increased expression of E-cadherin in cells. CRKL suppression induced early apoptosis of K562 through reducing BCL-X expression. The induced imatinib sensitivity of K562 cells by CRKL knockdown was correlated with *BCRP* reduction. The expression level of another CRK family member, CRKII, was increased following CRKL knockdown in K562 cells, which might compensate the malfunction of CRKL. The current work indicates the upregulation of CRKL potentially through activating PI3K/Akt, FAK/Src, transcription factors STAT5 and c-MYC, and inhibiting E-cadherin and apoptosis of CML cells, promotes CML malignant progression and imatinib resistance. CRKL is of great use as a diagnosis and therapeutic molecule in CML.

Key Words: CML, CRKL, proliferation, migration, invasion, apoptosis

A-3-071 FAM129A overexpression promotes ccRCC clinical progression by enhancing patient's TNM stage and Fuhrman grade

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Clear cell renal cell carcinoma (ccRCC) is the most aggressive RCC subtype. It is characterized with high rates of local invasion and metastasis, resistances to chemotherapy and radiotherapy. The pathogenesis, diagnosis and treatment target of ccRCC needs more attention. The member A of family with sequence similarity 129 (FAM129A) was originally identified in Eker rats with hereditary renal carcinoma induced by tuberous sclerosis 2 gene mutation (Tsc2). It is upregulated in thyroid cancer, head and neck squamous cell carcinoma and sporadic renal carcinomas. *FAM129A* was detected in sporadic RCCs, clear cell, granular cell and spindle cell carcinomas, implicating its potential use as ab indicator for renal carcinogenesis, while its function remains unclear in ccRCC. This work aimed to establish the deregulation and clinical association of

FAM129A expression with ccRCC. *FAM129A* levels in 32 paired tumorous and paracancerous normal tissues form ccRCC patients were measured by qRT-PCR. Compared with paired paracancerous tissues, *FAM129A* level was globally increased in patients' cancerous tissues by ~3.2-fold (P=0.001). Among clinicopathology parameters, compared with localized ccRCC group patients with p(Ta1NOMO), *FAM129A* level in patients with p(T1b-T2NOMO) increased by ~171% (*P*=0.0116). Compared with localized ccRCC patients, *FAM129A* level in tumorous tissues from patients with advanced ccRCC increased by 166% (*P*=0.018). *FAM129A* expression level was increased by 201% in tumorous tissues from patients with Fuhrman>2 than patients with Fuhrman≤2 (*P*=0.019). Current work shows *FAM129A* acts as a promoting factor in ccRCC. Its overexpression enhances ccRCC progression by promoting TNM stage and Fuhrman grade of ccRCC patients.

Key Words: ccRCC, FAM129A, Development, Progression

专题四：糖脂生物学

A-4-001 The inhibitory effect of 17β-estradiol on triglyceride synthesis is dependent on ERα, not ERβ in skeletal muscle cells

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Abstract: The aim of the study was to examine the inhibitory effects and the related mechanisms of 17β-estradiol (E2) on triglyceride synthesis and insulin resistance in skeletal muscle tissues and cells. Six-month-old female rats were subjected to sham surgery (SHAM), ovariectomy (OVX) and ovariectomy plus E2 treatment (OVX+E2). The serum biochemical parameters, pathological changes in uterus, liver and skeletal muscle, triglyceride (TG) content in muscle were detected. Differentiated myotubes C2C12 were treated with palmitic acid (PA) or pretreated with E2, estrogen receptor (ER) α and ERβ agonist propylpyrazole triol (PPT) and diarylpropionitrile (DPN), expression of ERα and ERβ, peroxisome proliferator-activated receptor α (PPARα), CD36, fatty acid synthetase (FASN), adipose differentiation-related protein (ADRP), phosphorylated acetyl-CoA carboxylase (p-ACC), phosphorylated protein kinase B (p-AKT) and phosphorylated c-Jun N-terminal kinase (p-JNK) in skeletal muscle or C2C12 were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and western blotting, respectively. Results of the present study demonstrated that E2 had suppressive effect on OVX-induced body weight gain, muscle TG deposition and insulin resistance. ERα, CD36, PPARα, p-ACC and p-AKT expression were decreased, while ERβ, ADRP, FASN, and p-JNK expression were increased in the OVX group; meanwhile E2 treatment reverted their expressions. In C2C12 cells, similarly, E2 and PPT inverted the changes induced by PA treatment, yet DPN had the opposite effects. In conclusion, E2 could bind with ERα, thus activated CD36-PPARα pathway to reduce muscle TG content and to improve insulin resistance in skeletal muscle and cells.

Key words: estrogen, fatty acid metabolism, ovariectomy, skeletal muscle, estrogen receptor.

A-4-002 白藜芦醇对高脂饮食诱导的肥胖小鼠脂代谢及肠道微生物群的调控作用

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研究目的：天然抗氧化剂白藜芦醇的生物利用度低，食用后能到达结肠，可与肠道微生物生态系统发生作用。已有研究发现摄取高脂饮食的同时食用白藜芦醇可预防肠道微生物群紊乱。然而，白藜芦醇是否可重塑已因肥胖饮食引起肥胖小鼠的肠道微生物群，并减轻肥胖相关疾病仍有待研究。本研究旨在探讨白藜芦醇干预高脂诱导肥胖的作用，及其与氧化应激和肠道微生物群调节的关系。

研究方法：将雄性C57BL/6小鼠分为5组：正常对照组始终饲喂低脂对照饲料；另外4组为高脂饲料（HFD，60%能量由猪油提供）饲喂组，分别为HFD对照组和三个剂量白藜芦醇干预组（50、75和100mg/kg，饮水给予），高脂饲料喂养8周后给予8周的白藜芦醇干预。

研究结果：中、高剂量白藜芦醇干预显著抑制过度体增重、肝重指数和脂肪组织指数（$p<0.05$）。所有剂量显著抑制了HFD所致血清甘油三酯、低密度脂蛋白胆固醇、葡萄糖和内毒素异常升高（$p<0.05$）。中、高剂量干预也抑制了以血清IL-1和TNF-α过量为标志的慢性炎症（$p<0.05$），并通过诱导过氧化物酶歧化酶、过氧化氢酶和谷胱

甘肽过氧化物酶活性抑制肝脏和脑组织氧化应激（p<0.05）。所有剂量都抑制了HFD诱导组织脂质过氧化产物丙二醛升高（p<0.05）。相对于HFD对照组，中、高剂量白藜芦醇处理显著升高了肠道菌群α多样性（Chao1指数和香浓指数）（p<0.05）。对于β多样性分析，仅中剂量组引起了明显的菌群构成改变（PCA分析）。高剂量组显著抑制了HFD引起红蝽菌科（*Coriobacteriaceae*）和脱硫弧菌科（*Desulfovibrionaceae*）细菌过度增殖($p < 0.05$)。这两个科的细菌与体重呈显著正相关关系（$r > 0.8$， $p < 0.00$）。

结论： HFD诱导的肥胖小鼠饮用白藜芦醇后，内毒素血症、氧化应激和肠道菌群紊乱得到了抑制。

关键词：白藜芦醇；肥胖；肠道菌群；氧化应激

A-4-003　N-糖链在肿瘤干细胞中的功能和意义

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糖链是继核酸、多肽蛋白质后的第三条重要的生命分子链，而细胞内至少超过50%的蛋白质都是有糖链修饰的。糖链参与了包括细胞识别、细胞分化、发育、信号转导、免疫应答等各种重要生命活动。在肿瘤等人类现代重要疾病发生、发展中均伴随着蛋白质糖基化异常的发生，而糖基化异常调控肿瘤细胞恶性行为，并成为临床诊断和治疗的靶点。阐释N–糖链在肿瘤干细胞中的机制有助于明确肿瘤特性维持的分子机制。 本团队长期从事N–糖链、干细胞标志物CD133蛋白的糖链结构、功能和信号通路研究，如发现CD133具有唾液酸化修饰、CD133和Src、P85相互等促进肿瘤干细胞自我更新等（相关研究发表于PNAS、J Biol Chem、Mol Cell Biol等杂志）。本团队近期利用糖链结构分析平台，解析了肿瘤干细胞中的N–糖链结构特征，阐释合成该结构的关键糖基转移酶。发现在肝癌等干细胞中，主要是高甘露糖型糖链结构，包括CD133蛋白在肝癌干细胞中具有高甘露糖型结构。高甘露糖型糖链结构可以维持肝癌干细胞静态和抑制分化。并且高甘露糖型糖链结构可以通过和微环境中的其他细胞表面的甘露糖受体相互作用，促进IL–17的表达。IL–17通过和其受体的相互作用可以促进肿瘤干细胞自我更新和成瘤。抑制高甘露糖型聚糖和受体相互作用可以抑制IL–17的分泌。本研究阐释了N–糖链与凝集素相互作用的机制、 调控因素及其在肿瘤干细胞微环境中的作用与机制。 从而为阐释微环境调控干细胞命运的机制提供糖生物学思路。本研究获得国家自然基金（31770856、81773164和81472724）资助。

关键词：肿瘤干细胞; CD133蛋白; N–糖基化; 微环境

A-4-004　Sulfatide inhibits m6A methylation of MTF1 mRNA by interfering the m6A writer complex formation

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Metal responsive transcription factor 1 (MTF1) is a zinc dependent transcription factor which is involved in the regulation of intracellular signaling pathways. We noted that the expression and half-life of MTF1 mRNA were increased after sulfatide treatment, and elevated MTF1 expression was significantly associated with low survival rate in HCC patients. We also found that sulfatide inhibited the N6-methyladenosine (m6A) methylation of MTF1 mRNA by MeRIP-seq. As the m6A modification is reversible due to the interplay of methyltransferase (MTase) 'writer' and demethylase 'eraser' enzymes, with the methyl group was recognized by m6A 'reader' proteins. We further detected whether sulfatide influenced the expressions of m6A binding proteins (YTHDF1, YTHDF2, YTHDF3), the m6A methyltransferases (METTL3, METTL14 and WTAP) and the m6A demethylases (ALKBH5 and FTO). Interestingly, even though sulfatide made no difference in the m6A 'writers,

erasers or reader' expression, but inhibited the METTL3-METTL14-WTAP complex interaction. In conclusion, this study demonstrated that sulfatide may regulated MTF1 m6A modification by interfering the formation of m6A writer complex.

Key words: MTF1; sulfatide; HCC; m6A methylation; m6A writer complex

A-4-005　阿尔茨海默病外周血脂肪酸组成及代谢的变化

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为了探明脂肪酸组成及代谢与阿尔茨海默病（AD）发病的关系，本研究对比分析了正常老年人（正常组）和AD患者（AD组）血清脂肪酸成分、脂肪酸代谢相关分子等的变化。按照AD诊断标准及排除标准筛选研究对象（AD组和正常组各30例），空腹抽取外周静脉血，分离血清及单个核细胞用于实验。气相色谱法分析血清脂肪酸组成，Western blot方法分析单个核细胞线粒体β–氧化关键酶肉碱脂酰转移酶Ⅰ（CPT1）、过氧化物酶体β–氧化关键酶脂酰辅酶A氧化酶1（ACOX1）及过氧化物酶体增殖剂激活受体α（PPAR α）的蛋白表达。结果发现，与对照组相比，AD组总饱和极长链脂肪酸（C22:0 、C24:0和 C26:0之和）含量及C24:0含量升高（P<0.01，P<0.01），单不饱和脂肪酸C16:1升高（P<0.05），总多不饱和脂肪酸含量降低（P<0.05），PPAR α蛋白表达增多（P<0.05），而CPT1、ACOX1蛋白表达无明显变化（P>0.05）。结果表明AD患者脂肪酸组成及含量存在异常，血清饱和极长链脂肪酸含量升高，多不饱和脂肪酸含量降低，脂肪酸代谢调控因子PPARα表达升高。脂肪酸代谢异常可能是引发AD的原因之一。

关键词：阿尔茨海默病；脂肪酸组成；脂肪酸β–氧化；PPAR α

A-4-006　ANGPTL3 association with lipid levels in obstructive sleep apnea patients

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Background: Obstructive sleep apnea (OSA) is a common sleep problem. It has been observed that many lipid/lipoprotein abnormalities are prevalent in OSA. Angiopoietin-like protein 3(ANGPTL3) is well acknowledged as a key regulator of lipid metabolism. However, no studies have investigated the involvement of ANGPTL3 in OSA. The purpose of this study was to evaluate whether high levels of ANGPTL3 are markers of risk of OSA.

