第三届中国生物物理学会代谢组学分会年会暨 2023 年代谢组学国际研讨会

会议摘要手册

邀请报告

Exploring Nutrition and Gut Health through Metabolic Phenotyping

Jia Li

Gut health encompasses the overall well-being and functionality of the gastrointestinal tract, a complex system profoundly shaped by both the gut microbiome and dietary habits. The gut microbiome, composed of microorganisms and their metabolites, undergoes significant modulation in response to our diet and has been associated with various digestive diseases. Yet, our understanding of the cause-and-effect relationships between the gut microbiome and gut health remains limited, despite their crucial roles in developing strategies for disease prevention and intervention.

In this presentation, I will delve into the application of metabolic phenotyping in dietary interventions and prevention studies for digestive diseases, including Inflammatory Bowel Disease (IBD) and colon cancer. Additionally, I will explore how surgical interventions for obesity, such as bariatric surgery, disrupt the gut microbiome environment in comparison to non-surgical approaches. These findings provide valuable insights into the long-term implications for gut health following bariatric surgery. Finally, I will shed light on the biological significance of these metabolic changes within the gut lumen resulting from diet-gut microbial interactions and their potential impact on overall gut health. These studies advance our understanding of nutrition and gut health and contribute to the exploration of the host-microbiota-diet interactome.

Deep Metabolome Analysis of Micro-Samples

Liang Li

The Metabolomics Innovation Centre of Canada, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

ABSTRACT: The growth of metabolomics in the past decade can be directly linked to the development of modern analytical techniques that are able to quantitatively profile a wide range of metabolites in a sample. Liquid chromatography mass spectrometry (LC-MS) has become a powerful tool for metabolomic profiling. To increase the sensitivity of the LC-MS platform, researchers are continually developing more sensitive mass spectrometers, new LC techniques and improving ionization efficiency of metabolites. The latter can be done using high-performance chemical derivatization methods, such as chemical isotope labeling (CIL). In this presentation, recent advances in CIL LC-MS for metabolome analysis of micro-samples are discussed. These include the analysis of dried urine and blood samples, finger blood, single cells, spheroids, and tiny tissues. Novel sample handling methods in dealing with micro-samples with limited amounts of starting materials will be described. LC-MS strategies for ultra-sensitive analysis of metabolites with high metabolomic coverage and accurate relative quantification accuracy will be presented.

KEY WORDS: Ultra-sensitive LC-MS, micro-sample processing, chemical isotope labeling, high coverage metabolomics

Gut Microbiome as Mediator of Chemical Exposome - Host Metabolism Crosstalk

Matej Orešič[a,b]
[a]School of Medical Sciences, Örebro University, Örebro, Sweden
[b]Turku Bioscience Centre, University of Turku, Turku, Finland

The origins of many non-communicable diseases occur often as a result of complex interactions of environmental and genetic factors. Growing evidence suggests that exposure to endocrine disrupting chemicals such as per- and polyfluoroalkyl substances (PFAS) interfere with host metabolism, however, the underlying mechanisms are poorly understood. Here, recent and ongoing studies will be presented that aim to elucidate the link between the exposures to harmful chemicals and metabolic health.

In a cohort of 264 Danes (121 men and 143 women, aged 56.6 ± 7.3 years, BMI 29.7 ± 6.0 kg/m2), we measured serum bile acids (BAs), PFAS and additional twenty-seven environmental toxicants as well as gut microbiome composition by shotgun metagenomic sequencing. We found that blood concentrations of widespread environmental toxicants such as PFAS associate with measures of body fat accumulation and insulin resistance in a sexually dimorphic manner. These associations may be mediated by gut microbiome-synthesized secondary BAs. These findings were substantiated by the outcome of the murine exposure study. In another study, we investigated potential role of exposure to PFAS in the developmental origin of metabolic disease. Human fetal livers from elective termination of pregnancies between 11-19 weeks of gestation (n = 78) were analyzed by both targeted and untargeted metabolomic analyses of lipids, polar metabolites, BAs and PFAS. Several amino acids, fatty acids and sugar derivatives in fetal livers were inversely associated with PFAS exposure, while the BA glycolithocholic acid was markedly positively associated with all quantified PFAS. Furthermore, 7α-hydroxy-4-cholesten-3-one (C4), a marker of BA synthesis rate, was strongly positively associated with PFAS levels and was detectable as early as gestational week 12. Identification of metabolic perturbations in the human fetus associated with PFAS exposure demonstrates that environmental exposure and its potential harmful impacts start *in utero*. These effects of PFAS in the fetus, particularly with respect to lipid and BA metabolism, might be responsible for reported adverse health effects during early life.

Mass spectrometry imaging - a novel technology to explore metabolic heterogeneity in liver cancer

Yulan Wang[1], Liang Xu[1], Jia Ying Joey Lee[2], Sin Chi Chew[3,4], Lit-Hsin Loo[2], Pierce Chow[3,4,5],
1.Singapore Phenome Center, Lee Kong Chian School of Medicine, Nanyang Technological University
2.Bioinformatics Institute, Agency for Science, Technology and Research, Singapore.
3.Department of Hepatopancreatobiliary and Transplant Surgery, Division of Surgery and Surgical Oncology, Singapore General Hospital and National Cancer Centre Singapore.
4.Program in Clinical and Translational Liver Cancer Research, Division of Medical Science, National Cancer Center Singapore, Singapore
5.Academic Clinical Programme for Surgery, Duke-NUS Medical School, Singapore.

Mass spectrometry imaging (MSI) provides spatially resolved metabolic information and enables the exploration of metabolic heterogeneity within and between tumors[1]. The two most common MSI techniques, matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI), have the potential to identify highly specific diagnostic and prognostic markers for different tumor niches or regions. Therefore, MSI has emerged as a key technology for label-free bioanalysis of the spatial distributions of biomolecules, pharmaceuticals, and/or other xenobiotics in tissue sections[2-3]. The technology offers huge diagnostic potentials by augmenting decision making in personalised treatment strategies[4].

The ability of MSI to generate spatial metabolic information is based on dividing a tissue section into many pixels, typically ~50 μm between pixels. Mass spectrum is generated from each pixel, which contain rich information on the molecular ions and their relative intensity levels that can be found in the pixel. The annotation and validation of the found putative metabolites are vital for translational applications of MSI, which is also the current bottleneck of clinical adoptions of MSI technology. In this presentation, solutions to metabolite annotation and validation in MSI will be demonstrated. In addition, multi-modal image analysis and data integration to discover the tumor niches or regions that produce these putative metabolites will also be presented. Using these technologies and methods, we have identified several putative metabolic markers for hepatocellular carcinoma.

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基于多组学的偏头痛患者代谢特征研究

白玉 [1]*

1. 北京大学化学与分子工程学院，北京分子科学国家研究中心，北京，100871

*E-mail: yu.bai@pku.edu.cn

偏头痛（Migraine）是临床最常见的原发性头痛类型，人群发病率较高，严重影响患者的生活质量。偏头痛的诱发因素复杂多样，发病机制不明，临床缺乏根治疾病的特效药；同时，偏头痛缺乏特异性诊断方式和诊断标志物，使其临床诊断与治疗面临巨大挑战[1-3]。菌群代谢异常与偏头痛发病密切相关，代谢组可在分子水平上反映疾病的发生发展过程。

我们利用靶标和非靶标代谢组学策略，对偏头痛队列患者血清和粪便样本开展分析，利用 16SRNA 开展肠道菌群测序。进一步将代谢组学与肠道菌群数据结合，对偏头痛患者代谢特征开展研究。研究结果显示偏头痛患者发病期间的代谢特征扰动，以及偏头痛患者胆汁酸代谢异常，为基于菌群和血清代谢异常的偏头痛发病机制研究奠定基础，为偏头痛生物标志物的发现乃至疾病的诊断与治疗提供全新的视角和研究思路。

关键词: 偏头痛，肠脑轴，代谢组，肠道菌群，代谢特征

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Identification of Metabolite Interference Is Necessary for Accurate LC-MS Metabolomics Analysis

Zhikun Jia[1], Qiongju Qiu[1], Ruiping He[1], Tianyu Zhou[1,2], **Li Chen**[1,2,*]

[1]Shanghai Key Laboratory of Metabolic Remodeling and Health, Institute of Metabolism & Integrative Biology, Fudan University, Shanghai, 200433, China.

[2]Shanghai Qi Zhi Institute, Shanghai, 200030, China

ABSTRACT: Targeted metabolomics has been broadly used for metabolite measurement due to its good quantitative linearity and simple metabolite annotation workflow. However, metabolite interference, the phenomenon that one metabolite generates a peak in another metabolite's MRM setting (Q1/Q3) with close retention time (RT), may lead to inaccurate metabolite annotation and quantification. Besides isomeric metabolites having the same precursor and product ions that may interfere with each other, we found other metabolite interferences as the results of inadequate mass resolution of triple-quadruple mass spectrometry and in-source fragmentation of metabolite ions. Characterizing the targeted metabolomics data using 334 metabolite standards revealed that about 75% of the metabolites generated measurable signal in at least one other metabolite's MRM setting. Different chromatography can resolve 65~85% of these interfering signals among standards. Metabolite interference analysis combined with manual inspection of cell lysate and serum data suggested about 10% out of ~180 annotated metabolites were mis-annotated or mis-quantified. These results highlight a thorough investigation of metabolite interference is necessary for accurate metabolite measurement in targeted metabolomics.

KEY WORDS: Metabolite Interference, LC-MS, Targeted metabolomics

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Metabolomic studies provide new mechanistic insights into the pathogenesis of esophageal squamous cell carcinoma

Xing Jin[1,2], Jia Wu[1,2], Lei Liu[3], Wei Wang[3], Dan Liu[1,2], Si-Liang Wang[1,2], Cong-Cong Wang[1,2], Chen-Tai Qin[1,2], Miao-Miao Zheng[1,2], Wen-Lian Chen[1,2,*]

1. Cancer Institute, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China

2. Shanghai Frontiers Science Center of Disease and Syndrome Biology of Inflammatory Cancer Transformation, Shanghai 200032, China

3. Department of Thoracic Surgery, The Affiliated Tumor Hospital of Nantong University, Nantong 226300, China

ABSTRACT: Esophageal squamous cell carcinoma (ESCC) is a major subtype of esophageal cancer, characterized by evident metabolic perturbations. However, the precise identification of key metabolic signatures and pathways underpinning the pathogenesis of ESCC remains elusive. In light of this, we have undertaken a multi-omics study using clinical paired tissue samples. Our findings illuminate a series of perturbed amino acid metabolic pathways within ESCC tumors, attributed to the specific upregulation of pertinent metabolic enzymes. Notably, upon conducting a rigorous differential analysis, the methionine cycle emerges as the foremost among these orchestrated metabolic pathways. Mechanistic investigation revealed that methionine fosters ESCC growth by upregulating the oncogene NA4A2 via a SAM-METTL3-NR4A2 cascade. In addition, we have conducted a serum metabolomic study, thereby leading to the identification of a panel of 12 serum metabolites intricately linked to ESCC tumors. Noteworthy among these metabolites is pipecolic acid, whose remarkable elevation in murine models at the stage of esophageal squamous dysplasia establishes it as a novel predictive biomarker for ESCC. Moreover, our investigations unveil the importance of pipecolic acid for mitigating oxidative stress-induced DNA damage and consequent cell proliferation arrest in ESCC cells. In conclusion, our comprehensive analyses offer a panoramic insight into the intricate landscape of ESCC pathogenesis. Specifically, the aberrant activation of amino acid metabolic pathways within tumors stands out as an enabler of oncogene expression, thereby expediting cell growth. Simultaneously, certain metabolites within the circulating blood assume a tumor-promoting role in the initiation and progression of ESCC.

KEY WORDS: Esophageal squamous cell carcinoma; Metabolomic study; Methionine cycle; NR4A2; Pipecolic acid

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Thermodynamics, control, and design principles of metabolic networks

Wenchao Fan[1], Weiyan Zheng[1], and Ziwei Dai[1*]

1. Department of Systems Biology, School of Life Sciences, Southern University of Science and Technology, Shenzhen 518055, China

ABSTRACT: Thermodynamics not only affect the feasibility and directionality of metabolic reactions, but also shape the pattern of metabolic control (i.e. how activity of metabolic enzymes in a pathway regulates the flux carried by it). However, it is unclear how the thermodynamic and kinetic parameters of metabolic reactions coordinate with each other for the optimal design of metabolic networks, largely due to the lack of quantitative kinetic and thermodynamic datasets of metabolic reactions at the genome-scale. To characterize the thermodynamic features of metabolic reactions and understand the design principles of metabolic networks, we develop a graph neural network-based deep learning model, dGbyG, for accurate prediction of Gibbs free energy change of metabolic reactions from molecular structure and features of metabolites. We then demonstrate that dGbyG can achieve higher accuracy and broader coverage compared to all existing methods and apply dGbyG to predict the standard Gibbs free energy change of 11,156 reactions in the human genome-scale metabolic network model, Recon3D. Furthermore, based on the theory of metabolic control analysis (MCA), we mathematically prove that an analytical relationship exists between the thermodynamic and kinetic parameters of metabolic enzymes when a metabolic pathway maximizes its efficiency. By integrating thermodynamic parameters predicted using dGbyG and several enzyme kinetics and cancer multi-omics databases, we validate that this principle is highly consistent with the abundance of metabolic enzymes in human cancer cells. Finally, we discover a general rule about the trade-off between the flux efficiency and controllability of metabolic networks. This study discovers a universal principle on how metabolic pathways optimize their enzyme allocation to achieve maximal efficiency and unravels a general trade-off between efficiency and controllability in the operation of cellular metabolism.