Methods: A total of 327 samples were used, including 271 OSA subjects and 56 controls. The concentration of serum ANGPTL3 was measured by enzyme-linked immunosorbent assay. Polysomnography was used to diagnose obstructive sleep apnea. The associations between ANGPTL3 and OSA were determined by multivariate regression analyses.

Results: Serum ANGPTL3 in patients with OSA was higher than that in the control group, and the difference was statistically significant (median: 40.25 ng/ml vs 29.48 ng/ml, respectively; P=0.001). After adjusting for confounding factors, circulating ANGPTL3 levels were an independent risk factor for OSA (odds ratio=1.022/ng ANGPTL3, 95% CI 1.001-1.044, P=0.04) and positively correlated with TC (r = 0.2111, P<0.001), TG (r = 0.2118, P<0.001) and LDL-C (r = 0.1576, P<0.001).

Conclusions: ANGPTL3 was significantly elevated in OSA and increased the risk of OSA.

Keywords: Obstructive sleep apnea, Angiopoietin-like protein 3, lipid metabolism

A-4-007　人参多糖与半乳凝素-3相互作用的分子机制

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人参多糖具有降血糖、抗疲劳、免疫调节和抗肿瘤等药理活性，但其作用的靶分子尚不清楚。我们前期工作显示，人参多糖能结合并且抑制半乳凝素-3（简称Gal-3）。Gal-3参与多种生理和病理过程，与肿瘤的发生、发展和转移密切相关，已经成为某些肿瘤的标志物并作为抗癌药物研发的靶点。本研究旨在深入探索人参多糖与Gal-3相互作用的分子机制。

人参多糖中含有不同种类和结构的多糖，为了研究分子互作的构效关系，我们首先对其系统地分离纯化，得到了一系列结构均一的级分；然后采用酸水解、酶解等生物化学实验方法，结合高效液相（HPLC）、离子色谱（HPAEC）以及核磁共振（1D/2D NMR）等仪器分析方法，研究了人参多糖各级分的结构。在此基础上，我们利用红细胞凝集阻抑、荧光偏振、等离子共振（SPR）、生物膜干涉（BLI）、微量热涌动（MST）等定性和定量分析方法，比较研究了各种人参多糖与Gal-3的亲和力，以及对Gal-3的抑制活性。研究结果如下：1）不同结构类型的人参多糖级分活性差别很大，RG-I型果胶是主要的活性成分，其中级分RG-I-4抑制Gal-3的活性最强；2）RG-I-4的高活性主要来源于侧链与侧链以及侧链与主链之间的协同作用；3）不仅含有半乳糖基的RG-I型果胶能结合Gal-3，不含半乳糖基的HG型果胶也能结合Gal-3；4）含有半乳糖基的多糖主要结合在CRD的S-face，即经典的糖结合位点。而不含半乳糖基的多糖主要结合在F-face，即在经典结合位点的背面；5）HG型和RG-I型果胶具有协同结合/抑制Gal-3的作用，其作用机制是HG和RG-I分子缔合导致更多的RG-I结合位点暴露于Gal-3的S-face。

本研究结果明确了人参多糖中抑制半乳凝素-3的活性成分及其结构特征，有助于阐明人参多糖的活性机理，为开发低毒、高效、选择性好的Gal-3抑制剂提供理论依据。

关键词：人参多糖；半乳凝素-3；分子互作；构效关系

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A-4-008　Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-Heptose

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Immune recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors often activates proinflammatory NF-kappaB signalling. Here we combined a transposon screen in *Yersinia pseudotuberculosis* with biochemical analyses and identified ADP-beta-D-manno-heptose (ADP-Hep), which mediates type III secretion system-

dependent NF-kappaB activation and cytokine expression. ADP-Hep, but not other heptose metabolites, could enter host cytosol to activate NF-kappaB. A CRISPR-Cas9 screen showed that activation of NF-kappaB by ADP-Hep involves an ALPK1 (alpha-kinase 1)-TIFA (TRAF-interacting protein with forkhead-associated domain) axis. ADP-Hep directly binds the N-terminal domain of ALPK1, stimulating its kinase domain to phosphorylate and activate TIFA. The crystal structure of the N-terminal domain of ALPK1 and ADP-Hep in complex revealed the atomic mechanism of this ligand-receptor recognition process. HBP was transformed by host adenylyltransferases into ADP-heptose 7-P, which could activate ALPK1 to a lesser extent than ADP-Hep. ADP-Hep (but not HBP) alone or during bacterial infection induced Alpk1-dependent inflammation in mice. Our findings identify ALPK1 and ADP-Hep as a pattern recognition receptor and an effective immunomodulator, respectively.

Keywords: innate immunity, bacterial infection, ALPK1, ADP-heptose.

A-4-009 Anti-tumor effect of the polysaccharides from cultivated *Dendrobium huoshanense stems* on lung carcinoma *in vitro* and *in vivo*

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Cancer is one of the major public health problems globally which remains a disease of high morbidity and mortality. At present, the conventional lung cancer therapeutic schedule is surgery supplemented with chemotherapy and radiotherapy, and endeavors to prevent postoperative metastasis in clinical practice. Unfortunately, these treatments produce high prevalence of metastasis and recurrence, furthermore, most adjuvant therapies are nonspecific and inevitably cause adverse effects. Therefore, there is a critical need to explore and evaluate more effective alternative strategies for the treatment of lung cancer. It has become a hot research field in the drug development area to find the anti-tumor agents with notably curative effect as well as less adverse reactions. *Dendrobium huoshanense*, a well-known edible traditional Chinese medicine herb, is wildly used in medicines and health supplement. Our previous study has demonstrated that the polysaccharides isolated from cultivated *Dendrobium huoshanense* (cDHP) can alleviate lung inflammation in cigarette smoke-induced mice. In this study, the present work was attempted to investigate the antitumor activity and possible underlying mechanism of cDHP on lung cancer using a murine Lewis Lung Cancer model *in vitro* and *in vivo.* Results showed that cDHP was capable of repressing Lewis lung cancer (LLC) cells growth *in vitro* and *in vivo.* cDHP provided a dose-dependent impairment of cell vitality, induction of apoptosis and cell cycle arrest of LLC cells. The results of animal experiments showed that cDHP not only inhibited the tumor growth, but also increased the immune organ indices, ConA-induced lymphocytes proliferation, and serum cytokine levels, as well as the activities of immune cells in spleen of LLC tumor-bearing mice. These findings suggested that *D. huoshanense* presented remarkable anti-tumor activity and could be a potential novel leading agent to inhibit lung cancer cell growth.

Keywords: *Dendrobium huoshanense*; Polysaccharides; Antitumor activity; Lung cancer

A-4-010 *Dendrobium officinale* polysaccharide modulates tumor-associated macrophages polarization and immune response in hepatocellular carcinoma microenvironment

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Abstract: Hepatocelluar carcinoma (HCC) is the most prevalent malignancy and the second-highest fatal neoplasm among worldwide. Chemotherapy remains the conventional method in treatment of tumor. However, chemotherapy of cancer generally causes various side reactions and immuno-suppression. Notably, cancer immunotherapy have rose hope for the successful treatment of advanced HCC. Tumor associated macrophages (TAMs) are a major tumor-infiltrating cells of tumor microenvironment (TME) and play critical roles in the progression of HCC. Switching tumor-promoting M2-like TAMs toward a tumor-inhibiting M1-like phenotype is a promising strategy for liver cancer immunotherapy. Nevertheless, the mechanism governing of macrophage polarization are still elusive. Here, we aimed to investigate the antineoplastic activity mechanism of a homogeneous *Dendrobium officinale* polysaccharide (DOP) on modulating the tumor-associated macrophages polarization and immune response in hepatocellular carcinoma microenvironment. Meanwhile, in vivo macrophage and *in vitro* cell coculture system were performed to investigate DOP regulated crosstalk between TAMs and tumor cells. M1 and M2 macrophage signatures were evaluated by qRT-PCR, IHC and flow cytometry. DOP treatment induced successful polarization of immunosuppressive M2 TAMs into a M1 macrophage phenotype with potent immunostimulating activity both from a series of in vitro and in vivo experiments. Transcriptome analysis demonstrates that oral administration DOP significantly altered differentiation expression genes networks of TAM phenotype and further regulate immune cell behaviors, which are associated with immunity and inflammatory. The expression of M1 associated genes and surface marker were significantly up-regulated, while M2 associated marker genes were down-regulated in tumor microenvironment by DOP. Moreover, DOP enhanced the secretion of immunostimulate cytokines and chemokines and diminished the releation of immunosuppressive anti-inflammatory factors. Taken together, DOP suppresses murine liver tumor growth via activating TAMs antitumor immune response. These results provide a new paradigm for macrophage polarization and immunosuppressive TAM conversion and shed light on the action mode of DOP immunotherapy for cancer.

Key Words: *Dendrobium officinale* polysaccharide, macrophage polarization, immune response, tumor microenvironment, hepatocellular carcinoma

A-4-011 Molecular hydrogen modifies dysregulated cholesterol metabolism in mouse liver

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Cholesterol dyshomeostasis is associated with increased risk of cardiovascular and brain diseases. Our previous studies demonstrated that long-term intake of hydrogen gas decreased plasma LDL-C level both in animal models and patients with potential metabolic syndrome. However, the underlying mechanism of such modulation was yet to be investigated. On account of the central role of liver in cholesterol metabolism, we speculated that hydrogen gas could modify the dysregulated cholesterol metabolism in liver. A western diet (WD)- and chemical-induced murine non-alcoholic steatohepatitis (NASH) model which was reported to resemble the key metabolic and histologic features of human NASH were applied. In addition to the WD and chemical treatment consistent with the model group, hydrogen intake in the experimental group was accomplished through inhalation by keeping mice in a box filled with 66% hydrogen and 33% oxygen for 1 h per day (mice from model group were kept in a box filled with air and additional 12% oxygen). After 12-week treatment, preliminary results showed that total serum cholesterol, triglyceride, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were decreased in hydrogen-treated group indicating the modulated cholesterol metabolism and alleviated liver injury. In addition, the dysregulated protein expression of SREBP-2, HMGCR, LDLR and PCSK9 were significantly ameliorated in hydrogen-

treated group. Following experiments would further investigate detailed information on the status of all cholesterol metabolic pathways in the liver, and explore the potential signaling pathways and mechanisms involved.

Key Words: Hydrogen, Cholesterol Metabolism, NASH.