KEY WORDS: metabolic networks, thermodynamics, metabolic control analysis, machine learning

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模拟和重塑微生物代谢秘诀的合成生物学创新之路

邓子新
上海交通大学/武汉大学

Role of CtBP in cancer: a central hub connecting growth, migration and death

Lifen Wang, Tianyu Liu, Li Wang &Li-jun Di*

1. Department of Biomedical Sciences, Faculty of Health Sciences, University of Macau

Contact: Lijundi@um.edu.mo

ABSTRACT: Since its initial discovery in the 1980s based on the finding that it can form a complex with the E1A protein of adenovirus, most studies have found that CtBP is involved in developmental regulation in different animal models. However, approximately 10 years ago, numerous studies began demonstrating that CtBP plays a promoting role in tumor initiation, progression, and metastasis. Through genome-wide ChIP-seq analysis combined with gene expression analysis, we have provided the first evidence of CtBP's global transcriptional regulatory role in cell stemness maintenance, DNA damage repair, migration and metastasis etc. Over the past few years, we have individually confirmed CtBP-mediated transcriptional repression of genes such as BRCA1, RAD51, SIRT4, SREBF2 etc., and thereby promoting chemotherapy resistance and metastatic progression in cancer cells. In our recent study, we further found that CtBP mRNA levels are induced by serum starvation, hypoxia, and even suspension culture, and RNA-seq analysis reveals significant enrichment of the ferroptosis pathway. CtBP KO breast cancer cells exhibit an accumulation of various types of fatty acids, and further analysis reveals that CtBP KO leads to increased fatty acid synthesis and reduced fatty acid oxidation. Functional analysis confirms that CtBP indeed has an inhibitory effect on ferroptosis. Previous studies have attempted to screen for compounds that inhibit CtBP dimerization in order to inhibit its function, but with limited success. Currently, we are exploring a protein complement assay (PCA) approach using Gaussia luciferase as a reporter gene, aiming to screen natural product libraries. Therefore, based on establishing CtBP as a therapeutic target, we are pursuing novel targeting strategies in cancer treatment.

KEY WORDS: CtBP, ferroptosis, breast cancer

基于网络的代谢组学数据分析新方法及应用

董继扬[1,*]，郭凡靖[1]，汪永佩[1]，林亘今[1]，邓伶莉[2], Daniel Raftery[3]

1. 电子科学系，厦门大学，厦门 361005，中国

2. 信息工程系，东华理工大学，南昌 330013，中国

3. Northwest Metabolomics Research Center, University of Washington, Seattle, WA 98109, USA

生物代谢是一个动态的复杂系统，包含大量化学反应和分子间相互作用，意味着系统中各代谢物的状态(浓度)互相影响且动态变化。因此，当前代谢组学试图通过分析少量代谢物(低覆盖度)的状态，推断机体的生理病理状况，必将给数据分析和解释提出巨大挑战。

复杂网络是近二十年迅速发展起来的研究领域[1]，它将复杂系统中的实体抽象成节点，将实体之间的关系抽象成连线，构造现实复杂系统的抽象模型。复杂网络分析关注系统中个体相互关联作用的结构，是理解复杂系统性质和功能的一种有效途径。近年来，复杂网络在代谢组学数据分析和解释中受到越来越多的关注[2]。

代谢组学研究涉及知识网络和统计网络两种模型。其中，知识网络是根据 KEGG、Reactome 等先验的知识库构建，网络的节点通常表示代谢物(包括检测和未检测)，而边则表示代谢反应；统计网络则是基于代谢表型的观测数据计算得到的检测代谢物之间的统计关系网络，网络的节点表示检测的代谢物，边表示两相应代谢物的统计关系(如相关系数)。两种网络模型反应机体代谢系统的两个侧面。本文提出三种基于网络的数据分析算法，分别用于代谢组学研究中的批次校正、代谢通路富集和疾病异质性分析。

一、将知识网络用于代谢通路富集分析中，提出药物协同作用分析新方法(iMSEA)[3]。构建代谢物-反应关联网络模型，将检测到的代谢物作为网络中的种子节点，并通过网络的传播将检测到的代谢物信息传播至未检测到的代谢物节点，以弥补目前代谢物检测范围较小的问题。并根据网路中通路的活性来计算协同系数，衡量药物相互作用对通路的协同影响。为研究药物联合作用的分子机制提供代谢组学的视角。

二、提出了一种基于数据结构一致性的批次效应校正新方法(CordBat)[4]。通过构建统一的高斯图模型，各个批次在代谢网络水平上向预设的参考批次对齐。传统的批次效应校正方法倾向于在不同批次之间实现代谢物浓度对齐，而非网络对齐。相比这些方法，CordBat 在去除批次效应的同时能更好地保留代谢相关性。

三、利用观测的表型数据对通用的知识网络模型进行修正，获得疾病特异性的代谢网络，并在此基础上进行通路富集分析 dci-MSEA[5]，以实现高灵敏、高特异的扰动通路识别。同时，引入单样本分析思路，从代谢层面剖析疾病异质性。

关键词: 网络分析，代谢组学数据分析，知识网络模型，统计网络模型

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Oligomer-Finder: non-target screening of polymer oligomers in environmental and human samples

Changzhi Shi[1], Mingliang Fang[1,*]

1. Department of Environmental Science and Engineering, Fudan University

ABSTRACT: Polymer production and contamination has become a serious environmental issue. However, traditional studies have focused on the polymers themselves such as microplastics and little information is available for their breakdown products. In this study, we developed a non-targeted tool and strategy, named "Oligomer-Finder," which utilizes the LC-HRMS method for the screening and annotation of polymer oligomers in complex environmental samples. This comprehensive strategy operates on four dimensions, relying on the structural characteristics of oligomers. Screening for oligomers was based on “seed oligomers” and other oligomers associated with “seeds” with different confidence levels can be found. These "adjacent oligomers" are further classified into homologues. Specifically, a database comprising oligomers from 170 polymer sources was established for the first time. This was accomplished by elucidating the correlation between oligomer structures and MS information, leveraging open-source polymer databases and pertinent literatures. Identification of seed oligomers was conducted through screening for repeated neutral losses (rNL) within MS/MS spectra. Subsequently, homologues were identified based on anticipated mass differences and retention times (RT), while congeners, referring to oligomers sharing the same polymer backbone but possessing distinct end groups, were screened using the end group database and MS information. To validate the effectiveness of this algorithm, real environmental samples, including lake water, soil, and landfill leachate, as well as samples simulating polymer contamination, were analyzed. With the assistance of self-developed R packages and the oligomer database, Oligomer-Finder has achieved rapid screening and annotation of oligomers derived from diverse polymers, all within minutes and with high confidence. This tool holds significant potential in simplifying research endeavors concerning oligomers as emerging pollutants.

KEY WORDS: non-targeted analysis; oligomers; polymers; chemical informatics

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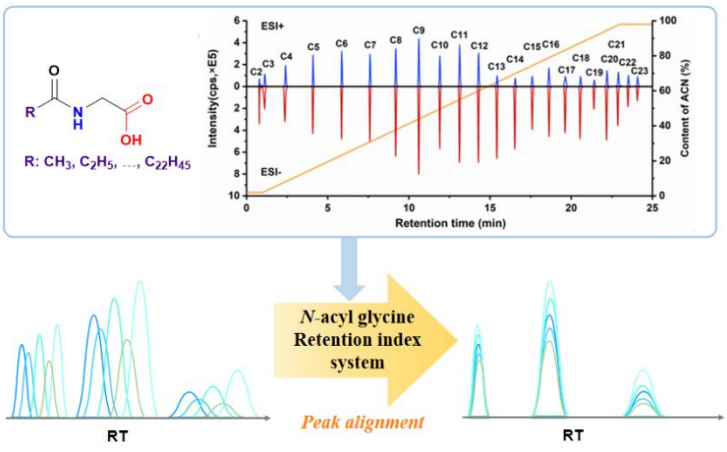
Retention Index for Metabolomics Analysis

Jun-Di Hao[1], Yao-Yu Chen[1], Quan-Fei Zhu[2], Yu-Qi Feng[1,2*]
1. Department of Chemistry, Wuhan University, Wuhan 430072, China
2. School of Public Health, Wuhan University, Wuhan 430071, China

ABSTRACT: Peak alignment is a crucial step in liquid chromatography-mass spectrometry (LC-MS)-based large-scale untargeted metabolomics workflows, as it enables the integration of metabolite peaks across multiple samples, which is essential for accurate data interpretation. Slight differences or fluctuations in chromatographic separation conditions, however, can cause the chromatographic retention time (RT) shift between consecutive analyses, ultimately affecting the accuracy of peak alignment between samples. Here, we introduce a novel RT shift correction method based on the retention index (RI) and apply it to peak alignment. We synthesized a series of Nacyl glycine (C2−C23) homologues via the amidation reaction between glycine with normal saturated fatty acids (C2−C23) as calibrants able to respond proficiently in both mass spectrometric positive- and negative-ion modes. Using these calibrants, we established an N-acyl glycine RI system. This RI system is capable of covering a broad chromatographic space and addressing chromatographic RT shift caused by variations in flow rate, gradient elution, instrument systems, and LC separation columns. Moreover, based on the RI system, we developed a peak shift correction model to enhance peak alignment accuracy. Applying the model resulted in a significant improvement in the accuracy of peak alignment from 15.5 to 80.9% across long-term data spanning a period of 157 days. To facilitate practical application, we developed a Python-based program, which is freely available at https://github.com/WHU-Fenglab/RI-based-CPSC.



KEY WORDS: ： Retention Index, Retention time shift, LC-MS

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High Spatial Resolution Mass Spectrometry Techniques for Single Cell Imaging

Wei Hang

Ministry of Education (MOE) Key Laboratory of Spectrochemical Analysis and Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China

ABSTRACT: Visual chemical identification by mass spectrometry imaging (MSI) approaches has become increasingly important in biological and chemical research and can help to intuitively understand complex molecular processes taking place within systems of life. For any imaging technique, high spatial resolution is the eternal pursuit. High spatial resolution can unambiguously illuminate differences and details, and provide an opportunity for diversiform fields, especially for single-cell analysis. Traditional single-cell analysis refers to stochastic average values masked by bulk measurements, resulting in the loss of information related to intercellular chemical heterogeneities in large cell populations. However, the development of single-cell imaging makes it possible to understand the differences among organelles. Herein, we will present several new mass spectrometry techniques for single cell imaging, including micro-lensed fiber laser desorption, near-field desorption, and tip-enhance desorption mass spectrometry techniques. With these new high-resolution imaging approaches, we can conduct visual detection of both molecules and elements at the organelle level, which is difficult to achieve with existing laser-based mass spectrometry techniques.

KEY WORDS: Mass Spectrometry, Spatial Resolution, Single Cell Imaging.

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高清质谱成像空间分辨代谢组学新技术与应用

周晏合[1]，黄建鹏[1]，郁萌[1]，王相宜[1]，李通[1]，高佳敏[1]，史俊稳[2]，贺玖明[1,*]

1. 中国医学科学院药物研究所;

2. 维科托（北京）科技有限公司

近年来，组学技术的发展极大地推动了人们对生命活动和疾病机理的认识和临床精准诊治[1]。然而，基于匀浆或单细胞解离的组学分析技术会破坏组织中细胞和分子所处的空间位置，无法获得高异质性组织（如脑、肿瘤等）中的分子和细胞的分布及相互作用信息。基于质谱成像（MSI）技术的空间代谢组学（SM）得到快速发展，并在探索发病机制，发现生物标志物方面显示巨大潜力[2, 3]。空间分辨率是 MSI 的重要参数之一，微米级分辨率的 MSI 实现了代谢物在细胞中的精准定位。然而，灵敏度和空间分辨率成反比关系，做好灵敏度和分辨率之间的权衡一直是 MSI 分析中亟待解决的问题。本研究在课题组研发的空气动力辅助解吸电喷雾电离质谱成像（AFADESI-MSI）的基础上，设计了精细喷雾探针，并对成像的关键参数进行了系统性的优化和改进，研发了一种针对具有复杂微区组织的高清空间分辨代谢组学方法，保证了检测的灵敏度的同时，使空间分辨率达到 30 μm，实现了小鼠脑和临床胃癌组织的高清空间分辨代谢组学分析，构建了空间代谢谱图及代谢网络。

关键词: 空间代谢组学，质谱成像，代谢网络，脑，肿瘤微环境.

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Metabolite profiling and identification in single living cell

Guangming Huang*
School of Chemistry and Materials Science, University of Science and Technology of China, 230026, Hefei, China
E-mail: gmhuang@ustc.edu.cn

ABSTRACT: Direct observation of metabolites in single living cell by mass spectrometry offers a bright future for biological studies but also suffers severe challenges. For example, the highly variable response rates to immunotherapies underscore our limited knowledge about how tumors can manipulate immune cells. We demonstrated that the membrane topology of natural killer (NK) cells from patients with liver cancer showed that intratumoral NK cells have fewer membrane protrusions compared with liver NK cells outside tumors and with peripheral NK cells. Dysregulation of these protrusions prevented intratumoral NK cells from recognizing tumor cells, from forming lytic immunological synapses and from killing tumor cells. The membranes of intratumoral NK cells have altered sphingomyelin (SM) content and dysregulated serine metabolism in tumors contributed to the decrease in SM levels of intratumoral NK cells. Furthermore, we developed a method combining stable isotope tracing and induced electrospray mass spectrometry for more accurate metabolites identification. By using 13C6-glucose and ammonium chloride-15N as the sole carbon and nitrogen sources for cell culture, Escherichia coli synthesized metabolites with 15N and 13C elements. As a result, the identification confidence of metabolites achieved a universal improvement based on carbon/nitrogen labelling and filtration.

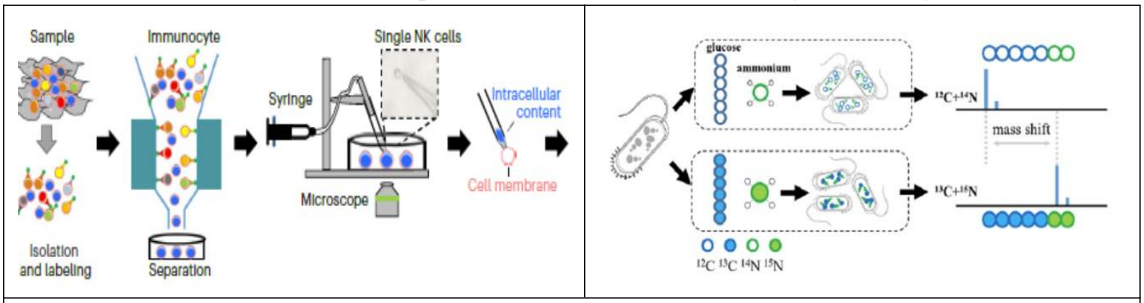

Fig.1 Single-cell mass spectrometry reveals membrane topology of NK cells and stable isotope protocol for more accurate metabolites identification.

KEY WORDS: Single cell metabolism，Mass spectrometry，Induced electrospray ionization

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肠道菌源酶在代谢性疾病中的作用

姜长涛
北京大学

The metabolic disorder of T cells leads to neurological and bowel diseases

Li Zhang[1], Hao-li Wang[1], Xin-tao Mao[1] , Ting-ting Wu[2], Yi-yuan Li[3, *], Jin Jin[1, 2, *]

[1]The MOE Key Laboratory of Biosystems Homeostasis & Protection, Zhejiang Provincial Key Laboratory for Cancer Molecular Cell Biology, Life Sciences Institute, Zhejiang University, Hangzhou, Zhejiang 310058, China

[2]Department of Gastroenterology, Sir Run Run Shaw Hospital, College of Medicine Zhejiang University, Hangzhou 310016, China

[3]Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Jiangsu Province High-Tech Key Laboratory for Bio-Medical Research, Southeast University, Nanjing 210096, China

*Correspondence: jjin4@zju.edu.cn (J.J.), 103200067@seu.edu.cn (Y.-y.L.)

ABSTRACT:

Diarrhea irritable bowel syndrome (IBS-D) is a continuous or intermittent disorder involving intestinal dysfunction associated with bowel movements. The pathogenesis of IBS-D is not been well understood, but its incidence is thought to be related to various factors, including gastrointestinal permeability, abnormal regulation of the brain-gut axis, inflammation, and psychological stress. However, the molecular mechanism of these factors-induced IBS remains poorly investigated. In this study, we found that T-cell-derived xanthine induced by chronic stress directly leads to the occurrence of IBS-D. Xanthine acted on intestinal epithelial cells and promoted the formation of exosome. Hemoglobin containing in exosomes specifically led to an abnormal expansion of probiotic *Lactobacillus*, which produces excessive colonic spermidine that further suppresses the expressions of interferon-stimulated genes and triggered IBS-D. Our study provides a theoretical basis for the pathological mechanism of psychological stress-induced IBS-D and highlights new targets for the treatment of IBS-D.

KEY WORDS: Diarrhea irritable bowel syndrome; Psychological stress; Xanthine; AdorA2B; Hemoglobin; *Lactobacillus*; Spermidine.

机体肝脏中脂酰辅酶 A 硫酯酶调节乙酸生成以对抗能量危机

王锦阳[1], 温雅馨[1], 赵文涛[1], 张妍[1], 林福荣[1], 欧阳聪[1], 王慧慧[1], 姚丽铮[1], 马欢欢[1], 卓岳[1], 黄慧英[1], 石秀林[2], 冯柳宾[3], 林东海[4,*], 江彬[1,*] 李勤喜[1,*]

1. 厦门大学医学与生命科学学院生命科学学院，细胞应激生物学国家重点实验室
2. 厦门大学第一附属医院，厦门市糖尿病研究所内分泌与糖尿病科，福建省糖尿病转化研究重点实验室
3. 厦门大学化学化工学院高场核磁共振中心
4. 厦门大学化学化工学院化学生物系

论文摘要：越来越多的证据表明，在糖尿病和长期饥饿等能量危机情况下，血液中乙酸水平明显增加。然而，关于血液中乙酸的产生机制、来源和其生物学意义仍存在许多未解之谜。我们通过综合运用糖尿病小鼠模型、GEO 数据库差异分析、腺病毒介导的小鼠肝脏基因特异性敲低、代谢物同位素追踪等技术开展了一系列研究发现，在糖尿病、饥饿等情况下 ACOT12 和 ACOT8 在肝脏中表达水平显著上调，产生非肠道菌群来源的乙酸；且 ACOT12 和 ACOT8 将脂肪酸来源的的乙酰辅酶 A 转化为乙酸和还原型辅酶 A，不仅提供了大量的乙酸，为肝外组织如大脑提供能量，而且还维持了持续脂肪酸 β 氧化和生酮过程所需的还原型辅酶 A。综上所述，血液中的乙酸水平可能是糖尿病的一个重要指标，不仅能反应脂肪酸 β 氧化的情况及机体能量代谢模式，而且能为糖尿病的精准分类提供重要依据。

关键词: 糖尿病；脂酰辅酶 A 硫酯酶 12；脂酰辅酶 A 硫酯酶 8；乙酸

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Metabolic Regulation of Critical Metabolites: Insights from NMR-based Metabonomic Analysis in Skeletal Muscle

Lin Donghai[1,*], Huang Caihua[2]， Zhou Yu[1], Zhou Lin[3], Zhou Hong[4],
Lu Ruohan[1], Zhang Shuya[1], Li Yantong[1], Xu Wenqi[1], Qiu Xu[1]
1. College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, China;
2. Research and Communication Center of Exercise and Health,
Xiamen University of Technology, Xiamen, China;
3. First Affiliated Hospital of Gannan Medical University, Ganzhou, China；
4. Physical Education Department, Xiamen University, Xiamen, China.

ABSTRACT: Metabolites play diverse regulatory roles in metabolic and signaling pathways, influencing gene expression, protein activity and cellular functions. Maintaining proper metabolite regulation is critical for cellular homeostasis and energy balance, as dysregulation can lead to metabolic disorders. In recent years, our team has used NMR-based metabonomic analysis to investigate the effects of lactate, 3-hydroxybutyric acid, α-ketoglutarate, taurine, creatine, trehalose and trimethylamine N-oxide on various biological samples. In particular, we focused on the metabolic profiling of lactate treatment in myoblasts. Our results show the significant effects of lactate treatment on myoblast proliferation and differentiation, as indicated by significantly increased expression levels of proteins related to cellular proliferation and differentiation, including p-AKT, p-ERK, MyoD and myogenin. Lactate treatment facilitates lactate uptake and intracellular utilization, activating the TCA cycle and enhancing energy production. Notably, lactate stimulates the activation of AMPK, as indicated by increased levels of p-AMPK and p-ACC. Our study sheds light on the underlying metabolic mechanisms associated with these metabolic treatments and contributes to a comprehensive understanding of their physiological implications and potential therapeutic targets in the human body, particularly in skeletal muscle.

KEY WORDS: NMR; Metabonomics; Metabolites; Metabolic regulation; Skeletal muscle.

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微流控质谱联用细胞药物代谢分析方法研究

林金明
清华大学化学系
E-mail: jmlin@mail.tsinghua.edu.cn

细胞是组成生物结构的基本单位，同时细胞研究是生命科学的基础。质谱分析技术是一种强大的分析方法，它可以用于细胞标志物和细胞内代谢物的鉴定与表征。质谱的单次样品分析就可以提供样品中大量化合物的信息，从而可以实现单一样品的多种物质的同时测定。同时，通过串联多级质谱检测器的二级离子谱图还可以提供所检测的物质的结构信息。我们通过将微流控平台与质谱进行联用，实现了细胞样品样本预处理和在线检测的同时进行，在微流控芯片上进行细胞的动态培养，研究细胞的药物代谢过程。本次报告将重点介绍细胞缺氧代谢、红景天抗缺氧以及维生素 D3 的细胞代谢研究的最新结果，拓展新型微流控芯片质谱联用仪器在细胞药物代谢研究中的应用领域。

关键词：微流控，细胞分析，药物代谢分析，质谱检测

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Mass spectrometry-based metabolite discovery and functional studies

Shuhai Lin[1],*

1. State Key Laboratory for Cellular Stress Biology, School of Life Sciences, Faculty of Medicine and Life Sciences, Xiamen University, Fujian 361102, China.

* E-mail: shuhai@xmu.edu.cn

ABSTRACT: Mass spectrometry-based metabolomics identifies small molecular metabolites as the biomarkers for disease diagnosis. The metabolites could also serve as signaling molecules for disease treatment and mechanism studies. In our first work, we found that tryptophan catabolite 3-hydroxyanthranilic acid (3-HAA) is downregulated in liver tumor. The following experiments demonstrated 3-HAA can induce liver cancer cell apoptosis via binding YY1-DUSP6 and inhibition of ERK transcription. Additionally, in the metabolic interactions between virus and host, we found that glycylproline accumulation in plasma samples of COVID-19 convalescent patients with rapidly faded antibodies compared with COVID-19 convalescent patients with antibody. Further interrogations revealed that glycylproline can impair generations of T follicular helper cells, GC B cells and plasma cells upon SARS-CoV-2 vaccination, resulting in reduced antibody levels. Due to glycylproline catalyzed by DPP4, we found that DPP4 inhibitor Sitagliptin, a reducing blood glucose drug, can rescue the SARS-CoV-2-specific antibody levels caused by glycylproline, suggesting that Sitagliptin could be not only utilized in type 2 diabetic patients but also benefit for maintaining antibody levels after vaccination. In another unpublished work, we performed metabolomics and lipidomics to identify that the metabolite H is positively correlated with body weight changes of Syrian hamsters infected with SARS-CoV-2. We further figured out that the metabolite H can abrogate pulmonary inflammation by regulating ECHS1 ubiquitination and Treg fragility, remodeling lung immune microenvironment. The studies reveal that the small molecular metabolites identified by mass spectrometry-based metabolomics, can function and even treat the specific diseases.

KEY WORDS: Small Molecular Metabolite; Metabolomics; Tumor Metabolism; Metabolic Interactions between Virus and Host

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AI-driven Mass Spectrometry-based Identification of Small Molecule

Hongmei Lu*, Zhimin Zhang, Qiong Yang, Ting Xie
College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China

ABSTRACT: Mass spectrometry (MS) is a convenient, sensitive, and reliable method for the analysis of complex mixtures, which is vital for life sciences fields such as metabolomics and proteomics, and organic synthesis in chemistry. The identification of small molecules from chromatography-mass spectrometry data remains a major challenge in complex matrices, due to the enormous chemical and compositional diversity of small molecules and the limit of standard mass spectra in databases. Over the past years, we developed several data and artificial intelligence (AI) approaches to enable small molecule identification by improving coverage and accuracy, such as FastEI, DeepMASS, GNN-RT and SigmaCCS so on. They include molecular fingerprint prediction, in-silico mass spectral prediction, mass spectral match and scoring algorithms, retention time (RT) prediction and collision cross-section (CCS) prediction. These methods take advantage of big data of public databases or datasets, and multidimensional orthogonal information of modern instrumental data. The latest research methods of deep learning have been introduced to build relevant models, including DNN, GCN, Transformer, etc. Finally, we open source all our algorithms and softwares.

KEY WORDS: Big data; Artificial intelligence; Small molecue; Identification

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基于数字细胞模型的酵母菌株表型定量预测研究

鲁洪中
上海交通大学，微生物代谢国家重点实验室，上海，200240

关键词:数字细胞模型、组学约束、酵母定量生物学

近年来，基因组尺度代谢网络模型 (GEMs) 得到飞速发展，已用于多组学数据整合分析和菌株设计等领域。但传统的 GEMs 属于计量学模型，不包括详细的酶动力学和基因调控等信息，这就造成传统代谢模型预测能力差，无法在系统层面上精确预测提升工业菌株生产效率所需的代谢改造策略，成为工业底盘菌株高效开发和持续升级的关键瓶颈之一。围绕“如何构建高性能细胞代谢模型以提升菌株定量表型预测的精准性”这一关键科学问题，我们开展了以酵母为主要对象的菌株特异性代谢模型构建和多维信息约束的高精度代谢模型构建。基于自研的算法平台，能够一次性从头构建 1800 个以不同酿酒酵母菌株的 GEMs；其次通过整合酶动力学和蛋白质 3D 结构数据，申请人成功构建新一代酵母多尺度细胞代谢模型，显著提升了传统代谢模型预测能力，正成为酵母系统生物学和合成生物学研究中不可或缺计算平台。

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植物代谢组多样性的遗传解析

沈双欠[1]，占传松[2]，袁弘伦[1]，罗杰[2,1*]
1. 海南大学; 2. 崖州湾国家实验室

摘要：植物为应对各种内外胁迫产生大量化学结构和生理功能各异的代谢产物。了解与代谢相关的基因并解析相关代谢途径，对于提高植物对环境胁迫的适应性、改善食品质量和提高主要作物的产量品质至关重要。我们基于 LC-MS/GC-MS 建立了广泛靶向代谢组学分析方法，并结合正向遗传学手段，通过代谢数量性状位点（mQTL）分析和代谢全基因组关联分析（mGWAS）剖析了植物代谢组的遗传和生化多样性。我们证明了这些策略适用于研究植物物种内和物种间代谢的自然变异。进一步的研究表明，植物代谢多样性在很多情况下受到基因簇（代谢途径的结构和/或调控基因在基因组中相邻或相邻）控制。我们的研究为大规模的基因鉴定、途径阐明和基于知识的作物遗传改良提供了有力的工具。

关键词: 植物代谢组；遗传多样性；基因簇；作物遗传改良.