专题五：应用生物化学与新技术

A-5-001 Effects of viral promoters, WPRE and weakened antibiotic resistance markers on transgene expression in CHO cells

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Abstract

The product yield and transgene instability of CHO cells need to be further increased and solved. Promoter and selection maker are essential elements on transgene expression. We investigated the effects of promoters, WPRE, and selection marker on transgene expression efficiency in CHO cells to enhance the transgene expression. In the present study, the CMV and SV40 promoters were combined with a weakened form of the Neo resistance gene NPT, and with the WPRE cis-acting element to generate expression vectors. Those vectors were transfected into CHO cells then use Flow cytometry, qRT-PCR, qPCR, Western blot and ELISA to detect the expression of the transgene. The results showed that the weakened Neo resistance gene enhanced eGFP expression driven by the CMV and SV40 promoters. Moreover, a vector that WPRE combine with the Neo resistance gene considerably enhanced eGFP expression in CHO cells. However, the WPRE showed no enhancing effects when combined with the weakened Neo resistance gene. Furthermore, when the CMV promoter combined with the WPRE could result in higher cetuximab expression levels than the CMV promoter alone and could be useful for improving recombinant protein production.WPRE could not enhance the transgene expression under the circumstance of CMV and weakened Neo resistance gene. However, WPRE can improve transgene expression levels when CMV combined with the Neo resistance gene. That suggested that effects of the WPRE were selection marker-specific. The conclusion has a significant implication to industrial production of therapeutic proteins.

Keywords: Chinese hamster ovary; WPRE; Promoter; Weakened Resistance gene; Transgene

A-5-002 仿生蜘蛛丝的研究

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蜘蛛丝有强度高、弹性大、抗辐射、热稳定、耐疲劳、可降解等特性，是一种优质的天然生物材料，在军工、航空航天以及生物医学工程领域有着巨大的潜在应用价值。我们通过建立基因组文库，在世界上首次筛选获得了大腹园蛛编码捕获丝蛋白MiSp（编码基因为10.9kb，包含5.6kb单一基因内含子，整个基因编码序列为5298bp,编码1766个氨基酸）、 包卵丝蛋白Tusp1（（编码基因为5766bp,编码1921个氨基酸，包含有N段和C端非重复区和9个重复区）、包裹丝蛋白AcSp（编码基因为10335bp,编码3444个氨基酸，包含N段和C端非重复区和15个重复区）以及附着丝蛋白质PySp1（编码基因为11931bp,编码3976氨基酸）四个完整基因；解析了蜘蛛捕获丝MiSp蛋白CT和NT的结构与功能的关系，破解了pH可驱动蜘蛛丝蛋白质二聚化和稳定性以及可控制蛛丝纤维的快速形成机理；获得了蜘蛛丝蛋白质模块数量与丝纤维性能的关系；借助蜘蛛包裹丝蛋白质AcSp1四个重复蛋白质片段的表达，研究了AcSp1液态蛋白质与成丝的结构以及成丝机理；我们构建蛛丝蛋白的N端和C端及中间重复单元三部分嵌合体，成功获得了可以在大肠杆菌中高效表达高溶解性的微型重组蛛丝蛋白NT2RepCT，表达的蜘蛛丝蛋白质在生理溶液中即可达到天然蜘蛛腺体中同样高浓度的溶解性重组蛛丝蛋白；利用建立的高效表达蛋白质连接系统，我们体外合成了蜘蛛丝蛋白质；通过静电纺和水纺技术，获得了基因工程仿生蜘蛛丝纤维，纤维的杨氏模量可达到6.7 ± 1.8GPa；通过静电纺丝

技术我们制备了小血管和神经导管，细胞培养实验证实制备的血管支架对细胞无毒性，有相容性，有利于细胞的粘增殖附。

关键词：编码蜘蛛丝蛋白质基因，丝蛋白质结构与功能，成丝机理，蛛丝纺丝仿生，小血管和神经导管支架。

A-5-003　多基因编辑与多基因联合干预技术

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目的：动物性状特别是多基因性状的改良需要多基因联合干预。针对人类疾病的靶向药物治疗十分有效，那么针对其靶点的基因干预治疗效果也明显，因而多基因靶向干预将更加有效。本研究旨在寻找高表达、安全靶位点，建立高效多基因编辑与联合干预技术。

方法：寻找高表达、安全靶位点：将外源基因定点插入到人类细胞的rDNA基因间隔序列（ITS）和动物体内细胞的ITS，检测外源基因的表达稳定性。

建立多位点基因打靶和多基因编辑联合干预技术：建立以动物rDNA基因间ITS为靶位点的稳定表达的多位点基因打靶技术，多位点基因打靶结合锌指核酸酶（ZFN）介导的高效基因编辑技术，结合CRISPR/Cas9介导的多基因编辑技术，结合TALEN介导的多基因编辑与多基因联合干预技术，并对三种基因编辑技术进行比较 。

结果：高表达、安全靶位点：研究发现插入到rDNA基因间隔序列（ITS）的外源基因能够随着细胞的分裂稳定遗传给子代细胞，能够稳定表达，克服了毒性整合等安全性问题。

建立了多位点基因打靶和多基因编辑联合干预技术：建立了以ITS为靶位点的多位点基因打靶技术，打靶效率从10^{-6}提高到15%，结合锌指核酸酶（ZFN）介导的基因编辑技术效率提升到23%，结合CRISPR/Cas9介导的多基因编辑技术效率再提高到65%，结合TALEN介导的多基因编辑与多基因干预技术效率又提高到78%，基因干预效果得到明显提高。

结论：ITS为基因定点插入的理想位点，外源基因稳定表达，克服了毒性整合等安全性问题。ZFN、CRISPR/Cas9、TALEN介导多基因编辑大大提高了基因定点插入的效率和基因干预效果。

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关键词：多位点，基因打靶、ZFN、CRISPR/Cas9、TALEN

A-5-004　微流控芯片单细胞分泌分析

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摘要：细胞是生命存在的基础，探索生命健康与疾病常需要以细胞研究为基础。由于细胞与细胞之间存在差异，群体细胞的研究结果只能得到一群细胞的平均值，这往往会掩盖个体差异信息。为更全面的了解细胞以服务人类健康、疾病研究，单细胞分析就变得尤为必要[1, 2]。细胞通讯是多细胞生物为执行各种生物学功能的必需手段，其中最普遍的是通过化学、生物分子的分泌、接收来进行细胞通讯。细胞通过复杂、稳定、动态的细胞分泌网络协同实现组织、器官、人体的各种生理功能。利用单细胞分析工具解析细胞之间的多维、动态分泌信息，可以精准监测细胞的状态及其执行的功能。在过去的几年中，我们开发了一系列的基于抗体条形码微流控芯片的高通量、高内涵单细胞细胞分泌分析工具，实现了如对数以千计的活体单细胞所分泌的42种蛋白分子分别进行同时检测，单细胞外囊泡免疫分型，单细胞–单细胞自分泌/旁分泌分析，单细胞分泌蛋白动态分析，单细胞三维培养/分析等诸多应

用，大大加深了人们对细胞分泌异质性的认识，并尝试将其服务临床实现个体化、精准医疗[3-9]。

关键词：单细胞分析、细胞通讯、细胞异质性、分泌分析、微流控芯片

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A-5-005　用于增强阳离子聚合物转染效率的pH敏感肽的设计

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背景：阳离子聚合物/DNA复合物从溶酶体内释放效率低是其转染效率低的主要原因，抗菌肽通过裂解酸性条件下的溶酶体膜能够增加转染复合物的释放从而提高基因转染效率，通过谷氨酸残基替换天然抗菌肽中带正电荷的氨基酸残基，我们设计了新的pH敏感肽，该多肽在酸性条件下膜裂解能力明显增强，能显著增强聚乙烯亚胺（PEI）介导的转染效率。**方法**：用谷氨酸残基取代抗菌肽melittin (Mel)和RV-23 (RV)序列中带正电荷的氨基酸残基，合成了两个pH敏感肽aMel和aRV。测定了多肽在不同pH条件下的膜裂解能力、多肽对PEI/DNA转染复合物理化特性的影响、多肽对PEI转染效率和细胞毒性的影响。**结果**：谷氨酸残基取代的多肽在低pH条件下的溶血能力增强，表示其在低pH条件下膜裂解活性强。谷氨酸残基取代的多肽掺入不影响PEI复合的DNA结合能力，但影响PEI/DNA复合物的理化特性。谷氨酸残基取代的多肽能提高PEI介导的转染效率，在HeLa细胞中其转染效率相比单独PEI提高了约42倍。**结论**：本研究结果表明，谷氨酸残基取代抗菌肽序列中带正电荷的残基可用于设计pH敏感肽，作为转染增强剂提高PEI/DNA复合物在哺乳动物细胞系中的转染效率。

关键词：抗菌肽，pH敏感肽，谷氨酸，基因递送

A-5-006　连翘、丁香、川芎复合涂膜保鲜剂对杏子果实采后品质及适宜保鲜浓度筛选

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以灵台牛心杏为研究对象，用3种中药材的乙醇提取液与基质液配伍成涂膜保鲜剂，在温度为-1℃～0℃、相对湿度为80%～90%的条件下贮藏牛心杏，比较对其贮藏品质和生理生化指标的影响。结果表明：以2.0mg/ml连翘、1.5mg/ml丁香、1.5mg/ml川芎提取液涂膜处理牛心杏后，可延缓牛心杏果实的衰老和品质的劣变，大大减少牛心杏果实的失重率、腐烂率，减缓可溶性固形物含量、可滴定酸含量、VC含量的下降速度；并对牛心杏相对电导率、PPO的活性的有较强的抑制抑制作用，与1-MCP(对照)无明显差异(P>0.05)；而保持牛心杏果实CAT、POD较高活性，能有效清除牛心杏果实衰老自由基，对牛心杏有很好的保鲜效果。

关键词：连翘；川芎；丁香；提取液；牛心杏；保鲜；品质

A-5-007 Molecular docking reveals fucoxanthin regulates Ran to inhibit gastric cancer lymphangiogenesis and metastasis

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Gastric cancer is one of the most lethal cancer types in the world. Its typical malignant behavior is metastasis, and lymph node metastasis is the main metastasis route of gastric cancer. Lymphangiogenesis is an important step in the process of lymph node metastasis. Currently, no specific drugs for lymph node metastasis of gastric cancer have been applied in clinical practice. Fucoxanthin is a material found in brown algae that holds promise in the context of drug development. Previous studies have confirmed that fucoxanthin has an inhibitory effect on human lymphatic endothelial cells (HLEC), and confirmed its inhibitory effect on lymphangiogenesis in nude mice induced by breast cancer (MB-MDA-231) for the first time. In this study, gastric cancer cell lines (SGC7901) were selected to further confirm the inhibitory effect of fucoxanthin on tumor-induced lymphangiogenesis and lymph node metastasis, elucidate the mechanism and analyze the target. The results of experiments show that fucoxanthin, extracted from Undaria pinnatifida (Wakame), inhibits the proliferation, migration, invasion and the phosphorylation of PI3K/Akt/NF-κB signaling pathway in SGC-7901 cells. Gastric cancer microarray showed higher expression of Ran protein in lymph node metastatic tissue, and molecular docking simulation showed that fucoxanthin binds to Ran protein by hydrogen bond. In addition, fucoxanthin also suppressed tumor-induced lymphangiogenesis and metastasis in vitro and in vivo via VEGFC/VEGFR3 axis depend on Ran expression. These findings will provide the basis and new ideas for the treatment of tumor lymph node metastasis, as well the research and development of new active ingredients of marine drug, which has important theoretical significance and clinical application value.

Key Words: Fucoxanthin, Gastric cancer, Lymphangiogenesis, metastasis, Molecular docking, Ran

A-5-008 A novel multiplex xMAP assay for generic detection of avian, fish and ruminant DNA in feed and feedstuffs

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The identification of animal species in feed and feedstuffs is important for detecting contamination and fraudulent replacement of animal components that might cause health and economic problems. A novel multiplex assay, based on xMAP technology and the generic detection of closely related species, was developed for the simultaneous differential detection of avian, fish and ruminant DNA in products. Universal primers and probes specific to avian, fish or ruminant species were designed to target a conserved mitochondrial DNA sequence in the 12S ribosomal RNA gene (rRNA). The assay specificity was validated using samples of 27 target and 10 nontarget animal species. The limits of detection of the purified DNA were determined to be 0.2 pg/μL-0.1 ng/μL by testing the meat samples of six species and four feedstuffs. The detection sensitivities of the assay on target ingredients in three experimental ternary mixtures and a quaternary mixture with plant matrix base were demonstrated to be 0.01% (weight percentage). The assay's suitability for practical application was evaluated by testing a variety of feed samples with different ingredients; unlabeled animal ingredients were detected in 32% of the 56 samples. The

assay differentially detected the three targeted categories of animal species in less than 2 h, reflecting improvements in speed and efficiency. Based on these results, this novel multiplex xMAP assay provides a reliable and highly efficient technology for the routine detection of animal species in feed and other products for which this information is needed.