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多模态交叉分子科学驱动的功能代谢组学生物医药转化应用研究

吕海涛
香港浸会大学

基于生物流体代谢物的质谱相关疾病早筛早诊

刘会会[1]，熊彩侨[1]，聂宗秀[1,*]（‘*’通讯作者;）
1. 中国科学院化学研究所

肿瘤困扰着千千万万的患者和家庭，为提高肿瘤的生存率，早筛早诊已成为公认的最佳方法，然而早期病变的症状非常隐匿，很难在更易治疗的阶段对肿瘤进行筛查和早诊，因此研究和开发低成本、快速和非侵入型的早诊方法迫在眉睫。

生物流体（血液、呼出气体、尿液、脑脊液等）包含的代谢物分子信息能够实时反映机体健康状况，通过检测其中代谢小分子生物标志物的含量变化并解析相关代谢途径，有望预测肿瘤早期发生。质谱已成为蛋白质组学、代谢组学、脂质组学等新兴“组学”研究的利器，然而传统的质谱方法需要对复杂样品进行严格的预处理和分离富集，阻碍了其在临床研究中的应用。

近年来，课题组开发了用于分析呼出气体中醛类分子的即时检测试纸，研究发现肺癌患者的呼出气体中醛类标志物的含量显著增加；开发了一款解吸分离电离质谱（DSI-MS）平台和多官能团差异化衍生策略，用于含有酸、醛、酮等官能团的小分子化合物的结构鉴定，研究了膀胱癌患者和健康对照者尿液样品的代谢轮廓；并结合机器学习模型，利用基质辅助激光解吸电离质谱（MALDI-MS）高通量获取尿液的代谢指纹图谱，实现了对泌尿生殖系统癌患者的识别和相关代谢通路研究；最后，通过高效提取尿液及血清样本代谢指纹图谱，实现了膀胱癌和良性泌尿系统疾病的快速诊断及肝癌临床筛查。

针对样本前处理简便、分析速度快、灵敏度高的大规模疾病筛查需求，课题组开发了用于分析患者呼吸气、尿液和血清样本中代谢物分子差异的质谱新方法，具有应用于临床中相关疾病早筛早诊的潜力。

关键词: 生物流体，质谱，DEI，MALDI.

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Mechanisms underlying sensing fumarate for tumor progression

Xin Ge[1,2], Jianxin Yin[2], Mengdie Li[1,2], Zhumei Shi[2], Xu Qian[1,2,*]

[1]Department of Nutrition and Food Hygiene of School of Public Health, Nanjing Medical University, Nanjing 211166, China

[2]Institute for Brain Tumors, Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing Medical University, Nanjing 211166, China

*Correspondence: xqianmedres@njmu.edu.cn

ABSTRACT: Fumarate is a shared metabolite that links multiple metabolic pathways including Krebs cycle, *de novo* purine synthesis, urea cycle, and tyrosine catabolism. As an oncometabolite, the functions and mechanisms of fumarate and its metabolic pathways in tumor progression are largely unknown. Our work demonstrated that fumarate generated by argininosuccinate lyase (ASL), a urea cycle enzyme, inhibits KDM5C and subsequently facilitates H3K4me3 enrichment at promoter-mutant regions of *TERT*, resulting in recruitment of c-Myc for TERT transcription and malignant progression of glioblastomas (*Molecular Cell* 2022a, PMID: 36270249). In addition to its role on inhibiting KDMs, we revealed that accumulation of fumarate in fumarate hydratase (FH)-deficient type 2 papillary renal cell carcinoma is responsible for succination modification of PTEN at cysteine 211 (C211), which abrogates the binding of PTEN with the cellular membrane, leading to activation of PI3K/AKT signaling pathway and tumor growth (*Molecular Cell* 2022b, PMID: 35216667). Recently, our ongoing project found that PTEN directly binds with MMS19 and competitively disrupts MMS19-based cytosolic iron-sulfur (Fe-S) cluster assembly (CIA) machinery in the differentiated glioma cells (DGCs). The highly activated *de novo* purine synthesis pathway in glioma stem cells (GSCs) fuels fumarate to promote PTEN C211 succination. This modification abrogates the interaction between PTEN and MMS19, thereby reactivating CIA machinery pathway for GSC maintenance. Intriguingly, consuming fumarate by N-acetylcysteine (NAC), an FDA-approved prescription drug, inhibits PTEN C211 succination and reactivates PTEN to impair GSC maintenance by inhibiting CIA machinery, therefore sensitizing GSC-derived brain tumors to temozolomide and irradiation therapies (*Science Translational Medicine*, in revision). In conclusion, fumarate promotes tumor progression through distinct mechanisms: 1) epigenetic reprogramming by competitively inhibiting activities of α -ketoglutarate-dependent KDMs, and 2) protein succination modifications. These studies highlight that fumarate is potentially targetable for cancer treatment.

Key words: fumarate; KDMs; protein succination modification; PTEN; tumor progression.

代谢流技术解析抑郁症有氧糖酵解代谢转变

赵云昊[1,2]，王贤贤[1,2]，田俊生[1,2]，秦雪梅[1,2*]

1. 山西大学 中医药现代研究中心，山西 太原 030006;

2. 地产中药功效物质研究与利用山西省重点实验室，山西 太原 030006

抑郁症是一种严重且反复发作的情感障碍疾病，已有研究证实患者所表现出的食欲不振、精力不足、缺乏动力等症状与能量代谢障碍有关，但主要集中于抑郁症线粒体功能障碍，重点关注线粒体内代谢（TCA 循环），却忽视了由葡萄糖分解代谢途径所提供的线粒体能量底物供应[1,2]。因此，阐明葡萄糖分解代谢途径异常对于解析抑郁症能量代谢障碍具有重要意义、并为抗抑郁药物研发提供新思路。

本课题组聚焦于抑郁症葡萄糖分解代谢通路异常，在国家自然基金项目支持下，采用更安全的稳定同位素示踪技术研究，建立了一套在整体动物体内的稳定同位素引入、样本制备分析和数据处理方法；通过对正常与抑郁模型大鼠引入 $^{13}C_6$-葡萄糖同位素丰度对比分析，发现其模型动物的葡萄糖分解代谢途径在代谢流向上发生变化即丙酮酸进入 TCA 循环受阻，由氧化磷酸化转变为有氧糖酵解（Warburg 效应）供能，被称为代谢转变；且与另一疾病肾病综合征比较，代谢流向的疏解路径有差异，表明示踪代谢组学技术可用于不同疾病的代谢途径差异分析。然而，关键代谢物代谢流量的变化也是表征疾病异常代谢通路的重要指标，还有助于确定关键靶点。

在本研究中，利用多成分同位素标记加数学计算的代谢流分析技术，以明确异常通路上关键代谢物的代谢流量变化，并锁定关键靶点。基于流平衡分析理论构建细胞代谢流数学模型[4]，采用公认的抑郁症细胞模型—皮质酮损伤原代星形胶质细胞模型[3]，使用 $^{13}C_6$-葡萄糖、$^{13}C_3$-乳酸、$^{13}C_5$-谷氨酰胺 3 种稳定同位素对星形胶质细胞孵育 24h，经样本采集和预处理、仪器分析、数据处理，将各代谢产物同位素丰度代入数学模型计算分析。结果表明模型细胞内丙酮酸转化为乳酸流量显著升高，而丙酮酸进入 TCA 循环流量显著降低。使用柴归颗粒含药血清及文拉法辛干预模型细胞，发现两种药物对流量变化均有回调作用，但回调的流量大小及流向存在差异，表明两药作用靶点不同。以上结果进一步说明，代谢流向和代谢流量是解析抑郁症能量代谢障碍的重要参数，为治疗抑郁症和开发基于提高能量的抗抑郁理想药物提供新路径。

关键词:抑郁症，稳定同位素示踪代谢组学，代谢流分析，Warburg 效应

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多组学分析助力功能脂质及脂质代谢调控新发现

税光厚
中国科学院遗传与发育生物学研究所

生物样本中代谢组的多样性和复杂性激发了各种分析技术创新和改进，以满足无数农业和生物医学等研究和应用的需要。过去 20 年来，代谢组学、脂质组学研究领域的快速发展主要归功于相关技术尤其质谱技术的进步和各种创新方法的涌现与发展。代谢组学与脂质组学推动了生命科学中的许多重要发现，尤其是其与其他组学数据（转录组、蛋白组等）的联合分析为相关机制的深入研究提供了重要线索。我们团队在过去多年来开发了一系列用于脂质组/代谢组精确定量和精确分析的可靠的组学策略，并将其成功应用于多种人类疾病与模式动物果蝇研究中，并结合分子生物学、遗传筛选等多项技术对生物体中相关代谢扰动实现系统解码，发现了一些具有重要生理、病理功能的脂质，揭示了影响脂质代谢的一些关键基因的调控新功能，为相关疾病生物标志物及治疗新靶点探索提供了新的策略与思路。

关键词: 脂质组学，代谢组学，蛋白质组学，转录组学，脂质代谢

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Metabonomics: Advances and Challenges

Huiru Tang
Fudan University

完整活单细胞电发射电离质谱方法用于单细胞代谢组学分析

刘元星, 赵瑶瑶,汪夏燕*
北京市，北京工业大学化学系

单细胞质谱（SC-MS）能够揭示细胞异质性和细胞内生化反应的分子机制，但目前SC-MS 的发展由于基质干扰、样品稀释和样品浪费而受到检测灵敏度不足的阻碍。为了克服这些问题，本研究提出建立一种完整活单细胞电发射电离质谱（ILCEI-MS）方法，以显著提高单细胞检测的灵敏度。该方法使用窄内径毛细管（内径略小于细胞系的平均细胞直径）来实现有效的单细胞分离和运输，同时避免引入大量流动相而造成样品稀释和基质干扰。通过自行设计并构建的在线可视化高速显微摄像平台用来探究 ILCEI 技术的离子化机理。ILCEI-MS 采用的等内径薄壁石英毛细管质谱喷针可产生连续的单细胞液滴，该液滴包含一个完整活细胞和包裹于细胞外围的缓冲液薄层，保证了单细胞质谱进样过程中细胞成分几乎不被稀释；细胞在脱离发射器进入质谱进样口的过程中保持了完整和存活，并在质谱离子传输管内发生了胞内组分的离子化，消除了样品离子在大气环境中的损失，显著提高了单细胞样品的利用率，这也是与传统电喷雾离子化技术的明显差异。在提高检测灵敏度的同时，ILCEI-MS 实现了约 51 个细胞/分钟的高通量单细胞检测。使用 ILCEI-MS 方法，在一次检测中从 A549 细胞中鉴定了 368 种代谢物，提高了单细胞分析的代谢物覆盖度。该方法对来自多个细胞系的 2800 多个活细胞进行了快速的单细胞代谢指纹谱分析并成功实现了不同细胞系的细胞区分。此外，使用该方法对来自非小细胞肺癌荷瘤小鼠模型的心脏、肝脏、肺脏等多个小鼠器官的 4072 个原代单细胞进行了高通量分析，并研究了肺肿瘤组织细胞和正常肺组织细胞的单细胞代谢组差异，说明了该方法对于实际复杂体系的单细胞样品具有普适性，且具有发现新标志物的潜力。

关键词:单细胞质谱，单细胞代谢组学，原代细胞，非小细胞肺癌

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肠道微生物调控人体健康和疾病的代谢免疫分子机制研究

王保红 [1], *

1. 浙江大学医学院附属第一医院传染病诊治国家重点实验室，杭州 310003；

wangbaohongzju@zju.edu.cn;

摘要：

宿主-微生物组的密切关联调控人类健康和疾病中的免疫、代谢和神经元的反应，但尚未开发用于疾病生物标志物或干预。因此，我们的目标是阐明肠道微生物代谢物在重大疾病中的改变，包括肝病、代谢性疾病和精神或心理疾病。我们利用多组学分析和生物信息学分析，我们揭示了肠道生态失调介导的代谢物如何影响健康和系统稳态的潜在机制。我们发现通过“肠-脑”轴的肠源代谢物在脑功能障碍疾病中发挥作用。此外，我们最近的研究结果为临床实践提供了新的治疗方法，如基于肠道微生物群的靶向治疗与营养干预、微生物移植、益生菌和益生元补充，以及微生物群相关代谢物等。综上所述，我们的研究结果进一步阐明宿主-微生物相互作用中的代谢免疫信号，将有助于开发新的人类疾病诊断和治疗方法。

关键词: 代谢组、微生物组、重大疾病、免疫、代谢机制。

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Spatially-resolved Metabolic Flux Analysis: Pitfalls and Promises

Lin Wang[1,*]

1. State Key Laboratory of Common Mechanism Research for Major Disease, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China 100730

ABSTRACT: The accumulation of metabolites can arise from both increased production and decreased consumption. Hence, measurement of metabolite concentration tells only half the story. An equally significant aspect to consider is the understanding of pathway activity, which can be quantified in terms of metabolic flux. Isotope tracing together with imaging mass spectrometry has been developed to determine the metabolic activities of major organs. However, in contrast to untargeted metabolomics analysis using LC MS, metabolite analysis based on imaging mass spectrometry lacks the capability for separation. Resolving and profiling many hundreds to thousand of metabolites with varying chemical properties in a biological sample thus presents unique challenges or pitfalls, which hampering the quality of the data and the subsequent biological interpretation. Here we discuss step by step the potential pitfalls inherent in the process and propose some tips that can mitigate the problems. Additionally, we employ stable isotope-labelled nutrient infusion to MALDI-imaging mass spectrometry to quantitate metabolic activity in mammalian tissues in a spatially resolved manner. Distinct differences in the utilization of TCA substrate can be observed in the murine kidney, particularly in different renal areas. It is evident that there is a preference for the utilization of glutamine and citrate in the cortex, whereas fatty acids are predominantly utilized in the medulla. In the brain, we observe spatial gradations in carbon inputs to the TCA cycle and glutamate selectively under ketogenic diet: in carbohydrate-rich diet, glucose predominates throughout; in ketogenic diet, 3-hydroxybutyrate contributes most strongly in hippocampus and least in the midbrain. Brain nitrogen sources also vary spatially: branched-chain amino acids contribute most in the midbrain, while ammonia contributes preferentially in the thalamus. Consequently, we have built an efficient platform *iso-imaging* that enables the elucidation of the spatial organization of metabolic activity within tissues.