Keywords: Animal species identification; Multiplex xMAP assay; Universal primers and probes; Feed and feedstuff

A-5-009　细胞微环境调控的蛋白质化学修饰及其肿瘤靶向治疗

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蛋白质是生命活动的主要执行者，其对生命过程的调控多通过种类复杂、时空分辨的化学修饰实现。设计对细胞微环境具有响应性的化学修饰蛋白可以在分子水平上揭示蛋白质调控生命过程的化学本质，并发展疾病诊断和治疗的分子工具。近来，我们发展了系列对疾病细胞微环境（如高浓度活性氧、还原性微环境、以及特异表达的蛋白等）具有刺激响应性的蛋白质化学修饰新方法，实现了化学修饰蛋白质功能的选择性激活。进一步利用纳米载药技术，实现了细胞和活体动物层次蛋白质的递送以及蛋白质活性原位、特异性调控，并发展了由细胞微环境调控的高效CRISPR/Cas9基因编辑方法，有望进一步用于发展基于蛋白质的肿瘤靶向治疗新策略。

关键词：蛋白质化学修饰；细胞微环境；蛋白质递送；肿瘤靶向治疗

A-5-010　Transcriptomic profiling of microglia and astrocytes throughout aging

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Activation of microglia and astrocytes, a prominent hallmark of both aging and Alzheimer's disease (AD), has also been suggested to contribute to aging and AD progression. However, the underlying cellular and molecular mechanisms are largely unknown. Hence, to address this issue, we performed RNA-seq in microglia and astrocytes freshly isolated from Wild-type(WT)/APP-PS1(AD) mice brains across the lifespan to analyze the longitudinal genes expression profile. Our results have shown that, compared to the young adult mice (2-month old), a set of age-related genes in microglia and astrocytes (termed age-up or age-down) showed consistent up- or down-regulation from 4 months onwards. Further bioinformatic analysis revealed that Age-up genes in microglia are associated with inflammatory response, while those in astrocytes include well-known AD risk genes, synaptic transmission or elimination-associated genes and peptidase inhibitors. Interestingly, in the late onset of AD, most of those detectable transcripts are perturbed in both microglia and astrocyte, regardless of their cellular identities. Overall, our RNA-seq data provides a valuable resource for future explorations of the role of microglia and astrocytes in aging and Aβ induced AD pathologies.

Key Words: Microglia, Astrocyte, Aging, Alzheimer's Disease.

A-5-011 Aurora Kinase inhibitor VX-680 in combination with cisplatin exerts a synergistic anti-tumor effect on esophageal carcinoma cells

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Cisplatin is one of the most effective chemotherapeutic drugs for esophageal carcinoma treatment. However, its considerable side effects and the development of drug resistance are becoming major limitations for its application. The serine/threonine kinase Aurora includes three members, Aurora A, B and C. They are frequently overexpressed in many human cancers and correlate with poor prognosis. VX-680, which specifically targets Aurora kinase, decreases their activity and inhibits tumorigenesis. However, whether VX-680 and cisplatin can could present synergistic effects on esophageal carcinoma cells remains unclear. In this study, we found that the combinative treatment could inhibit cell growth and promote apoptosis of the esophageal carcinoma cells by increasing cleavages of Caspase3 and PARP in a synergistic way. Furtherly, used in combination with cisplatin, VX-680 impaired cell migration ability, increased cell-cell adhesion ability and weakened the adhesion ability of the cells to the extracellular matrix in a synergistic way. Moreover, the combinative treatment could reduce HUVEC tubule formation, indicating inhibition of angiogenesis. Further studies revealed that VX-680 and cisplatin decreased the expression of the MMP-2, VEGF and increased E-cadherin expression, reduced phosphorylation of AKT and ERK proteins, while the combination of VX-680 and cisplatin showed the most significant difference. Taken together, these results suggest VX-680 can enhance anti-tumor effectiveness of cisplatin in esophageal carcinoma cells.

Key Words: VX-680, cisplatin, esophageal carcinoma

A-5-012 The profile of *Bombina maxima*'s skin microbes

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Amphibian skins play lots of important roles, such as respiration, metabolism, and antimicrobial, etc. It is very urgent to understand the skin microbes for elucidating the functions of skin. Amphibian species of *Bombina maxima* lives in dirty environments rich in microbes. However, the skin microbe profile of *B. maxima* was unclear up to now. The 16S rRNA analysis and ITS analysis are two powerful methods to explore the consist of bacteria and fungi, respectively. We carrying out both 16S rRNA analysis and ITS analysis of *B. maxima* skin microbe using wild individuals (16S rRNA, n=5; ITS, n=3). After that, we analyzed the Alpha diversity of the skin microbe by Rarefaction Curve, Venn Graph, and Ternaryplot Analysis. We analyzed the Beta diversity by Weighted Unifrac, Unweighted Unifrac, PCoA, PCA, NMDS, Simper, UPGMA, MRPP, T-test, MetaStat, and LEfSe. The interaction of bacteria to fungi contained in the skin of *B. maxima* was investigated through the database published on esa (http://esapubs.org/archive). Totally 249 different kinds of bacteria were found in 5 used skin samples, most of the determined microbes were *Bacteroidetes, Proteobacteria,* and *Firmicutes.* Meanwhile, 79 kinds of fungi existed in the tested animal skins and *Ascomycota, Basidiomycota*, and *Chytridiomycota* represented the most diversified species. Importantly, amphibian threatening fungi of *Batrachochytrium dedrobatidis* were found in all the 3 test animals. Our data suggested that there were no significant differences in the skin microbes between dorsal and ventral, and we first found that *B. dedrobatidis* was existed on the skin of *B. maxima*.

Key Words: *Bombina maxima* , skin microbes, 16S rRNA, ITS

A-5-013 The recombinant expression of gamma-bungarotoxin

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Bungarus multicinctus is one of the most deadly snakes in the world. The lethality of biten by B. multicinctus was ranked first in China. The main components responsible for the death of the victims are attributed to neurotoxins contained in B. multicinctus venoms, including alpha-bungarotoxin, γ-bungarotoxin,beta-bungarotoxin and kappa-bungarotoxin. Previous researches were mainly focused on α-bungarotoxin and γ-bungarotoxin, while the study on γ-bungarotoxin was rare because of it's lower content in crude venom. To obtain enough γ-bungarotoxin for investigation, we inserted the γ-bungarotoxin cDNA into expression vector of pMAL-p2X, and the MBP- γ-bungarotoxin recombinant protein was successively generated in the periplasm of Escherichia coli (TB1) after IPTG inducing and 18h culture at 18°C. According to the method of periplasmic extract provided by NEB, the recombinant protein was collected in soluble form and at least accounts for 90% of total periplasmic proteins. The recombinant protein MBP- γ-bungarotoxin was isolated by one step affinity chromatography on an MBP-affinity column. The recombinant protein collected from affinity chromatography can be further fractionated by using a source 15S cationic ion-exchange chromatography column. Finally, an electrophoretic purity recombinant MBP-γ-bungarotoxin was prepared for the next step of factor Xa enzyme digestion to release γ-bungarotoxin. This method is simple and economical, it might provide enough γ-bungarotoxin to investigate the biological activities of γ-bungarotoxin.

Key words: Bungarus multicinctus, γ-bungarotoxin, pMAL-p2X, prokaryotic vector.

A-5-014 Pharmacology, pharmacokinetics and toxicology of a novel antithrombotic peptidomimetic inhibitor ωRWR in animal model

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Abstract: ωRWR is a new patented antithrombotic small peptide which exhibits anti-coagulation effects by competitively inhibiting arginine-glycine-aspartic acid (RGD) on fibrinogen binding with αIIbβ3 integrin on the surface of platelets. In this study, ωRWR were synthesized by solid phase peptide synthesis. This peptide could inhibit platelet aggregation and thrombus formation in a dose-dependent manner. Through establishment and optimization of liquid chromatography and mass spectrometry conditions, pharmacokinetic property of ωRWR in rat was also analyzed. We further investigated the toxicity of ωRWR. The data showed that the maximum tolerance dose of ωRWR was larger than 192mg/kg. After the injection of ωRWR for 26 weeks, the body weight and food utilization rate of the rats were normal. Organs had no pathological changes through H&E staining. In conclusion, ωRWR is a promising antithrombotic peptide for the treatment of thrombosis-related disease.

Keywords:ωRWR, αIIbβ3 integrin, RGD

A-5-015 Inhibition of melanin formation by kojic acid derivative KAD3

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Melanogenesis is the process of producing melanin pigments that are responsible for the color of skin, eyes and hair and provide protection from ultraviolet radiation. However, excessive levels of melanin formation can lead to pigmentation disorders such as freckles, melasma and age spots, in which tyrosinase plays an important role. Kojic acid was well known by its de-pigmenting activity, however it has been banned to use in cosmetic due to its instability and side effect. In this study, a novel kojic acid derivative KAD3 (5-hydroxy-2-(((4-(((1Z,2Z)-3-phenylallylidene)amino)-5-(2-((E)-3-phenylallylidene) hydrazinyl)-4H-1,2,4-triazol-3-yl)thio)methyl)-4H-pyran-4-one) was synthesized, which molecular weight is 498. The inhibitory mechanism of KAD3 on melanin formation was evaluated by enzyme assay, cell assay and zebrafish assay. The results showed that KAD3 could effectively inhibit the diphenolase activity of mushroom tyrosinase with IC_{50} of 10.00 μmol/L and it belongs to revisible mixed inhibitor. Then, the antimelanogenic activity of KAD3 was further confirmed by assessing the inhibition of melanin content and intracellular tyrosinase activity in B16F10 cells and zebrafish model. It demonstrated that KAD3 suppressed the expression of microphthalmia-associated transcription factor (MITF), tyrosinase (TYR), tyrosinase related protein-1 and 2 (TRP-1 and TRP-2) in a concentration-dependent manner. Otherwise, we found KAD3 could inhibit the pigment formation of zebrafish embryo and the effect stronger than kojic acid. Our study could lay the theoretical foundation for the application of kojic acid derivatives in the fields of cosmetics and medicine and provide new ideas for the development of new anti-melanogenesis agents.

Key words: KAD3; melanin; tyrosinase

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A-5-016 TREX2 enables efficient genome disruption mediated by paired CRISPR/Cas9 nickases that generate 3'-overhanging ends

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Abstract: Paired CRISPR/Cas9 nickases are an effective strategy to reduce off-target effect in genome editing. However, this approach is only efficient with 5'-overhanging ends in genome disruption, not with 3'-overhanging ends, thus limiting its editing applications. In order to expand utilization of paired CRISPR/Cas9 nickases in genome editing, we tested the effect of the TREX2 exonuclease on repair of 3'-overhanging ends generated by paired CRISPR/Cas9 nickases. We found that overexpression of TREX2 stimulates the efficiency of paired CRISPR/Cas9 nickases in genome disruption with 3'-overhanging ends up to 80-fold. Upon TREX2 overexpression, TREX2-mediated end processing preferentially deletes entire 3'-overhangs for genome disruption but has no significant effect on 5'-overhangs. Length of 3'-overhangs affected by TREX2 overexpression could extend over 100 nucleotides. TREX2 overexpression also stimulates genome disruption by paired CRISPR/Cas9 D10A nickases that generate short 3'-overhanging ends at overlapping CRISPR/Cas9 target sites. These results indicate that TREX2 overexpression could be used to promote efficient genome disruption by paired CRISPR/Cas9

nickases generating 3'-overhanging ends. Moreover, analysis of off-target effect revealed that TREX2 overexpression causes little stimulation in off-target editing by paired CRISPR/Cas9 nickases. We also found that TREX2 acts on XRCC4-dependent non-homologous end joining for 3'-overhangs induced by paired CRISPR/Cas9 nickases to promote genome disruption. Taken together, this study provides an approach in cells to process 3'-overhanging ends generated by paired Cas9 nickases for efficient genome disruption.