KEY WORDS: Spatially-resolved metabolomics analysis, metabolic flux, imaging mass spectrometry

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方证代谢组学驱动的中医药治疗疾病原理解读

孙 晖[1]，杨 乐[2]， 王喜军[1, 2*]
1 黑龙江中医药大学，经方与现代中药融合创新全国重点实验室，
2.广东省中医院 省部共建中医湿证国家重点实验室

中药有效性在临床上是以方剂为药物针对证候而表达的。由于证候的模糊性及方剂组成的复杂性使得有效性的研究一度陷入僵局，中医药治疗疾病的原理难以说清楚说明白。

基于此，本世纪初我们将代谢组学技术与中药血清药物化学相整合，以证候为切入点，以方剂为研究对象，建立了有效性研究新策略-中医方证代谢组学：利用代谢组学技术发现并鉴定证候生物标记物，以证候生物标记物为参数精准评价方剂疗效及阐释其作用机制；在有效状态下，利用中药血清药物化学鉴定方剂体内的显效成分；进而将证候生物标记物与方剂体内显效成分相关联，发现与生物标记物轨迹变化高度关联的体内成分，从而鉴定表达方剂临床疗效的药效物质基础，阐明效应、效应成分及效应靶点等中药有效性机制。通过黄疸证及肾虚证等 9 个证候及 14 个相关方剂的系统研究实践，形成原创的中医方证代谢组学理论及技术。中医方证代谢组学英文被笔者定义为 ***Chinmedomics***， 2012 年首次发表在 Omics 杂志；2015 年 **Nature** 专题推介其为“揭示中药有效性的强有力手段，是沟通中医与西医的生物学语言”。应用该理论及技术，揭示 19 个经方及 11 个上市药品的有效性机制，解读其治疗疾病的原理，提升国际社会对中医药的认知度。

关键词: 方正代谢组学，中医药，有效性，有效成分.

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活细胞线粒体能量代谢的原位测量技术

王旭东[1,*]，姜睿[1]，杨恩来[1]，徐影[1]

[1]复旦大学人类表型组研究院，上海市浦东新区张衡路 825 号，200300

线粒体是细胞的供能中心，它还参与了细胞内多项重要生理活动如与钙离子信号通道协同作用、自由基生成、细胞增殖与凋亡等。线粒体的功能失调已经被发现与心脑血管疾病、癌症、神经性疾病等重大疾病有密切的联系[1]。对活细胞线粒体的能量代谢进行原位、实时、分析对理解线粒体的功能与代谢具有十分重要的意义[2]。报告人将从线粒体的功能、现有的线粒体能量代谢分析技术和现状出发，讲解活细胞线粒体能量代谢分析的难点和突破口，最后汇报报告人的课题组在活细胞线粒体能量代谢分析领域的部分工作。报告中包含课题组研发的用于线粒体中氧气浓度变化、pH 值、三磷酸腺苷 ATP 和谷胱甘肽等代谢物浓度测量的纳米传感器设计、合成和在活细胞中的应用。

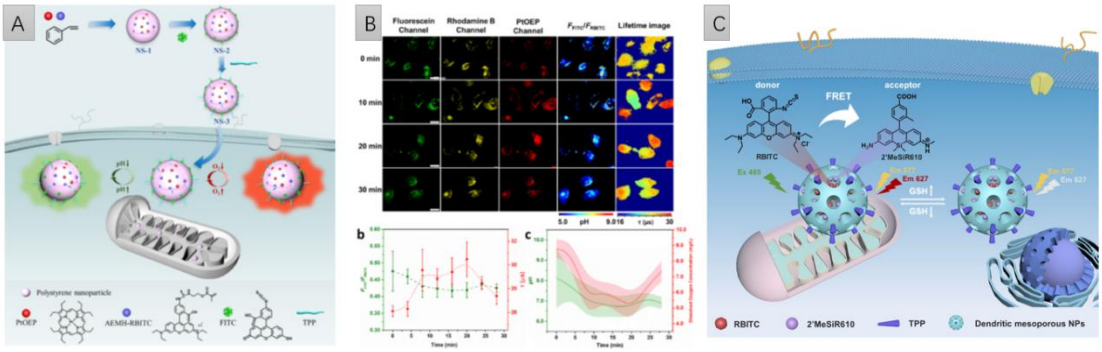

图 1 用于活细胞线粒体内pH和氧气(A)浓度测量的纳米传感器及其荧光成像和实时分析数据(B)，（C）用于活细胞线粒体内谷胱甘肽GSH测量的纳米传感器

关键词：活细胞；线粒体；能量代谢；荧光成像；纳米传感器

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MS-XDF 系列软件工具助力临床诊疗生物标志物的发掘

袁彩霞[1]，王苏萍[1]，董继扬[2,*]，吴彩胜[1,*]
1. 厦门大学药学院
2. 厦门大学电子科学与技术学院

代谢组学研究中，很多内源性小分子化合物因为色谱行为差、质谱响应低或受其它内源性物质干扰等原因，直接进行液质联用分析常无法获得理想的结果。根据分析对象不同的结构类型，针对性的进行衍生化，将液质联用与衍生化标记技术组合使用是代谢组学定量和表征生物样品中小分子化合物所采用的一种重要策略。然而，庞大的数据量和生物基质的干扰给后期数据处理分析带来了巨大挑战。前期基于窄范围质量亏损过滤原理（NMDF，Narrow mass defect filter），我们研发了全新的非靶向代谢组学数据处理软件工具 MS-IDF（包括 IsoFinder 和 MDFinder 两个功能模块）。该工具巧妙地运用了稳定同位素之间质量亏损的准确差异设计了 MDFinder 功能，旨在峰提取过程中根据稳定同位素之间的准确质量亏损差异，设置合适的过滤间隔，有效筛选出目标代谢物，同时消除其他内源性成分的干扰。在此基础上，我们进一步提出多维组合衍生化整合智能质谱数据处理技术研究策略：将多种衍生化试剂有机组合，利用不同衍生化试剂之间的差异（色谱行为差异、质量亏损数差异、质量数差异、同位素丰度匹配和特征质谱碎片等），研发 MS-XDF 系列质谱数据处理算法软件工具，包括基于三维组合衍生化策略的 MS-TDF， 基于背景扣除原理的 MS-BDF，以及针对寡糖分析的 MS-GDF 等。研究结果表明，我们所建立的 MS-XDF 系列质谱数据处理算法软件工具与现有软件或平台相比具有多项优势，可显著提升内源性代谢物的搜索效率、简化谱图、准确发现目标代谢物的准分子离子峰等。基于该软件建立的非靶向内源性代谢物识别策略，可高效全面的挖掘目标内源性代谢物，有助于临床诊疗生物标志物的探寻。目前该研究策略已经成功应用于精神分裂症患者的伯胺类生物标志物及肺癌患者诊疗生物标志物的研究。

关键词: 高分辨质谱，数据后处理技术，MS-XDF 软件工具，多维组合衍生化，生物标志物

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衍生化液质联用代谢组学分析新方法开发及其应用

张培[1]，焦宇[1,2]，唐恬[1]，张尊建[1]，许风国[1*]

1. 中国药科大学药物质量与安全预警教育部重点实验室、天然药物活性组分与药效国家重点实验，江苏 南京 210009

2. 中国药科大学理学院有机化学教研室，江苏 南京 210009

针对低丰度、极性大、难离子化等结构新颖、性质特殊的内源性小分子代谢物 LC/MS 检测难题，报告人及其团队从“增加可靠性、提高灵敏度、加强整合转化”出发，在“理论”“试剂”“方法”“应用”等四个层面开展了“化学衍生化与 LC/MS 结合应用”的创新探索。提出了“配对衍生化”、“组合衍生化”新策略；开发出了拥有自主知识产权且具有显著质谱增敏优势的新型衍生化试剂 TMT-PP；解决了代谢组和脂质组同步提取、同步分析难题；建立了涵盖 10 大类 13 个通路 800 余种目标代谢物的衍生化 LC/MS 定量分析方法。化学衍生化技术创新赋能质谱仪器性能新高度，把“不可测”变为“可测”。极大提高了检测灵敏度、拓宽了检测覆盖面、有效解决代谢组学分析结果实验室间可比性差的问题。

关键词: 代谢组学，衍生化，液质联用

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从小分子看健康

许国旺*，郑福建，由蕾，王宇婷，王鑫欣，郑思佳，徐天润，丰迪生，胡春秀，石先哲，赵欣捷，路鑫，刘心昱

中国科学院大连化学物理研究所 大连 116023

生物分子可简单地分成大分子和小分子。小分子有无机小分子（H2O、CO2、Ca2+、Mg2+等）、代谢产物（丙酮酸、柠檬酸、苹果酸等）和构件分子（氨基酸、核苷酸、单糖等）等，简称代谢组。现实世界的中人会接触到很多外源性物质，包括食品、营养、药物，以及农药、兽药和化学污染物，统称为暴露组。同时，体内的菌群不仅代谢上述物质，而且也产生自己的代谢产物。这 3 类小分子的变化直接反映了人类的身心健康。因此，通过采用高分辨质谱和三重四级杆质谱技术分析组织或体液中暴露组的含量及代谢组的改变，可揭示这些物质与疾病发生发展的关系。

代谢组和暴露组中涉及的内源性和外源性化合物至少 50 万种，化学性质各异，浓度差别巨大。在体内，外源性化合物比内源性代谢物的浓度要低 1-2 个数量级，因此，研究的关键是在更大的浓度范围内实现内源性和外源性化学物质的“全”覆盖检测。同时，未知物结构鉴定是研究中又一必须解决的关键问题。不仅如此，为揭示相关的机制和在人群中的丰度及其变化，研究的对象从单细胞到大规模人群、从时间到空间、从宿主到菌群等对分析仪器和方法的灵敏度、重复性等一系列分析问题提出了挑战。

为解决上述科学问题，本研究组开发了从单细胞、菌群、动物到大规模人群样品中小分子研究的一系列新方法。先后建立基于多维色谱-高分辨质谱联用的新方法，以提高色谱峰容量，实现人类代谢组、菌群代谢组和暴露组的高覆盖检出和重要物质的精准定量。针对“暗”物质定性困难的问题，建立了包含保留时间、MS1、MS2 等信息的内、外源化合物数据库，并基于分子结构特征、色谱保留规律及分子网络的定性方法拓展数据库的信息量。将建立的方法用于恶性肿瘤、代谢性疾病等重大慢病的研究，试图揭示与这些重大疾病相关的风险因子、预警标志物及代谢水平上的分子机制。本报告将给出我们最新的一些研究结果。

关键词: 代谢组学，暴露组学，单细胞，菌群代谢组，空间代谢组学

稳定同位素示踪揭示代谢可塑性

杨立峰 [1,*]

1. 慢病代谢示踪与营养干预课题组，中国科学院上海营养与健康研究所

代谢组学反映生物化学反应中小分子的静态丰度，代谢流则可揭示其动态变化。两者相辅相成，绘制代谢全貌。相比于发展较为成熟的代谢组学，代谢流检测技术的开发及其在生物问题中的应用亟需探索挖掘。^{13}C, ^{2}H, ^{15}N,^{18}O 等稳定同位素营养标记体系及其标记位置的多重选择可帮助描绘组织器官中特定代谢参与不同代谢途径的反应过程以速率。本课题组在体外同位素示踪的基础之上构建了颈静脉插管活体同位素示踪体系，揭示了慢病发展过程中能量代谢的可塑性。 包括 ^{13}C、^{15}N 体外示踪揭示肿瘤微环境中成纤维母细胞可获取环境中的营养物质合成大量谷氨酰胺，进而支持癌细胞谷氨酰胺代谢，促进肿瘤增殖；^{13}C 活体示踪绘制组织器官在不同生理状态下的能量代谢以及胰腺癌在不同膳食结构下的代谢变化，为临床用药提供新思路；^{2}H 活体示踪挖掘新型 NADH 活性氢来源，通过阻断一碳代谢促进电子传递链受损细胞的增殖。因此稳定同位素示踪方法的不断开发和应用可为认知生物代谢反应提供新视角，并为临床新治疗手段的开发提供新的理论依据。

关键词: 代谢流、活体同位素示踪、代谢可塑性、氨基酸和通体代谢

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Phosphatidic acids regulate chloroplast development and isomeric identification of plant galactoglycerol lipids

Hong-Yan Yao[1,#], Yao-Qi Lu[2,#], Xiao-Li Yang[3,#], Xiao-Qing Wang[2], Zhipu Luo[2], De-Li Lin[4], Jia-Wei Wu[2,*] and Hong-Wei Xue[4,*]

[1]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai 200438, China

[2]Institute of Molecular Enzymology, School of Biology & Basic Medical Sciences, Suzhou Medical College of Soochow University, Suzhou, Jiangsu 215123, China

[3]National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai 200032, China

[4]Shanghai Collaborative Innovation Center of Agri-Seeds, Joint Center for Single Cell Biology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China

Abstract:

Lipids establish the specialized thylakoid membrane of chloroplast in eukaryotic photosynthetic organisms, while the molecular basis of lipid transfer from other organelles to chloroplast remains further elucidation. Here we revealed the structural basis of *Arabidopsis* Sec14 homology proteins AtSFH5 and AtSFH7 in transferring phosphatidic acid (PA) from endoplasmic reticulum (ER) to chloroplast, and whose function in regulating the lipid composition of chloroplast and thylakoid development. Crystal structures of AtSFH5-Sec14 domain in complex with L-α-PA and DPPA (1,2-dipalmitoyl-sn-glycero-3-phosphate) revealed that two PA ligands nestled in the central cavity with different configurations, elucidating the specific binding mode of PA to AtSFH5, different from the reported PE/PC/PI binding modes. Quantitative lipidomic analysis of chloroplast lipids showed that PA and monogalactosyldiacylglycerol (MGDG), particularly the C18 fatty acids at *sn*-2 position in MGDG were significantly decreased, indicating a disrupted ER-to-plastid (chloroplast) lipid transfer, under deficiency of AtSFH5 and AtSFH7. Our studies identified the role and elucidated the structural basis of plant SFH proteins in transferring PA between organelles, and suggested a model for ER-chloroplast inter-organelle phospholipids transport from inherent ER to chloroplast derived from endosymbiosis of a cyanobacterium, providing a new mechanism involved in the adaptive evolution of cellular plastids.