Key Words: TREX2, paired CRISPR/Cas9 nickases, genome disruption, off-target effect

A-5-017 Tracking copy number variations of tumor suppressor mutations induced in CRISPR/Cas9-mediated mouse liver tumorigenesis

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CRISPR/Cas9-based targeted somatic multiplex-mutagenesis is a powerful tool for development of mouse liver cancer models. Here, using hydrodynamic tail vain injection of a validated single guide RNA plasmid library targeting 34 tumor suppressor genes together with an SpCas9 expression plasmid, we efficiently induced autochthonous liver tumors in mice within 30-60 days. Analysis of CRISPR/Cas9-induced tumors by next generation sequencing (NGS) revealed heterogeneous mutations at multiplex target sites, suggesting each tumor nodule is developed from multiple clonal cells carrying different spectra of mutations. We isolated single cells/clones from tumor nodules, analyzed targeted mutations, and found significant mutation heterogeneity between different single cells/clones. In individual cells/clones, many target sites frequently displayed over two types of variations, indicating possible polyploidy of a target allele or over two copies of a target site. Surprisingly, subclones from the same parental clone exhibited different genetic variations at certain target sites. Even proliferation of a subclone overtime in cell culture and in mouse subcutaneous xenograft induced targeted alterations in mutation spectra of target sites, suggesting a possible new extrachromosomal source for evolution of genetic heterogeneity in tumors. Karyotyping revealed abnormally high levels of micronuclei, nuclear buds, nuclear fragments, gross chromosomal rearrangements and chromosomal breaks in tumor cells. Sequencing analysis of extrachromosomal circular DNA further showed significant frequency alterations of targeted mutations in comparison with those of genomic targets. These findings indicate an extremely unstable genome in CRISPR/Cas9-induced multiplex-mutagenesis mouse liver cancer models and an important contribution of the extrachromosomal DNA elements in the evolution of liver tumor heterogeneity.

Key Words: CRISPR/Cas9; somatic multiplex-mutagenesis; liver tumor heterogeneity; extrachromosomal DNA

A-5-018 基于结构变异的香猪抗病基因的资源挖掘

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摘要：香猪作为贵州地方特色猪种，以肉质香嫩、基因纯合、富含微量元素、纯净无污染等独特优点而著称，具有良好的经济效应。与此同时，香猪适应性好、抗病力强、耐粗饲，故而挖掘香猪的抗病基因具有重要的意义。基因组水平的结构变异能够影响基因的功能，以致影响个体表型性状，在物种进化中也起着重要作用。本研究利用基因组重测序策略，完成6头香猪以及12头贵州地方猪种的重测序，包括关岭猪、柯乐猪、黔北黑猪、江口萝卜猪，结合36头中国地方猪种和欧洲猪种的基因组序列进行了结构变异的分析。利用Pindel和Soft SV软件对54头猪种在全基因组水平上共鉴定出39166个结构变异。其中，贵州地方猪种特有的结构变异有4650个，香猪特有结构变异有588个，富集到217条KEGG通路，2289条GO条目中，从中筛选出与抗病相关的KEGG通路51条，GO条目202个，涉及免疫细胞、免疫分子、病原菌感染以及炎症等，包含抗病基因59个，对应64个结构变异，以缺失类型为主。进一步筛选出与香猪抗病性状相关的重要候选基因23个，其中10个基因编码膜上蛋白，与CD14分子、MHC Ⅱ类分子以及T细胞诱导等相关，在免疫相关代谢通路中起着及其重要的作用。本结果为下一步研究香猪的提供了基因资源和分子理论基础。

关键词：贵州地方猪种；结构变异；抗病基因

A-5-019 Reconstitution of human eukaryotic translation initiation factor 3 using a ribozyme-mediated polycistronic expression system

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Eukaryotic initiation factor 3 (eIF3) is the largest factor involved in mRNA translation initiation, consisting of 13 different subunits. The ~800 kilodalton (kDa) mammalian eIF3 forms interactions with many other factors, such as eIF4F, the cap-binding factor, and eIF1, eIF5, etc. It is also responsible for recruiting the small ribosomal subunit by direct association with 40S. eIF3 has been implicated in playing many key roles in the regulation of translation, but its molecular mechanism is scarcely known. To obtain a functional eIF3 sample suitable for structural and functional investigation, we developed a polycistronic expression system mediated by ribozymes to co-express subunits of eIF3 in *Escherichia coli*. This strategy has successfully enabled us to obtain the 8-subunit core of eIF3, while peripheral subunits can be expressed individually. Biochemical characterization of the recombinant eIF3 is currently underway.

Key Words: translation initiation, eIF3, polycistronic.

A-5-020 条纹斑竹鲨单域抗体的结构及其V区特点的研究

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条纹斑竹鲨是一种浅海底栖小型鲨鱼，能够产生免疫球蛋白新抗原受体（ Ig New Antigen Receptor IgNAR ）其特点是仅由重链组成，是目前已知的尺寸最小的抗体分子。我们通过对鲨鱼脾脏及淋巴细胞进行转录组测序，比对分析获得数条鲨鱼IgNAR序列，针对已知序列设计特异性引物。提取鲨鱼脾脏及淋巴细胞RNA，进行RT-PCR，对鲨鱼抗体进行鉴定。通过对IgNAR全长进行检测，我们发现鲨鱼单链抗体除已经报导的含有一个可变区和5个恒定区的形式之外，还有一种缺少第二个恒定区和第三个恒定区的更小尺寸的抗体IgNAR-S，且通过对其可变区进行扩增子测序，确定IgNAR-S与正常抗体相同，具有多种多样的可变区，可以执行抗体的功能。对两种尺寸的抗体可变区进行检测后，根据半胱氨酸的位置，可以将条纹斑竹鲨的IgNAR主要分成两类：第一类是26位、87位和33位含半胱氨酸，相对应的这一类中有90%的抗体的CDR3区含有1个半胱氨酸；第二类是26位、87位含半胱氨酸，这种类型的抗

体中有80%的抗体的CDR3区含有2个半胱氨酸。这样4个半胱氨酸的组成似乎更有利于二硫键的形成，促进抗体结构的稳定，使其能够更好的与抗原结合。

关键词：条纹斑竹鲨；IgNAR；IgNAR-S；二硫键

A-5-021　基于鲨鱼免疫系统的PD-1抗原单域抗体的初步研究

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鲨鱼单域抗体 (single-domain antibodies, sdAbs) 是目前已知最小且能与抗原发生特异性结合活性的抗体分子，其分子量低至12kDa左右。因其具有分子量小、溶解能力强等特点，可以穿过血脑屏障，更有效的与抗原结合。鲨鱼单域抗体的FR1与CDR3和CDR3与FR3之间还存在两对非典型的二硫键，因此其作为药物能更加稳定地存在于机体内，更好发挥作用。而2018年我国因恶性肿瘤而死的人数仅次于心脏病，排在死因的第二位。而目前治疗癌症的医疗手段，均无法起到很好地治疗作用，由于血脑屏障，脑部癌症更是难以攻克。本实验将PD-1抗原作为免疫抗原，腹部皮下接种，首次接种量为1μg，分别在第1天、第4天、第7天、第14天和第21天，持续五次对条纹斑竹鲨(*Chiloscyllium plagiosum*) 进行免疫，免疫后取鲨鱼血清进行ELASE检测。对鲨鱼免疫前后的淋巴细胞进行转录组测序，在分子水平上比较免疫前后鲨鱼淋巴细胞mRNA的差异，并从中筛选出可能的抗体重链序列，通过体外合成，过柱纯化等步骤，筛选出特异性强、能透过血脑屏障的PD-1鲨鱼单域抗体，使其可用于免疫疗法或联合CAR-T技术治疗脑部肿瘤。

关键词：单域抗体；条纹斑竹鲨；PD-1抗体；免疫疗法

A-5-022　RNA Production using engineered ribozymes

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Large-scale RNA production is usually conducted by in vitro transcription using T7 RNA Polymerase (T7 RNAP). Oftentimes, ribozymes such as hammerhead or HDV are introduced to the transcript to obtain homogeneous ends of the RNA product. But they often suffer very low efficiency of cleavage. In addition, downstream purification of the RNA product still remains to be a tedious process. In this study we aim to improve RNA preparation in two ways. One is that we exploited recently discovered novel ribozymes to ensure highest cleavage efficiency. Second, we further engineered these ribozymes such that they are amiable for later separation from the mature RNA product. Our goal is to develop an efficient method to prepare a large quantity of RNA suitable for structural and functional studies.

Key Words: Engineered ribozyme, RNA aptamer, RNA production, RNA purification

专题六：生物化学教育

A-6-001　大学科研实验室科普开放“ 2+1 ”的实践与体会

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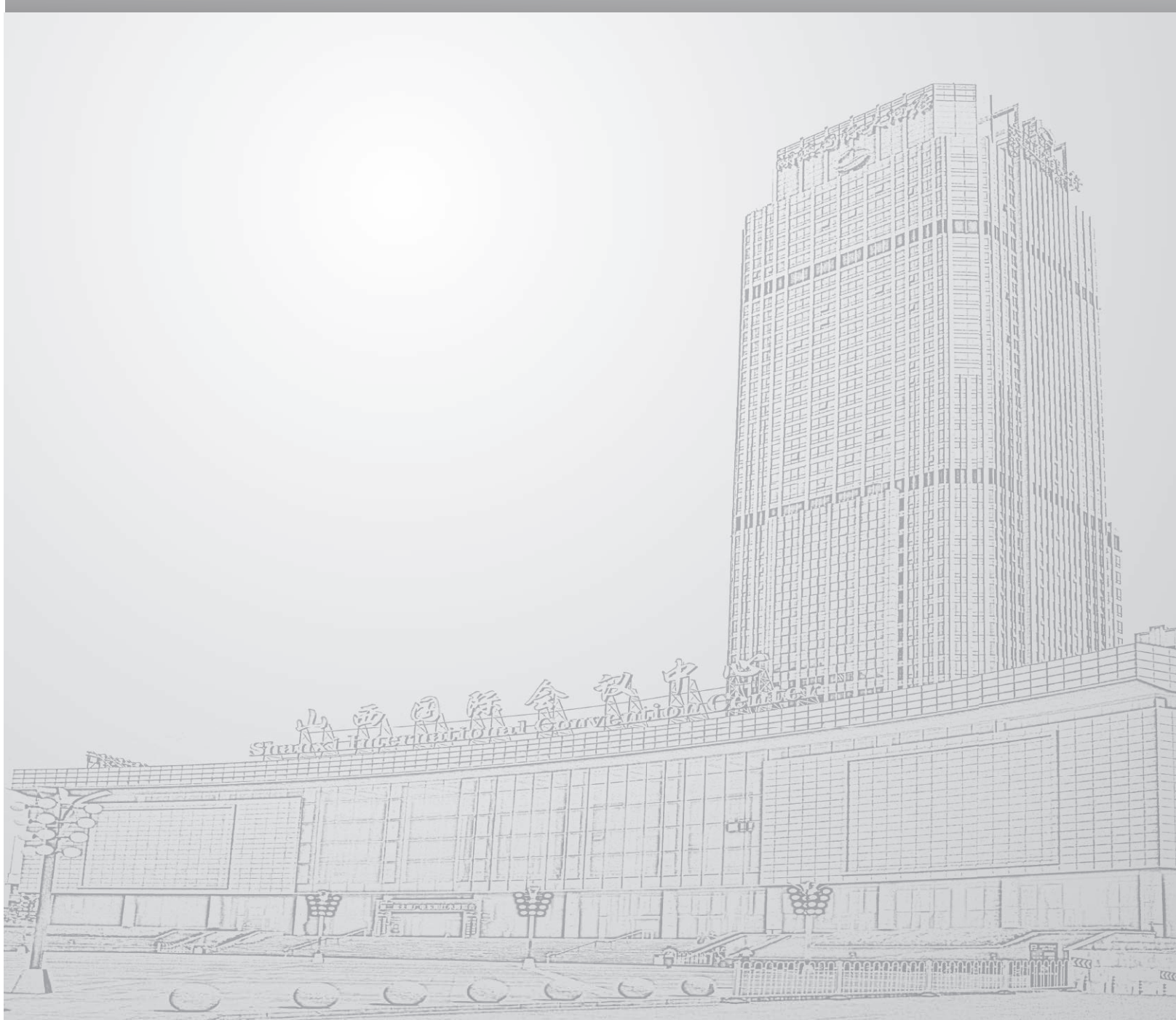
科教兴国的伟大战略需要全体国民科学素养、创新能力的整体提高。科学普及是提高公民素质、支撑科技创新驱动发展的基础性工程。高校不仅仅是教书育人的主阵地，科学普及上同样具有要发挥其积极的作用。大学科研实验室作为高校的重要科研基地，如何开展科普实验室开放。从2018年以来，作为河南省国际联合实验室，我们针对科普大众的特点，结合两次实验室开放的经验，提出了实验室开放的三个“2+1”方案，即“2+1的管理方法”，“2+1的实施方案”，“2+1的来访群体”。从前期宣传、活动实施、日常积累等方面讨论如何做好实验室开放，达到事半功倍的目的。

关键词：科学普及；实验室；开放；实践

青年科学家论坛

中国生物化学与分子生物学会2019年全国学术会议暨学会成立四十周年



01 Arginine methylation of SIRT7 couples glucose sensing with mitochondria biogenesis

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Sirtuins (SIRTs) are a class of lysine deacylases that regulate cellular metabolism and energy homeostasis. Although sirtuins have been proposed to function in nutrient sensing and signaling, the underlying mechanism remains elusive. SIRT7, a histone H3K18-specific deacetylase, epigenetically controls mitochondria biogenesis, ribosomal biosynthesis, and DNA repair. Here, we report that SIRT7 is methylated at arginine 388 (R388), which inhibits its H3K18 deacetylase activity. Protein arginine methyltransferase 6 (PRMT6) directly interacts with and methylates SIRT7 at R388 *in vitro* and *in vivo.* R388 methylation suppresses the H3K18 deacetylase activity of SIRT7 without modulating its subcellular localization. PRMT6-induced H3K18 hyperacetylation at SIRT7-target gene promoter epigenetically promotes mitochondria biogenesis and maintains mitochondria respiration. Moreover, high glucose enhances R388 methylation in mouse fibroblasts and liver tissue. PRMT6 signals glucose availability to SIRT7 in an AMPK-dependent manner. AMPK induces R388 hypomethylation by disrupting the association between PRMT6 and SIRT7. Together, PRMT6-induced arginine methylation of SIRT7 coordinates glucose availability with mitochondria biogenesis to maintain energy homeostasis. Our study uncovers the regulatory role of SIRT7 arginine methylation in glucose sensing and mitochondria biogenesis.

Key Words: arginine methylation, glucose sensing, mitochondria biogenesis, PRMT6, SIRT7

02 *Musa balbisiana* genome reveals subgenome evolution and functional divergence

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Banana cultivars (*Musa* ssp.) are diploid, triploid and tetraploid hybrids derived from *Musa acuminata* and *Musa balbisiana.* We presented a high quality draft genome assembly of M. balbisiana with 430 Mb (87%) assembled into 11 chromosomes. We identified that the recent divergence of *M. acuminata* (A-genome) and *M. balbisiana* (B-genome) occurred after lineage-specific whole genome duplication (WGD) and that the B-genome may be more sensitive to the fractionation process compared to the A-genome. Homoeologous exchanges occurred frequently between A- and B-subgenomes in allopolyploids. Genomic variation within progenitors resulted in functional divergence of subgenomes. Global homoeolog expression dominance occurred between subgenomes of the allotriploid. Gene families related to ethylene biosynthesis and starch metabolism exhibited significant expansion at the pathway level and wide homoeolog expression dominance in the B-subgenome of the allotriploid. The independent origin of 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) homoeolog gene pairs and tandem duplication-driven expansion of ACO genes in the B-subgenome contributed to rapid and considerable ethylene production after harvest in allotriploid banana fruits. The findings of this study provide greater context for understanding fruit biology and aid the development of tools for breeding optimal banana cultivars.

Key Words: Banana, Genome, Subgenome, Evolution, Functional divergence

03 A *Salmonella* Effector Reveals the V-ATPase-ATG16L1 Axis that Initiates Bacterial Autophagy

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Antibacterial autophagy (xenophagy) is an important host defense, but how it is initiated is unclear. Here we performed a bacterial transposon screen and identified a T3SS effector SopF that potently blocked *Salmonella* autophagy. SopF was a general xenophagy inhibitor without affecting canonical autophagy. S. Typhimurium Δ*sopF* resembled *S. flexneri* Δ*virA*Δ*icsB* with the majority of intracellular bacteria targeted by autophagy, which permitted a CRISPR screen to identify host V-ATPase as an essential factor. Upon bacteria-triggered vacuolar damage, the V-ATPase recruited ATG16L1 onto bacteria-containing vacuole, which was blocked by SopF. Mammalian ATG16L1 bears a WD40 domain that was required for interacting with the V-ATPase. Inhibiting autophagy by SopF promoted S. Typhimurium proliferation *in vivo.* SopF targeted Gln124 of ATP6V0C in the V-ATPase for ADP-ribosylation. Mutation of Gln124 also blocked xenophagy but not canonical autophagy. Thus, the discovery of SopF reveals the V-ATPase-ATG16L1 axis that critically mediates autophagic recognition of intracellular pathogen.

Key Words: *Salmonella* effector, bacterial autophagy, V-ATPase, ADP-ribosylation

04 Acetylation regulates ribonucleotide reductase activity and cancer cell growth

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Abstract: Ribonucleotide reductase (RNR), composed of RRM1 and RRM2 subunits, catalyzes the rate-limiting step in the de novo synthesis of deoxyribonucleotides triphosphate (dNTP) needed for DNA synthesis. Although allosteric regulation has been defined to control its activity to manipulate the dNTP pool balance and genomic integrity, insight into fine regulation is still limited. Here, we report that acetylation and deacetylation of the RRM2 subunit of RNR acts as a molecular switch that impacts RNR activity, dNTP synthesis, and DNA replication fork progression. Acetylation of RRM2 at K95 abrogates RNR activity by disrupting its homodimer assembly. RRM2 is directly acetylated by KAT7, and deacetylated by Sirt2, respectively. Sirt2, which level peak in S phase, sustains RNR activity at or above a threshold level required for dNTPs synthesis. We also find that radiation or camptothecin-induced DNA damage promotes RRM2 deacetylation by enhancing Sirt2-RRM2 interaction. Acetylation of RRM2 at K95 results in the reduction of the dNTP pool, DNA replication fork stalling, and the suppression of tumor cell growth in vitro and in vivo. This study therefore identifies acetylation as a regulatory mechanism governing RNR activity.

Keywords: Ribonucleotide reductase; Acetylation; Cancer nucleotide metabolism

05 LARP7-Mediated U6 snRNA Modification Ensures Splicing Fidelity and Spermatogenesis in Mice

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U6 snRNA, as an essential component of the catalytic core of the pre-mRNA processing spliceosome, is heavily modified post-transcriptionally with 2'-*O*-methylation being most common. The role of these modifications in pre-mRNA splicing, as well as their physiological function in mammals, has remained largely unclear. Here, we report that the La-related protein LARP7 functions as a critical cofactor for the 2'-*O*-methylation of U6 in mouse male germ cells. Mechanistically, LARP7 promotes U6 loading onto box C/D snoRNP, thereby facilitating U6 2'-*O*-methylation by box C/D snoRNP. Importantly, ablation of LARP7 in the male germline causes defective U6 2'-*O*-methylation, massive alterations in pre-mRNA splicing, and spermatogenic failure in mice, which can be rescued by ectopic expression of wildtype LARP7 but not an U6-loading deficient mutant LARP7. Our data uncover a novel role for LARP7 in regulating U6 2'-*O*-methylation and demonstrate the functional requirement of such modification for splicing fidelity and spermatogenesis in mice.

Key Words: U6 snRNA, 2'-*O*-methylation, LARP7, box C/D snoRNP, pre-mRNA splicing, spermatogenesis

06 MIAT silencing inhibited Hepatocellular carcinoma by inducing cellular senescence

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Hepatocellular carcinoma (HCC) is a leading cause of cancer deaths and lacks effective therapies. Cellular senescence acts as a barrier against cancer progression and plays an important role in tumor suppression. Cellular senescence associated long noncoding RNAs (SAL-RNAs) are thought to be critical regulators of cancer development. Here, the long noncoding RNA (lncRNA) myocardial infarction-associated transcript (MIAT) was first identified as an HCC specific senescence-associated long noncoding RNA (SALncRNA) that plays an important role in promoting cellular senescence and inhibiting hepatic progression. In addition, SAL-MIAT acts as a competitive endogenous RNA (ceRNA) that upregulated the expression of SIRT1 by sponging miR-22-3p. MIAT silencing alleviated HCC progression by inducing HCC cellular senescence and stimulating senescent cancer cells to secrete senescence-associated secretory phenotype (SASP) by activating the p53/p21 and p16/pRb tumor suppressor pathway. In summary, we identified a tumor-promoting and senescence-suppressing role of MIAT in HCC tumorigenesis. MIAT silencing-induced HCC cellular senescence served as a typical tumor-suppressive mechanism that restricts the proliferation of malignant cells, and the anticancer function of SASP contributes to tumor cell clearance, which might offer a potential therapeutic strategy for HCC treatment.

Key Words: Long noncoding RNA MIAT, ceRNA, miR-22-3p, SIRT1, hepatocellular carcinoma, cell senescence

07 Structure and degradation of circular RNAs regulate PKR activation in innate immunity

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Summary

CircRNAs produced from back-splicing of exons of pre-mRNAs are widely expressed, but current understanding of their functions is limited. These RNAs are stable in general and are thought to have unique structural conformations distinct from their linear RNA cognates. Here we uncover that endogenous circRNAs tend to form 16-26 bp imperfect RNA duplexes and act as inhibitors of double-stranded RNA (dsRNA)-activated protein kinase (PKR) related to innate immunity. Upon poly(I:C) stimulation or viral infection, circRNAs are globally degraded by RNase L, a process required for PKR activation in early cellular innate immune responses. Augmented PKR phosphorylation and circRNA reduction are found in peripheral blood mononuclear cells (PBMCs) derived from patients of autoimmune disease systemic lupus erythematosus (SLE). Importantly, over-expression of the dsRNA-containing circRNA in PBMCs or T cells derived from SLE can alleviate the aberrant PKR activation cascade, thus providing a connection between circRNAs and SLE.