KEY WORDS:

Sec14p homolog protein, chloroplast development, phosphatidic acid transfer, ER-chloroplast inter-organelles

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Fatty Acid Oxidation-induced HIF-1 Activation Facilitates Hepatic Urate Synthesis Through Upregulating NT5C2 and XDH

Ningning Liang [1,2], Huiyong Yin [1,2,3*]

[1] CAS Key Laboratory of Nutrition, Metabolism, and Food Safety, Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences (CAS), Shanghai, China, and University of Chinese Academy of Sciences, Beijing, China;

[2] Department of Biomedical Sciences, City University of Hong Kong, Hong Kong SAR, China

[3] School of Life Science and Technology, ShanghaiTech University, Shanghai, China 201210

ABSTRACT:

Objectives Dyslipidemia affects approximately half of all people with gout, and prior Mendelian randomization analysis has suggested a causal role for elevated triglycerides in hyperuricemia (HU) but the underlying mechanism remain elusive. We hypothesize that dyslipidemia promotes hepatic urate biosynthesis in HU and gout, and that fatty acid (FA) oxidation drives this process.

Methods Targeted metabolomics based on liquid chromatography-mass spectrometry was developed and applied to quantify major metabolites in purine metabolic pathways in sera of men with HU, gout, and normouricemic controls. FAs in serum and liver tissues were measured by gas chromatography-mass spectrometry. A stable isotope labeled metabolic flux assay tracked transport of hypoxanthine and biosynthesis of urate *in vitro* and *in vivo*. A high fat-induced HU mouse model mimicked human HU. ChIP-qPCR profiled promoter regions of HIF-1 downstream target genes.

Results Serum levels of major purine metabolites and multiple FAs were significantly elevated in men with HU and gout, compared to normouricemic controls, whereas hypoxathine showed the opposite trend. Furthermore, multiple serum FA levels positively correlated with urate, xanthine and inosine but negatively with hypoxanthine, findings also observed in murine high fat diet-induced hyperuricemia. FA oxidation induced HIF-1 activation, which upregulated NT5C2 and XDH levels to facilitate hypoxanthine transport from blood to liver and activation of hepatic urate biosynthesis. Our findings were validated in human hepatocytes and 50 paired serum and liver tissues from liver transplant donors.

Conclusions FA oxidation promotes hepatic urate synthesis by activating HIF-1 -NT5C2/XDH pathways. This novel mechanism directly links lipid metabolism to hyperuricemia.

KEY WORDS: dyslipidemia, hyperuricemia, metabolomics, hypoxanthine, metabolic flux

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RNA 修饰的分析鉴定及代谢通路研究

游雪娇，冯钰锜，袁必锋*
武汉大学公共卫生学院，武汉，430071
* 通讯作者 Email：bfyuan@whu.edu.cn

近年来，表观遗传学的重点研究内容之一为RNA化学修饰，这些修饰可以调控RNA稳定性、细胞增殖、细胞分化以及细胞代谢等多种生理过程。目前，RNA的化学修饰已经发现150多种，主要存在于细胞的mRNA、tRNA和rRNA上。RNA甲基化是哺乳动物细胞中最普遍的修饰形式之一，这些修饰在调节细胞增殖、细胞分化和细胞凋亡等过程中有重要的生物学功能。其中，腺苷的甲基化修饰可以发生在N6位和N1位分别形成N6-甲基腺苷（m^6A）和N1-甲基腺苷（m^1A）。腺苷上除了发生单一的甲基化修饰外，还能发生双重甲基化修饰，例如N6,N6-二甲基腺苷（$m^{6,6}A$）。然而，生物体内是否存在其它形式的腺苷双重甲基化修饰仍未可知。我们通过合成标品对比的方式在哺乳动物tRNA中发现并鉴定了一种新型的腺苷双重甲基化修饰：N1,N6-二甲基腺苷（$m^{1,6}A$）。通过细胞代谢标记实验证明了tRNA中的$m^{1,6}A$是一种以SAM作为甲基供体的内源性修饰，且在哺乳动物细胞中广泛存在。其中，哺乳动物细胞tRNA中$m^{1,6}A$的的含量为0.0049% ~ 0.0084%，组织tRNA中的含量为0.019% ~ 0.047%，乳腺癌组织中的含量比癌旁组织的含量高。此外，我们通过DNA探针杂交结合S1核酸酶消化实验证明了该修饰位于tRNA的A58位上。体内体外的实验证明了该修饰是一种动态的可逆性修饰，体外生化实验证明了该修饰的生成途径之一是其位点上保守的m^1A修饰可以重排成m^6A，再通过该位点的甲基化复合物TRMT6/61A进一步形成$m^{1,6}A$；体内和体外实验均表明了该修饰可以被ALKBH3去甲基化。$m^{1,6}A$的发现和其动态修饰过程扩大了RNA修饰的多样性，提供了tRNA修饰介导的基因调控的新机制。

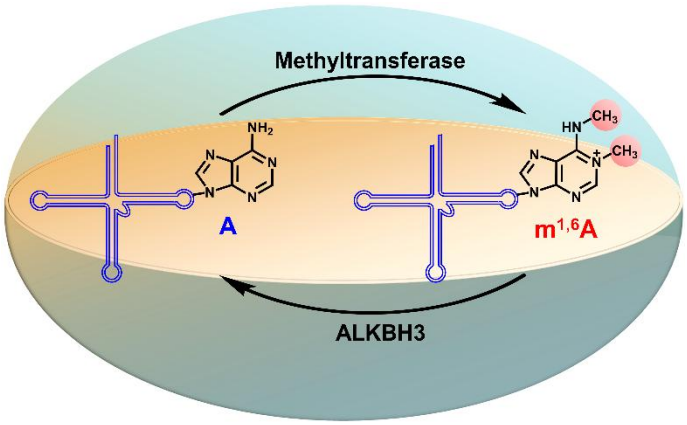

图 1：tRNA 上 $m^{1,6}A$ 动态修饰过程。

关键词: 色谱-质谱分析；RNA 修饰；稳定同位素代谢示踪

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质谱成像技术与空间分辨代谢组学研究进展

再帕尔·阿不力孜[1,2]

[1]中国医学科学院&北京协和医学院药物研究所，天然药物活性物质与功能国家重点实验室，北京 100050

[2]中央民族大学药学院，质谱成像与代谢组学国家民委重点实验室，北京 100081

当今，代谢组学及其应用取得迅速发展，已成为生命组学的重要组成部分，并在化学、生物学、生命科学、医学及药学等领域显示出巨大发展前景。本研究团队长期开展代谢组学分析新方法及其应用研究，创建了全面、系统的代谢组学分析技术平台与研究体系。近年来，开展基于 LC-MS/MS 技术的代谢组学分析方法研究的同时，我们还致力于质谱成像新技术的研发，并推动空间分辨代谢组学方法及其在生物医药等领域的应用研究。我们自主研发出新型常压敞开式离子化技术及其免标记、便捷、高灵敏的质谱分子成像技术（AFADESI-MSI），先后建立了无需切割的整体动物体内药物分析质谱成像方法，高灵敏、高覆盖的代谢物质谱成像分析新方法[1]和水凝胶辅助组织原位衍生化的质谱成像分析方法等一系列成像分析新方法。与此同时，将 AFADESI-MSI 技术与代谢组学相结合，系统开展了空间分辨代谢组学分析方法及其应用研究[2]，发展了大鼠脑代谢网络表征方法[3]、器官特异性质谱成像代谢物注释方法[4]、整合空间分辨代谢组学与同位素示踪分析方法[5]、肿瘤内氧化脂质代谢异质性可视化方法等一批创新方法。运用建立的方法在肿瘤代谢改变研究及发现食管癌异常表达的关键代谢酶[2]、候选新药的药效作用机制[5]及中药活性成分的毒性机制研究、肿瘤代谢标志物的原位筛查和糖尿病肾病/脑病发病机制等研究方面取得重要进展。本次学术报告将重点介绍上述研究进展。

关键词: 代谢组学分析方法；敞开式质谱成像技术；空间分辨代谢组学；疾病代谢机制；药物原位分析.

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胆汁酸干血斑 UHPLC-MS/MS 分析方法研究

马子英# 生宁# 张金兰*
中国医学科学院药物研究所

摘要：干血斑具有方便储存、运输、采血量少的特点，已经应用于新生儿疾病筛查中，具有广泛的应用前景。胆汁酸的代谢异常与很多代谢性疾病相关，例如脂肪肝、胆汁淤积性肝损伤、肝癌、高脂血症等，也与一些药物的不良反应相关，我们探索了胆汁酸干血斑分析新方法，克服了胆汁酸难以从干血斑滤纸洗脱的难点，建立了 UHPLC-MS/MS 分析方法，探索了替代基质，为实际转化应用提供了技术支撑，已经申请专利。

关键词: 胆汁酸， 干血斑，UHPLC-MS/MS

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单细胞代谢物质谱流式分析方法研究

姚欢[1,2], 潘星宇[1], 沈子征[1]，杨金磊[1]，潘思远[1]，张四纯[1,*]，张新荣[1]

1. 清华大学化学系;

2. 中国计量科学研究院

流式细胞仪是单细胞分析的有力工具，已在细胞生物学和临床研究实验室获得广泛应用。但是，现有的流式细胞仪大多依据检测细胞膜表面的蛋白质进行细胞区分，难以提供单细胞代谢物的信息。鉴于代谢物作为细胞新陈代谢的终端产物能更直观地反映细胞表型，加之肿瘤微环境中代谢物的影响成为肿瘤免疫治疗的研究热点，因此迫切需要研制可用于单细胞代谢物高通量检测的分析工具。

本课题组借鉴流式技术高通量单细胞分析的特点，提出了免标记质谱流式原理的单细胞代谢物分析方法，命名为 CyESI-MS [1]。这种方法从原理上继承了流式技术高通量单细胞分析的特点，又具备有机质谱能够同时检测多种代谢物分子的能力。通过该装置实现了高通量检测单细胞中的数百种代谢物，包括核苷酸、氨基酸、多肽、糖类、脂肪酸、甘油脂、甘油磷脂和鞘脂。目前的检测通量可以达到 40 个细胞/分钟左右，能够检测到单个哺乳动物细胞中上百种代谢物。通过各种细胞的代谢物质谱图，我们成功区分了四种不同类型的肿瘤细胞，并区分了肿瘤细胞的不同亚型[1]。我们将这种方法用于白血病临床样品的细胞分析，证明了用细胞代谢物的质谱图可以区分血液中五种白血病细胞，并通过分析五种细胞间的代谢物差异，筛选出 36 个存在显著性差异的代谢物[2]。我们还开展了免疫细胞与肿瘤细胞相互作用代谢物变化的研究，得到了 NK 细胞攻击癌细胞后单细胞代谢物的变化情况。结果表明，即使是同一种 NK 细胞在相同条件下，由于细胞的异质性和肿瘤细胞所处的微环境不同，NK 细胞杀伤肿瘤细胞的能力也存在差异。该工作有助于从代谢物角度加深对 NK 细胞杀伤肿瘤细胞机理的理解，为在单细胞水平评价 NK 细胞的杀伤能力提供新方法[3]。

综上所述，我们发展了一种基于有机质谱检测单细胞中代谢物的流式细胞术，实现细胞内代谢物小分子在单细胞水平的高通量分析。

关键词: 单细胞代谢组学，流式细胞术，质谱检测.

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Mass spectrometry imaging-based multi-modal technique for tumor metabolism

Chao Zhao[1,2,3 *]

1 Institute of Biomedical and Health Engineering, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

2 University of Chinese Academy of Sciences, Beijing, China

3 Shenzhen Key Laboratory of Precision Diagnosis and Treatment of Depression, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

ABSTRACT: A majority of the biological and chemical imaging techniques depend on "inherent properties" to visualize the target compounds and their interactions from different forms of samples. However, no single technique to date is capable of capturing the overall chemical and biological information simultaneously from complex biological processes. Multi-modal strategy, which intends to overcome the limitation of individual technique and to acquire more "hidden" information, provides a fresh and valuable perspective on investigating the biological processes with higher spatial or spectral resolutions. Therefore, comprehensive methodologies with two or more analytical techniques display a great attraction. By integrating mass spectrometry imaging (MSI) and others multiplex analytical techniques, MSI-based multi-modal technique is able to help us clarity the metabolic pathway of biomolecules, as well as disease progress [The work was supported by the National Natural Science Foundation of China (22176195), the Natural Science Foundation of Guangdong Province, China (2021A1515010171), the Guangdong Province Zhu Jiang Talents Plan (2021QN02Y028), the Key projects of basic research in Shenzhen (JCYJ20210324115811031)].

KEY WORDS: Mass spectrometry imaging; Tumor heterogeneity; Environmental toxicology.