Keywords

Circular RNAs (circRNAs), circRNA structure, circRNA degradation, double-stranded RNAs (dsRNAs), RNase L, PKR, innate immune responses, autoimmune disease, SHAPE-MaP, systemic lupus erythematosus (SLE)

08 CRISPR/Cas9-mediated gene knockout for DNA methyltransferase Dnmt3a in CHO cells displays enhanced transgenic expression and long-term stability

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Aims: DNA methyltransferase Dnmt3a was knocked out by CRISPR/Cas9 technology to construct Dnmt3a-deficient CHO cell expression system. The molecular mechanism of DNA methylation affecting the expression stability of recombinant protein in CHO cells was also revealed. Methods: The sgRNA target of Dnmt3a KO was designed to construct plasmid. Selection and identification of monoclonal Dnmt3a KO CHO cells by PCR, Western blotting and sequencing. Cell proliferation was detected by CCK-8, and apoptosis was detected by Annexin V-FITC /PI double staining. Transgene expression vectors driven by two commonly used CMV and EF1a promoters were constructed and transfected into CHO

cells. Polycolonies of the 3a-30 and control CHO-K1 cells stably transfected with CMV or EF1a were passaged under selection pressure in the presence (G418+) or absence (G418) of G418 for 60 passages. The MFIs of eGFP in the recombinant CHO cells were detected to evaluate the intensity values of the expressed eGFP at 10, 20, 30, 40, 50 and 60 passages. The total DNA methylation level in CHO cells was analyzed by flow cytometry. Detection of CpG island methylation in different promoter regions of recombinant CHO cells by MALDITOF mass array. Results: Six Dnmt3a-deficient CHO cell monoclonal clones were identified by PCR and sequencing. The results of cell proliferation and apoptosis showed that the biological characteristics of Dnmt3a defective CHO cell line were not significantly different from those of normal CHO cells. The Dnmt3a-deficient 3a-30 cell line transfected with CMV promoter displays the most stable and the highest expression levels regardless of the presence or absence of G418, suggesting that the Dnmt3a KO in CHO cells can enhance the long-term stability of recombinant protein expression by using CMV promoter for at least 50 passages. The results of DNA methylation analysis revealed that the rate of DNA methylation in the analysed 10 CpG sites of CMV promoter at high passage (P50) was lower in 3a-30 cell line than in natural CHO cell line. By contrast, Dnmt3a KO exerted no influence on the reduction in the DNA methylation of EF1a promoter in the transfected CHO cells. Conclusion: The Dnmt3a gene in CHO-K1 cells was knocked out by CRISPR/Cas9 technology, screened and obtained the Dnmt3a defective CHO-K1 cell line. Dnmt3a KO positively affected the maintenance of recombinant protein production in stably transfected CHO cells. The stability of transgene expression during long-term cultivation could be distinctly enhanced in Dnmt3a-deficient CHO cells transfected with CMV promoter, presumably because of the distinct reduction in the DNA methylation of CMV promoter in stably transfected Dnmt3a-deficient CHO cells.

Key Words: Chinese hamster ovary cell, DNA methylation, DNA methyltransferase Dnmt3a, gene knockout, transgene expression

09 Structural insights into trans-histone regulation of H3K4 methylation by unique histone H4 binding of MLL3/4

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Abstract

MLL3 and MLL4 are two closely related members of the SET1/MLL family of histone H3K4 methyltransferases and are responsible for monomethylating histone H3K4 on enhancers, which are essential in regulating cell-type-specific gene expression. Mutations of MLL3 or MLL4 have been reported in different types of cancer. Recently, the PHD domains of MLL3/4 have been reported to recruit the MLL3/4 complexes to their target genes by binding to histone H4 during the NT2/D1 stem cell differentiation. Here we show that an extended PHD domain (ePHD6) involving the sixth PHD domain and its preceding zinc finger in MLL3 and MLL4 specifically recognizes an H4H18-containing histone H4 fragment, and modifications of residues surrounding H4H18 modulate H4 binding to MLL3/4. Our In vitro methyltransferase assays and cellular experiments further reveal that the interaction between ePHD6 of MLL3/4 and histone H4 is required for their nucleosomal methylation activity and MLL4-mediated neuronal differentiation of NT2/D1 cells.

Key words: MLL3, PHD domain, histone H4

10 Dynamic Methylome of Internal mRNA N^7-methylguanosine and Its Regulatory Role in Translation

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Over 150 types of RNA modifications are identified in RNA molecules. Transcriptome profiling is one of the key steps in decoding the epitranscriptomic panorama of these chemical modifications and their potential functions. N^7-methylguanosine (m^7G) is one of the most abundant modifications present in tRNA, rRNA and mRNA 5'cap, and has critical roles in regulating RNA processing, metabolism and function. Besides its cap position in mRNAs, m^7G is also identified in internal mRNA regions. However, its transcriptome-wide distribution and dynamic regulation within internal mRNA regions remain unknown. Here, we have established m^7G individual-nucleotide-resolution cross-linking and immunoprecipitation with sequencing (m^7G miCLIP-seq) to specifically detect internal mRNA m^7G modification. Using this approach, we revealed that m^7G is enriched at the 5'UTR region and AG-rich contexts, which are well-conserved across different human/mouse cell lines and mouse tissues. Strikingly, the internal m^7G is dynamically regulated under both H_2O_2 and heat shock treatments, with both remarkable accumulations in the CDS and 3'UTR regions and function in promoting mRNA translation efficiency. Consistently, PCNA 3'UTR minigene reporter harboring m^7G site displays both enriched m^7G modification and increased mRNA translation upon H_2O_2 treatment compared to m^7G site-mutated minigene reporter (G to A). Taken together, our findings unravel the dynamic profiles of internal mRNA m^7G methylome and highlight the m^7G as a novel epitranscriptomic marker with regulatory roles in translation.

Key Words: N^7-methylguanosine, miCLIP-seq, Translation, Stress response

11 Subcellular transcriptome profiling by APEX2-mediated proximity-dependent RNA labeling

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The RNA localization is closely linked to its biological functions. Asymmetrically localized RNAs are vital to chromatin architecture, local translation, and RNA homeostasis. Current methods for studying RNA localization either lack throughput or suffer contaminations. In this study, we have developed a novel technique to profile local transcriptome. Our method, termed APEX2-mediated proximity-dependent RNA labeling (APEX-RNA labeling), utilizes reactive radicals generated by the reaction of the Btn-An probes with H_2O_2 at the catalysis of APEX2 to label proximal RNA. As a demonstration of the spatial specificity and depth of coverage in mammalian cells, we applied APEX-RNA labeling in the mitochondrial matrix, capturing all 13 mitochondrial mRNAs and no cytoplasmic RNAs. We then extended the methodology to open subcellular regions including nuclear lamina, nucleolus, and cell membrane. APEX-RNA labeling enriched 125 RNAs at nucleolus and 662 RNAs at nuclear lamina, with little overlapping between two sets of RNAs. The 119 mRNAs enriched by APEX-RNA labeling at cell membrane encode proteins that are involved in cell junction, motility, and anchoring. APEX-RNA labeling is thus a promising method for mapping the subcellular transcriptome, which could shed light on their functions in cell physiology.

Key Words: APEX2, proximity-dependent labeling, Biotin-aniline probe, subcellular transcriptome

12 Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-Heptose

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Immune recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors often activates proinflammatory NF-kappaB signalling. Here we combined a transposon screen in *Yersinia pseudotuberculosis* with biochemical analyses and identified ADP-beta-D-manno-heptose (ADP-Hep), which mediates type III secretion system-dependent NF-kappaB activation and cytokine expression. ADP-Hep, but not other heptose metabolites, could enter host cytosol to activate NF-kappaB. A CRISPR-Cas9 screen showed that activation of NF-kappaB by ADP-Hep involves an ALPK1 (alpha-kinase 1)-TIFA (TRAF-interacting protein with forkhead-associated domain) axis. ADP-Hep directly binds the N-terminal domain of ALPK1, stimulating its kinase domain to phosphorylate and activate TIFA. The crystal structure of the N-terminal domain of ALPK1 and ADP-Hep in complex revealed the atomic mechanism of this ligand-receptor recognition process. HBP was transformed by host adenylyltransferases into ADP-heptose 7-P, which could activate ALPK1 to a lesser extent than ADP-Hep. ADP-Hep (but not HBP) alone or during bacterial infection induced Alpk1-dependent inflammation in mice. Our findings identify ALPK1 and ADP-Hep as a pattern recognition receptor and an effective immunomodulator, respectively.

Keywords: innate immunity, bacterial infection, ALPK1, ADP-heptose.

13 靶向糖链结合受体LOX-1抑制食管癌发生的作用机制研究

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食管癌是一种地域性分布很强的恶性肿瘤，世界各国和地区的发病率相差很大。我国高发，排在恶性肿瘤的第4位，年新发病例15万以上，占世界年发病例总数的一半多。食管癌准确的病因仍不完全清楚，多数认为，食管粘膜上皮肿瘤的发生是由于多种因素联合作用、长期慢性刺激的结果，包括热饮、高盐饮食等对食管黏膜的慢性理化刺激，可致局限性或弥漫性上皮增生，形成食管癌的癌前期病变。在过去一个多世纪里，经过外科专家的不懈努力，手术技术、手术设备的不断进步以及各种辅助放化疗和生物免疫治疗等综合治疗方案的不断推陈出新，食管癌治疗较过去得到了很大的提高，但5年生存率在很长一段时间里仍无明显提高。化疗是食管癌治疗常用的方法，但是化疗药物对靶点的选择性差，在杀伤肿瘤细胞的同时对机体的正常细胞也会具有杀伤作用，因此亟待寻求一种针对消化道系统癌症疗效显著、副作用小的抗肿瘤药物和治疗方法。

氧化性低密度脂蛋白受体-1（LOX-1）是胞外段含有典型的C型凝集素样结构的模式识别受体，在动脉粥样硬化、高血脂和糖尿病等代谢性疾病中表达上调，而这些代谢性疾病都与肿瘤发生高度相关。在本研究中，我们通过分析TCGA数据库中不同肿瘤的LOX-1 mRNA表达数据发现，LOX-1在消化道肿瘤组织中均呈现上调趋势，其中在食管癌组织中上调水平最显著。而在4-NQO化合物诱导的食管癌小鼠模型中，相较于其他C型凝集素超家族分子，LOX-1在小鼠食管癌组织中的表达最高，提示LOX-1在食管癌发生发展中可能发挥重要作用。细胞水平分子生物学实验证实，LOX-1下调诱发细胞发生自噬性死亡，进而显著抑制食管癌细胞的活力以及克隆形成能力。进一步利用蛋白质谱组学技术和磷酸化激酶芯片筛选，我们阐明缺乏细胞内信号结构域的LOX-1通过招募接头分子RACK1调控

ERK1/2信号通路来实现下游效应事件的分子机制。此外，天然褐藻中提取的细胞壁聚糖Fucoidan可以促使食管癌细胞中LOX-1蛋白稳定性下降，诱导细胞发生自噬性死亡。我们的工作对C型凝集素样受体LOX-1介导食管癌发生的功能以及分子作用机制进行深入研究，并探讨海洋天然产物Fucoidan通过作用该靶标的抗肿瘤生物学活性，为食管癌靶向治疗提供新的有效策略。

14 hCINAP regulates the DNA damage response and mediates the resistance of acute myelocytic leukemia cells to therapy

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Maintenance of genomic stability is critical for the proper function of organisms. When DNA damage occurs and is detected, a series of repair proteins sequentially accumulate at the damaged site and induce signal transduction pathways to initiate the subsequent repair of double-stranded DNA breaks (DSB). Acute myeloid leukemia (AML) is a genetically heterogeneous malignant disorder of the hematopoietic system, characterized by the accumulation of DNA-damaged immature myeloid precursors. In this study, we find that hCINAP is involved in the repair of DSB and that its expression correlates with AML prognosis. hCINAP is a highly conserved adenylate kinase that plays essential roles in the growth of eukaryotic cells. Following DSB, hCINAP is recruited to damage sites where it promotes SENP3-dependent deSUMOylation of NPM1, which in turn results in the dissociation of RAP80 from the damage site and CTIP-dependent DNA resection and homologous recombination. NPM1 SUMOylation is required for recruitment of DNA repair proteins at the early stage of DSB, and SUMOylated NPM1 impacts the assembly of the BRCA1 complex.

Moreover, we find that knockdown of hCINAP also sensitizes a patient-derived xenograft (PDX) mouse model to chemotherapy. In clinical AML samples, low hCINAP expression is associated with a higher overall survival rate in patients. These results provide mechanistic insight into the function of hCINAP during the DNA damage response and its role in AML resistance to therapy. Meanwhile, the discovery of sumoylation/desumoylation pathways that function to control DNA damage repair highlights the possibility of modulating these PTM activities in order to protect healthy cells from the effects of genotoxic anticancer therapies, while still eliminating the cancer cells. As the analogous ubiquitination system can easily be targeted by drugs, such pleiotropic mechanisms can be of substantial use in cancer treatments, offering a number of possibilities for future applications.

Key Words: hCINAP, NPM1, double-stranded DNA breaks (DSB), SUMOylation, acute myeloid leukemia (AML)

Reference

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15 A Pandas complex adapted for piRNA-guided transposon silencing

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The repression of transposons by the Piwi-interacting RNA (piRNA) pathway is essential to protect animal germ cells. In Drosophila ovaries, Panoramix (Panx) enforces transcriptional silencing by binding to the target-engaged Piwi-piRNA complex, although the precise mechanisms by which this occurs remain elusive. Here, we show that Panx functions together with a germline specific paralogue of a nuclear export factor, dNxf2, and its cofactor dNxt1 (p15), as a ternary complex to suppress transposon expression. Structural and functional analyses demonstrate that dNxf2 binds Panx via its UBA domain, which plays an important role in transposon silencing. Unexpectedly, dNxf2 interacts directly with dNxf1 (TAP), a general nuclear export factor. As a result, dNxf2 prevents dNxf1 from binding to the FG repeats of the nuclear pore complex, a process required for proper RNA export. Transient tethering of dNxf2 to nascent transcripts leads to their nuclear retention. Therefore, we propose that dNxf2 may function as a Pandas (Panoramix-dNxf2 dependent TAP/p15 silencing) complex, which counteracts the canonical RNA exporting machinery and restricts transposons to the nuclear peripheries. Our findings may have broader implications for understanding how RNA metabolism modulates epigenetic gene silencing and heterochromatin formation.

16 Taurine-mediated browning of white adipose tissue is involved in its protective role against obesity in mice

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Taurine, a nonprotein amino acid, is widely distributed in almost all animal tissues. Ingestion of taurine helps to improve obesity and its related metabolic disorders. However, the molecular mechanism underlying the protective role of taurine against obesity is not completely understood. In this study, it was found that intraperitoneal treatment of the mice with taurine alleviated high-fat diet (HFD)-induced obesity, improved insulin sensitivity, and increased energy expenditure and adaptive thermogenesis of the mice. Meanwhile, administration of the mice with taurine markedly induced the browning of inguinal white adipose tissue (iWAT), with significantly elevated expression of PGC1α, UCP1 and other thermogenic genes in iWAT. In vitro studies indicated that taurine also induced the development of brown-like adipocytes in C3H10T1/2 white adipocytes. Knockdown of PGC1α blunted the role of taurine in promoting the brown-like adipocytes phenotype in C3H10T1/2 cells. Moreover, taurine treatment enhanced AMPK phosphorylation in vitro and in vivo, and knockdown of AMPKα1 prevented taurine-mediated induction of PGC1α in C3H10T1/2 cells. Consistently, specific knockdown of PGC1α in iWAT of the HFD-fed mice inhibited taurine-induced browning of iWAT, with the role of taurine in the enhancement of adaptive thermogenesis, the prevention of obesity and the improvement of insulin sensitivity being partially impaired. These results reveal a functional role of taurine in facilitating the browning of white adipose tissue, which is dependent on the induction of PGC1α. Our studies also suggest a potential mechanism for the protective role of taurine against obesity which involves taurine-mediated browning of white adipose tissue.

Key Words: Taurine, White adipose tissue (WAT) browning, Obesity, Energy expenditure, Adaptive thermogenesis, PGC1α, AMPK

17 Oncogenic lncRNA *TURBOR* promotes Warburg effect by enhancing LDHA enzyme activity

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Aerobic glycolysis, also known as the Warburg effect, is a hallmark of cancer and essential for malignancies, but its regulation and modulation in cancer cells remain poorly understood. Here, by large-scale functional screening, we identified a tumor-associated and broadly expressed oncogenic long noncoding RNA *TURBOR*, which is highly expressed in multiple types of human cancers. Notably, knocking down *TURBOR* significantly inhibits the proliferation of multiple types of cancer cells and reduces tumor growth in vivo. Mechanistically, *TURBOR* directly binds to lactate dehydrogenase A (LDHA), an essential glycolysis-associated enzyme, and enhances its enzymatic activity, thereby promoting glycolysis. Clinically, high expression of *TURBOR* is significantly associated with poor prognosis in many types of human cancers. Together, our results identified a new long noncoding RNA (lncRNA) that modulated a cancer-specific regulation mechanism for the Warburg effect and suggested a potential target against one of cancer's vulnerabilities for developing broad-acting anti-cancer therapies.

Key Words: Long noncoding RNA, Warburg effect, Tumor proliferation

18 IL-17B/IL-17RB signaling regulates lysine 63-linked Beclin-1 ubiquitination to strengthen self-renewal and tumorigenesis in gastric cancer

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Cancer stem cells (CSCs) display many malignant biological traits including tumorigenesis, metastasis, drug resistance and angiogenesis. Discovering new markers specific for CSCs and elucidating their regulatory mechanisms is a significant goal. Herein, we found that IL-17B /IL-17RB signaling promoted self-renewal and tumorigenesis of gastric cancer (GC) cells by activating autophagy. We identified this pathway by first determining that IL-17RB expression is significantly up-regulated in spheroid cells, which was closely associated with the degree of differentiation of patient-derived GC tissues. Exogenous recombinant IL-17B (rIL-17B) promoted the self-renewal capacity of GC cells in vitro and enhanced tumor growth and metastasis in vivo. Moreover, we found that rIL-17B induced autophagosome formation and cleavage-mediated transformation of LC3 in GC and 293T cells. Interestingly, inhibition of autophagy by ATG7 knockdown reversed rIL-17B induced self-renewal of GC cells. Further study revealed that rIL-17B promoted K63-linked ubiquitination of beclin-1 by mediating the binding of TRAF6 to beclin-1. Interfering with IL-17RB expression abolished all the effects of rIL-17B on ubiquitination of beclin-1 and autophagic activation of GC cells. Lastly, we discovered that IL-17B expression in the serum of patients was positively correlated with IL-17RB expression in GC tissues. In addition, rIL-17B increased IL-17RB expression in GC cells. Direct overexpression of IL-17RB in 293T cells mimics stimulated rIL-17B, which promoted K63-linked ubiquitination of beclin-1 and binding of TRAF6 to beclin-1. Together, these results revealed the novel action of IL-17B/IL-17RB signaling on CSCs and might provide new therapeutic targets against gastric cancer.

Key Words: IL-17B; IL-17RB; autophagy; K63-linked ubiquitination of Beclin-1; gastric cancer; cancer stem cell

19 Zinc Enhances the Formation of Liquid and Hydrogel Phases of Human Tau and thereby Increases Tau Toxicity in Neuronal Cells

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The homeostasis of zinc is fundamentally important in the CNS to ensure many physiological processes, but dyshomeostasis and accumulation of zinc are observed in the brain of patients with Alzheimer's disease. The transition between soluble intrinsically disordered Tau protein and Tau filaments from Alzheimer's disease is unknown. Liquid-liquid phase separation by low-complexity domains of proteins is the first step of protein aggregation, which can generate membrane-less organelles and drive the formation of pathological filaments. In this paper, we studied the influences of zinc on phase transition, filament formation, and neurotoxicity of a pathological mutant △K280 of full-length human Tau to elucidate the molecular mechanism underlying Alzheimer's disease. We report that zinc enhances the intrinsic ability of △K280 to complete the required phase transition to form pathological filaments. Substitution of Cys-291 and Cys-322 with Ala, however, essentially eliminates such enhancing effects of Zn^{2+} on the fibrillization and phase transition of △K280. Zinc dramatically accelerates hyperphosphorylation and abnormal aggregation of △K280 both in vitro and in SH-SY5Y neuroblastoma cells. Furthermore, zinc enhances the formation of liquid and hydrogel phases and elevated ROS production. These findings elucidate how zinc regulates Tau phase separation, aggregation associated with Alzheimer's disease.

Key Words: Tau protein, Zinc, Alzheimer's disease, Liquid-liquid phase separation, Protein aggregation, Tau toxicity, Phase transition

20 NRDE2 negatively regulates exosome functions by inhibiting MTR4 recruitment and exosome interaction

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The exosome functions in the degradation of diverse RNA species, yet how it is negatively regulated remains largely unknown. Here, we show that NRDE2 forms a 1:1 complex with MTR4, a nuclear exosome cofactor critical for exosome recruitment, via a conserved MTR4-interacting domain (MID). Unexpectedly, NRDE2 mainly localizes in nuclear speckles, where it inhibits MTR4 recruitment and RNA degradation, and thereby ensures efficient mRNA nuclear export. Structural and biochemical data revealed that NRDE2 interacts with MTR4's key residues, locks MTR4 in a closed conformation, and inhibits MTR4 interaction with the exosome as well as proteins important for MTR4 recruitment, such as the cap-binding complex (CBC) and ZFC3H1. Functionally, MID deletion results in the loss of self-renewal of mouse embryonic stem cells. Together, our data pinpoint NRDE2 as a nuclear exosome negative regulator that ensures mRNA stability and nuclear export.

Key words: NRDE2; the nuclear exosome; MTR4 recruitment; mRNA export

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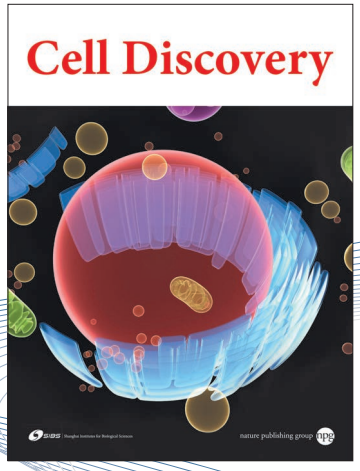

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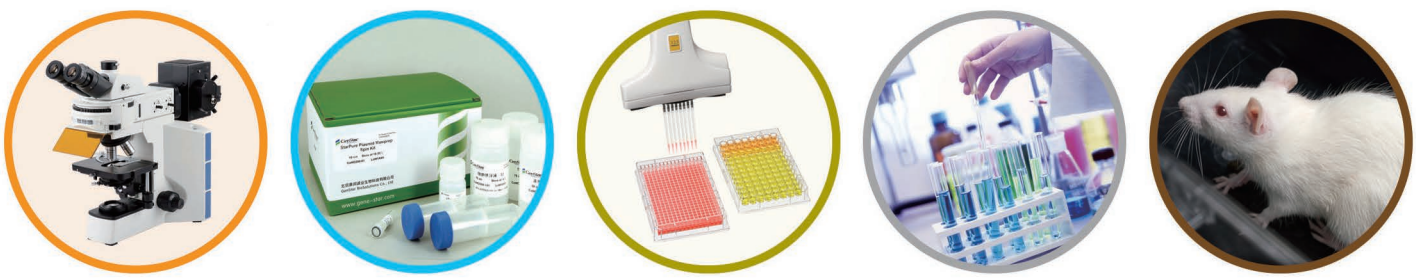

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