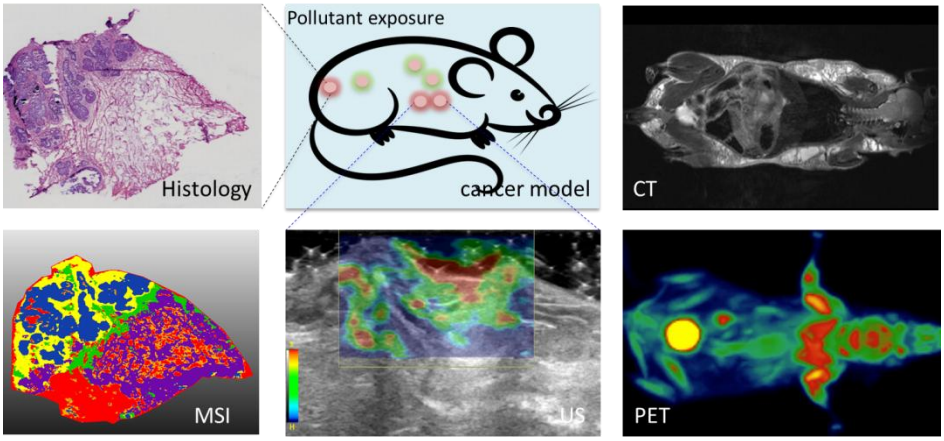

Fig.1. Take a typical cancer model for example to clarify the characteristics of MSI-based multi-modal technique

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代谢组学与心脑血管疾病研究

郑乐民[1]
[1]北京大学，北京，100091

在心血管疾病中，主动脉瘤与夹层（aortic aneurysm and dissection，AAD）是一种高死亡风险的主动脉疾病，为人类带来极大的生命威胁。后基因组时代带来了新的方法和机遇，研究心血管疾病风险预测和发病机理也能更加深入。代谢组学技术可以与上游的基因组、转录组和蛋白组变化、甚至环境因素进行功能性结合，反应最接近疾病状态的分子进程。本次报告将介绍我们通过代谢组学发现的琥珀酸、腐胺等 AAD 治疗靶点的科研成果，希望能推动代谢组学技术在 AAD 等心血管疾病研究中的应用。

线粒体缺陷型肾细胞癌的代谢重塑与临床应用

徐云则[1]，伍小宇[2]，王航[3]，孔文[2]，张进[2]，郑亮[1,*]

[1]上海交通大学医学院附属儿童医学中心，上海，200127

[2]上海交通大学医学院附属仁济医院，上海，200128

[3]复旦大学中山医院，上海，200032

[*]E-mail: lzheng@shsmu.edu.cn

关键词：肾细胞癌，肿瘤代谢，代谢组学，血液标志物，侵袭转移

摘要：肾细胞癌是泌尿生殖系统常见的恶性肿瘤，其中线粒体-缺陷型肾细胞癌属于预后极差的亚型，然而目前具有筛查/诊断功能的标志物仍是空白。延胡索酸酶是三羧酸循环重要的酶，它的胚系和体系突变会导致常染色体显性遗传综合征—即遗传性平滑肌瘤和肾细胞癌。FH 基因位点定位于染色体 1q42.3-q43，其突变存在多种类型，其中错义突变最为普遍，而移码、无义、插入或缺失、剪接位点突变则发生频率较低。临床上，II 型乳头状肾细胞癌在患者中比例最高，此外也偶发集合管癌和透明细胞癌。该型肿瘤常表现为单侧或单病灶，易呈囊性，或囊性、实性混合，且侵袭性远强于其他癌症综合征，在肾原发病灶较小且局限时（1.5 厘米），也可能出现淋巴结转移或远处转移，转移部位常见于肺，预后极差。由于 FH-缺陷患者队列研究存在不足，目前该疾病仍缺乏有效药物和标准治疗方法，由此我们组建了全国范围的多中心 FH 基因突变携带者队列。在多年临床代谢研究发现，琥珀酸型修饰代谢物在线粒体-缺陷型肾细胞癌患者血浆中显著升高。小鼠 CDX PDX 实验显示其血浆水平随肿瘤的生长而呈线性升高；细胞分子水平琥珀酸型代谢物可经 GGT1-DPEP1 轴心蛋白正向合成与反向分解生成, 该轴心级联蛋白位于肾小管上皮细胞和基底细胞外膜，由此可高效产生琥珀酸型代谢物；通过抑制 GGT 蛋白对该型肾细胞癌的侵袭和转移有显著影响。以上结果预示琥珀酸型代谢物可在临床上鉴别诊断线粒体-缺陷型肾细胞癌。为线粒体-缺陷型肾癌的新型诊断与治疗提供新策略。

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Bile acids and metabolic diseases

Xiaojiao Zheng[1,*], Junliang Kuang[1], Mengci Li[1], Aihua Zhao[1], Tianlu Chen[1], Weiping Jia[1], Wei Jia[1,2,*]

1. Center for Translational Medicine, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200233, China

2. School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

ABSTRACT: Hyocholic acid (HCA) is a major bile acid (BA) species in the BA pool of pigs, a species known for its exceptional resistance to spontaneous development of diabetic phenotypes. The clinical studies of five clinical cohorts underscore the association of HCA species with diabetes, and demonstrate the feasibility of using HCA profiles to assess the future risk of developing metabolic abnormalities. Both obesity and diabetes are associated with lower serum concentrations of HCA species. Serum HCA levels increase in the patients after gastric bypass surgery and can predict the remission of diabetes two years after surgery. Moreover, serum HCA species are found to be strong predictors for metabolic disorders in 5 and 10 years, respectively. The mechanistic study reveals that oral administration of HCAs improved serum fasting GLP-1 secretion and glucose homeostasis. HCAs upregulate GLP-1 production and secretion in enteroendocrine cells via simultaneously activating G-protein coupled BA receptor, TGR5, and inhibiting farnesoid X receptor (FXR), a unique mechanism that is not found in other BA species. Based on these research findings, we give perspectives of future research. HCAs are a class of non-12 hydroxylated bile acids synthesized in the bile acid alternative pathway. There is an important role of non-12 hydroxylated bile acids in treating hyperglycemia and fatty liver diseases. It is promising to target the alternative BA synthetic pathway for the treatment of metabolic diseases.

KEY WORDS: bile acids, gut microbiota, metabolic diseases, hyocholic acid species

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Skeletal muscle-secreted DLPC orchestrates systemic energy homeostasis by enhancing adipose browning

Xiaodi Hu[1,#], Mingwei Sun[2,#], Qian Chen[1], Yixia Zhao[1], Na Liang[1], Siyuan Wang[3], Pengbin Yin[4,5], Yuanping Yang[2], Sin Man Lam[6], Qianying Zhang[1], Alimujiang Tudiyusufu[1], Yingying Gu[1], Xin Wan[1], Meihong Chen[1], Hu Li[2], Xiaofei Zhang[2], Guanghou Shui[6,7], Suneng Fu[8], Licheng Zhang[4,5], Peifu Tang[4,5], Catherine C.L. Wong[3],Yong Zhang[1,2*], **Dahai Zhu**[1,2*].

[1]The State Key Laboratory of Complex, Severe, and Rare Diseases, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing, 100005. [2]Bioland Laboratory (Guangzhou Regenerative Medicine and Health Guangdong Laboratory), Guangzhou, 510005. [3]Clinical Research Institute, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, 100730. [4]Senior Department of Orthopedics, The Fourth Medical Center of Chinese PLA General Hospital, Beijing, 100853. [5]National Clinical Research Center for Orthopedics, Sports Medicine & Rehabilitation, Beijing, 100853. [6]LipidALL Technologies Company Limited, Changzhou, 213022. [7]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101. [8]Guangzhou Laboratory, Guangzhou, 510005.

*Correspondence: D.Z.: dhzhu@pumc.edu.cn; Y.Z.: yongzhang@ibms.pumc.edu.cn

Skeletal muscle is the largest metabolic and endocrine organ. It secretes various peptides that contribute to regulating body energy homeostasis by communicating with other metabolic organs. However, it is unknown whether muscle-secreted lipids exert a similar function. *Myod* is specifically expressed in skeletal muscle. Here, we report that genetic deletion of *Myod* in mice enhanced the oxidative metabolism of muscle and, intriguingly, rendered the mice resistant to HFD-induced obesity. By performing lipidomic analysis in muscle-conditioned medium and serum, we identified 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC) as a muscle-released lipid that is responsible for MyoD-orchestrated body energy homeostasis in *Myod* KO mice. Functionally, the administration of DLPC significantly ameliorated HFD-induced obesity in mice. Mechanistically, DLPC was found to induce white adipose browning via lipid peroxidation-mediated p38 signaling in mice. Collectively, our findings uncover DLPC as the first muscle-derived lipokine and suggest that it might have clinical potential for treating obesity in humans.

Keywords： Muscle-secreted lipokine, DLPC, MyoD, muscle-fat crosstalk, white-fat browning, systemic homeostasis

基于人工智能的超分辨空间代谢组学技术

朱洪影*
1. 中国科学技术大学

空间代谢组学（质谱成像，MSI）是一种无需在组织或器官切片中进行标记的表面分析方法，可以在一次实验中实现多种代谢分子空间分布图谱的绘制。该技术将质谱获得的代谢组学信息以及从组织切片获得的空间位置信息进行整合，是一种非常有价值的生物标本表征化学分析工具。目前，空间代谢组学技术被广泛应用于生理及病理机制解析、生物标志物的发现以及药代动力学研究，在生物医学研究和制药工业中越来越受欢迎。MSI 的空间分辨率是影响该技术评估精度的一个极其重要的参数，高空间分辨率使得研究人员能够在更精细的结构尺度上研究组织中的代谢分子变化，从而获得新颖而重要的发现。然而，对于所有的空间代谢组学成像技术，高空间分辨率则意味着需要很长的成像时间。因此，急需发展快速且保持高分辨率的空间代谢组学技术。

近年来，深度学习（DL）在生物图像（如荧光显微镜图像、电子显微镜图像和计算机断层扫描病理图像等）的超分辨率重建方面取得了巨大的进展。DL 需要大量的高分辨率数据集来训练，然而由于上述成像速度的限制，目前高分辨质谱成像数据集非常有限，很难将深度学习直接应用于空间代谢组学领域。我们团队提出了一种具有创新性的基于迁移学习的神经网络框架，称之为 MOSR（MSI from Optical Super-Resolution）。该 MOSR 模型可以先从生物样本光学图像中学习部分的低分辨-高分辨图片映射关系，然后将学习到的知识转移到 MSI 深度学习模型中，使得所需的高分辨 MSI 图像数量大大降低。经过训练后，MOSR 可以快速大幅提高 MSI 图像分辨率。更重要的是，与传统的深度学习方法相比，MOSR 在图像质量、训练效率和适用范围方面均表现得更好。并且，MOSR 目前已经可以实现跨物种和跨器官的质谱成像应用。

关键词: 空间代谢组学，人工智能，超分辨

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质谱驱动的精准代谢组学技术

朱正江 [1,*]

[1] 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，201210

*Email: jiangzhu@sioc.ac.cn

代谢组学技术主要研究生命体内小分子代谢产物的含量和动态变化规律。生命体的高度复杂性使得生命过程产生的代谢物具有数目众多、结构复杂、浓度分布范围广等特点。质谱技术能够在微量生物样本中同时检测数千种代谢物，具有选择性高和灵敏度高等优势，是代谢组学研究的主要技术之一。但由于生物样本微量、基质复杂、代谢物的理化性质差异大，目前基于质谱的非靶向代谢组学研究中代谢物精准定性和精确定量分析仍具有挑战。高分辨质谱技术能从微量生物样本中同时检测数万个代谢物离子峰，提供了丰富的代谢轮廓表征。但是由于质谱离子化过程的复杂性、代谢物标准二级质谱图库覆盖不足等挑战，如何大规模、准确地从质谱数据中识别代谢物并精准鉴定代谢物化学结构[1]，特别是发现未知代谢物，是目前代谢组学研究的核心挑战。

本报告主要介绍报告人近期在基于高分辨质谱的非靶向代谢组学领域的最新研究进展，主要介绍通过整合质谱技术和人工智能算法等交叉研究手段，通过发展“结构谱学关联、代谢网络迭代、生化信息演进”等一系列创新研究策略，系统开发了基于代谢反应网络的代谢组规模化精准定性技术 MetDNA[2,3]，实现了已知代谢物和未知代谢物大规模、自动化的精准鉴定和识别。该技术破解了利用质谱技术进行代谢组大规模精准鉴定的难题，显著提升了代谢组质谱分析的覆盖度、准确度和效率。进一步，从 MetDNA 鉴定的非标记代谢物出发，发展了“峰型模板、合并同类”的策略 MetTacer，实现了微量生物样本中超千个稳定同位素标记代谢物数的精准追踪和定量分析[4]，比同类技术的覆盖度提升了 2-4 倍，同时假阳性低于 5%。相关技术的发展极大地推动了质谱技术在代谢组学领域的应用，特别是有助于解析疾病相关的小分子代谢物动态变化规律[5]。近期，报告人进一步利用 MetTracer 技术在常见的模式细胞中实现了未知代谢反应和代谢反应网络的从头构建，发现了数百个新型代谢反应，并鉴定若干个全新未知代谢物。

关键词：质谱；代谢组学；代谢物定性；代谢反应网络

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口头报告

基于基质辅助激光解吸二次电离的高分辨率高灵敏空间代谢流成像

陈燕燕[1]，王佳宁[1]*， 蔡宗苇[1]*
香港浸会大学

对功能性代谢物在生物组织中的分布进行深入了解对于解释生物分子过程具有重要意义。然而，由于组织样本的复杂性和异质性，许多内源性代谢物的原位成像特别是高覆盖度检测仍存在挑战。为了克服这一难题，我们构建了一种基于基质辅助激光解吸二次电质谱成像方法，通过优化的基质与测试策略，揭示了组织中高覆盖度内源性代谢物的高分辨空间分布。

利用该方法，我们实现了包括糖酵解、三羧酸循环和嘌呤代谢等六条主要代谢通路内一百多种代谢小分子以及数百种脂质的质谱成像。这些结果展示了该方法具有广泛的质量覆盖范围和高灵敏度，并成功实现了不同组织中代谢物的高分辨率（10 μm）成像。

此外，通过 13C-同位素标记技术，展示了不同组织器官包括肾、脑、心脏和乳腺癌组织中的空间代谢流。结果显示了标记和未标记的糖酵解和三羧酸循环中间产物在小鼠肾脏和心脏的不同区域分布，揭示了代谢时空动态变化。在小鼠大脑中，成功可视化了神经递质谷氨酰胺和谷氨酸循环通路在大脑皮层、海马区等六个主要区域的分布。同时，在小鼠乳腺癌原位瘤中，本研究还揭示了 4 条代谢通路（包括糖酵解、三羧酸循环、糖原生成和戊糖磷酸化通路）的中间产物在肿瘤组织中的空间分布情况，进一步探明了这些通路分子在肿瘤增殖区的活跃状态。

综合来说，本研究所建立的空间代谢流成像方法为揭示代谢物在不同组织中的空间异质性提供了强有力的工具，为深入了解和探究细胞和组织层面的代谢活动开辟了新的途径。

关键词: 基质辅助激光解吸电离，二次电离，质谱成像，代谢流，空间代谢组

Sulfonylation Sites of Nucleobases, Nucleosides and Nucleotides

Xiaoyue Cheng, Pengcheng Li, CongCong Zhang, Li Xu, Qi Wang* and Huiru Tang*

State Key Laboratory of Genetic Engineering, School of Life Sciences and Human Phenome Institute, Metabonomics and Systems Biology Laboratory at Shanghai International Centre for Molecular Phenomics, Zhongshan Hospital, Fudan University, Shanghai 200438, China;

ABSTRACT: Nucleobases, nucleosides and nucleotides are the vital bricks of DNA and RNA exhibiting important physiological activities in storing and expressing genetic information, cell signal conduction and metabolism[1-3]. Dansyl chloride (DNS-Cl) and 5-(diethylamino)naphthalene-1-sulfonyl chloride (DEANS-Cl) have been widely used for sulfonylation and fluorescence labelling to quantitative nucleoside-related metabolites[4,5]. However, the exact sites for such sulfonation remained uncertain and our study suggested that such assumed sulfonation sites were not correct. To ascertain such sulfonation sites, here, we employed both high-resolution mass spectrometry (HRMS) and NMR to investigate the reaction products of 25 nucleosides-related metabolites, including nucleobases, nucleosides and nucleotides, with DNS-Cl and DEANS-Cl. Our results showed that DNS-Cl and DEANS-Cl could react with all these nucleoside-related metabolites. More specifically, our MS/MS spectra suggested the hydroxyl groups instead of exocyclic NH_2 groups of nucleosides, 2'-deoxynucleosides, except 2'-deoxyguanosine, and nucleotides as the sulfonylated sites for all sulfonyl chlorides. The nucleobase reaction sites are secondary amine on the imidazole ring or enolic hydroxyl group. Further, it indicated that nucleosides and monophosphate nucleotides were sulfonylated at ribose 2'-hydroxyl groups, except cytidine and CMP, whereas the sulfonylated sites with deoxynucleosides were the ribose 3'-hydroxyl group, except 2'-deoxyguanosine and 2'-deoxyinosine, by NMR. These findings clarified and provided detailed information about sulfonation sites for nucleobases, nucleosides and nucleotides and offered basic data for derivatization-based quantitative analysis and labelling.

KEYWORDS: nucleosides-related metabolites, sulfonylation sites, HRMS and NMR.

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Revealing Molecular Phenotypes for Chiral Carboxylic Acids in Multiple Human Matrices with the Probe-assisted Sensitivity-Enhanced Quantitative Metabolomics Using Liquid Chromatography with Tandem Mass Spectrometry

Yuting Sun[1,2], Yulan Wang[3,*], Huiru Tang[1,*]

1. State Key Laboratory of Genetic Engineering, School of Life Sciences, Human Phenome Institute, Zhangjiang Fudan International Innovation Center, Metabonomics and Systems Biology Laboratory at Shanghai International Centre for Molecular Phenomics, Zhongshan Hospital, Fudan University, Shanghai 200032, China.
2. Wuhan Institute of Physics and Mathematics, Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences, Wuhan 430071, China.
3. Singapore Phenome Centre, Lee Kong Chian School of Medicine, Nanyang Technological University, 639798 Singapore.

ABSTRACT: Many chiral carboxylic acids with multiple biological functions are concurrently present to form unique molecular phenotypes in biological matrices.[1] Their quantification is essential to reveal the biochemical details of physiology and pathophysiology but is challenging with their low levels in biological matrices and difficulty in enantiomer resolution. Here, we developed a method of sensitivity-enhanced quantitative metabolomics (Seq-Metabolomics) for chirality-resolved simultaneous quantification of chiral carboxylic acids. We designed and synthesized a hydrazide-based novel chiral probe, (*S*)-benzoyl-proline-hydrazide (*S*BPH), to convert chiral carboxylic acids into their amide diastereomers which were resolved on a C18 column. Using *S*BPH-d_5 labeled chiral carboxylic acid enantiomers as internal standards, we then developed a parameter-optimized ultrahigh-performance liquid-chromatography with tandem mass spectrometry (UPLC-MS/MS) method for simultaneous quantification of 60 enantiomers of 30 chiral carboxylic acids in a single run. The method showed excellent sensitivity (LOD<4 fmol on column), linearity (R^2>0.992), precision (CV<15%), accuracy (|RE|<20%), and recoveries (80-120%) in multiple biological matrices. By using this method, we managed to quantify sixty chiral carboxylic acids in human urine, plasma, feces, and a human cell line (A549) to define their metabolic phenotypes. This provides a powerful tool and promising approach to investigate interactions between the gut microbiome and host for human phenomics.

KEY WORDS: chiral carboxylic acids, sensitivity-enhanced quantitative metabolomics (Seq-Metabolomics) via probe-labeling, ultrahigh-performance liquid chromatography with tandem mass spectrometry

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单细胞/亚细胞代谢组学分析技术及应用

石先哲*，徐天润，丰迪生，常蒙蒙，李杭，胡春秀，许国旺
中国科学院大连化学物理研究所，大连，116023
E-mail: shixianzhe@dicp.ac.cn

细胞是生物体生命活动和形态结构的最基本单位，具有高度异质性。而且，细胞内外包涵许多亚细胞体，如线粒体、外泌体等。开展单细胞/亚细胞水平代谢组学研究，可以定性定量描述单细胞或亚细胞体中内源性代谢物，提供细胞表型最直接和动态的信息，有助于与疾病相关的生化过程的更深入的理解。然而，单细胞/亚细胞代谢组学研究对分析技术提出了巨大挑战。

在亚细胞代谢组学方面，针对目前纯化技术存在普适性低和纯化效率有限的问题。研制了一种抗体修饰的磁性亲和材料（MagG@PD@Avidin@TOM20），利用材料表面的抗体anti-TOM20 与线粒体外膜上的 TOM20 的亲和作用，实现了细胞中线粒体的选择性分离富集，并成功用于不同肝细胞中线粒体的代谢组学分析[1]。此外，研制了特异识别外泌体表面膜蛋白的核酸适配体功能化纳米复合材料（MagG@PEI@DSP@aptamer），发展了复杂生物样本中微量外泌体的高效分离纯化方法。结合高灵敏的非靶向液相色谱-质谱分析方法，实现了细胞外泌体的高覆盖代谢组学分析[2]。

在单细胞代谢组学方面，通过搭建用于细胞取样的多维度精密调节平台，结合纳升电喷雾直接进样的拼接式高分辨质谱采集方式，实现了20个细胞中19种脂质亚类的500多个脂质代谢物的高通量、高灵敏检测。该技术平台已成功应用于不同类型和亚类的癌细胞及不同病理组织靶细胞的分类，显示出巨大的临床应用潜力[3]。设计和研制了一种高通量细胞排序进样-低频脉冲感应纳升电喷雾电离源，结合高分辨质谱构建了高通量、高覆盖单细胞代谢组学分析平台。该技术平台不仅能耐受细胞高盐基质的干扰，从单个细胞中鉴定到120多种代谢物，而且具备80个细胞/分钟的分析通量，是目前已报道最高通量的2倍，可连续获取数千个单细胞的代谢组信息，为细胞异质性研究提供了有力工具[4]。

关键词：代谢组学；单细胞；亚细胞

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高覆盖率定量分子表型分析的代谢组学与脂质组学平台

李佳[1], 陈曦[1], 詹晶晶[1], 赵爽[1,*],
1. 厦门市迈理奥科技有限公司，福建省厦门市翁角西路 2052 号（厦门生物医药产业园）B2 栋 703 室

【目的】在各类型研究中全面且定量的分子表型分析的重要性日益突显，使用高性能的代谢组学和脂质组学方法能够有效获取准确、可靠且有意义的分子表型。这里我们将展示一种用于高覆盖率定量分子表型分析的深度代谢组学和脂质组学平台。

【方法】代谢组学分析采用 DeepMarker MT 代谢组学平台，该技术平台基于高效化学同位素标记（High Performance Chemical Isotope Labeling, HP-CIL）结合液相色谱-质谱联用（LC-MS）方法。不同于常规代谢组学方法中直接分析代谢物原型分子，我们首先使用一对同位素标记试剂对样本进行化学衍生化，再使用 LC-MS 检测衍生后的代谢物，最后使用 IsoMS Pro 软件对代谢物进行识别和分析。

脂质组学分析采用 DeepMarker LT 脂质组学平台，该技术平台将每个样本与一系列的稳定同位素内标混合，再进行脂质提取以及液相色谱-质谱联用（LC-MS、LC-MS/MS）分析，最后使用 Lipid Screener 软件对脂质进行识别和分析。

【结果】在 DeepMarker MT 代谢组学平台中，使用不同的衍生化试剂分别分析胺/酚类、羧酸类、羟基类和羰基类这四个次级代谢组。通过全套优化的衍生化试剂，有效实现液相分离、质谱检测和定量能力的大幅增强。此外，为了提升代谢物鉴定水平，我们还开发了三层级鉴定方法。通过检测灵敏度和鉴定能力的双重增强，实现全面代谢组的高性能分析。

在 DeepMarker LT 脂质组学平台中，通过一系列合理设计的同位素内标，增强了常见脂质类型的定量分析能力，以及利用 LC-MS 和 LC-MS/MS 的数据获取，增强了脂质的覆盖范围和鉴定能力。此外，为了提升脂质鉴定水平，我们也开发了三层级鉴定数据库。通过高覆盖率的检测和更精准的定量分析，实现高性能脂质组学分析。

目前，针对多类型常见样本分析的工作流程已经开发并完成。以血清样本为例，依托 DeepMarker MT 代谢组学平台，可以在每个样本中平均检测到 9179 ± 71 个代谢物。其中，超过 1200 个代谢物可以被高可信度地鉴定，覆盖了 100 多条代谢通路。依托 DeepMarker LT 脂质组学平台，可以在每个样本中检测到 8385 ± 266 种脂质，并有超过 800 种脂质可以被准确鉴定。结合这两种组学平台，共可以鉴定出超 2000 种代谢物，并实现高准确性和高精度的定量，最终实现超高覆盖率分子表型的分析。

【结论】可用于高效分子表型分析的代谢组学和脂质组学平台已开发完成。

关键词：代谢组学；脂质组学；高效化学同位素标记；液相色谱-质谱联用

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基于离子淌度质谱的四维代谢组学精准分析技术 Met4DX

罗名都[1,2], 尹岩东[1], 朱正江[1,*]

1. 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，200032

2. 中国科学院大学，北京，100049

离子淌度质谱相较于传统质谱增加了根据离子尺寸、形状以及电荷分离的离子淌度，有效提升了质谱的分离能力，特别是代谢物同分异构体的分辨能力，其跟液相色谱联用形成多维分离分析技术，能进一步提高复杂生物样本分析的分离度和峰容量。一次四维代谢组学分析能够同时对代谢物离子进行四个维度的表征，包括精确质量数、二级质谱图、色谱保留时间和离子淌度碰撞截面积，能有效提升对复杂生物样品中代谢物定性和定量分析的覆盖度和准确度。然而，四维代谢组数据的高度复杂性对数据的高效精准分析提出了巨大的挑战，尤其是四维质谱峰的检测仍然是难点。因此，四维代谢组数据分析技术和工具相对有限。目前少量工具，如 MS-DIAL[1] 与 MZmine[2] 等，均采用了自上而下压缩数据的降维策略进行峰检测。该降维策略可以降低数据的维数和复杂性，但降维过程也不可避免低引入了信号掩蔽以及干扰，显著降低了四维峰检测的灵敏度。

针对上述问题，我们开发了从一张质谱图出发的自下而上峰组装算法用于四维代谢组学数据中四维峰检测的技术（*Nature Communications*, **2023**）[3]。该算法的特点是将每一张质谱图作为四维数据中的最小数据单元，采用逆向工程的策略依次构建其在离子淌度和液相色谱上的峰形。自下而上的峰组装算法避免了数据压缩与降维，有效地提高了四维峰检测的覆盖度与灵敏度。以上述算法为核心，我们进一步开发了适用于四维代谢组学的端到端的精准数据分析技术 Met4DX，通过二级谱图去冗余模块、自下而上的峰组装模块、四维峰对齐以及分组模块、代谢物的多维匹配与鉴定模块等实现了的四维复杂代谢组的精准定性和精确定量分析。Met4DX 技术能够实现高覆盖的四维质谱峰检测，定量精密度高。与同类技术相比，Met4DX 能够提升四维峰检测的覆盖度 2-3 倍，提升准确定量代谢物的数目 2-5 倍。Met4DX 在代谢物同分异构体识别上具有优异的性能，以在小鼠肝脏代谢组为例，Met4DX 精准识别代谢物同分异构体数目高达 3033 对，比同类技术显著提升 3.6 倍，并且可准确识别出 CCS 差异为 1% 的共流出同分异构体。同时，本文还收集了 HMDB 和 KEGG 中的超过 13 万个代谢物，建立了目前最全面的四维代谢物数据库用于代谢物的多维匹配与鉴定。

关键词: 离子淌度质谱；四维代谢组；四维峰检测；代谢物四维匹配；代谢物同分异构体

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Plasma metabolomic profiling and biomarker panel selection using widely-targeted metabolomics and machine learning for patients in different stages of chronic kidney disease

Yao-Hua Gu,[1,2,3,†] Yu Chen,[4,†] Qing Li,[5,†] Neng-Bin Xie,[1] Xue Xing,[5] Jun Xiong,[1] Min Hu,[1] Tian-Zhou Li,[1] Ke-Yu Yuan,[1] Yu Liu,[6] Tang Tang,[4,*] Fan He,[5,*] Bi-Feng Yuan[1,3,6,*]

[1] School of Public Health, Research Center of Public Health, Renmin Hospital of Wuhan University, Wuhan University, Wuhan, 430071, China.

[2] School of Nursing, Wuhan University, Wuhan, 430071, China.

[3] College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China.

[4] Metware Biotechnology Co., Ltd., Wuhan, 430075, China.

[5] Tongji Medical College of Huazhong University of Science and Technology, Wuhan, 430030, China.

[6] Department of Radiation and Medical Oncology, Cancer Precision Diagnosis and Treatment and Translational Medicine Hubei Engineering Research Center, Zhongnan Hospital of Wuhan University, Wuhan Research Center for Infectious Diseases and Cancer, Chinese Academy of Medical Sciences, Wuhan 430071, China

Abstract

Chronic kidney disease (CKD) is an increasingly prevalent medical condition associated with high mortality and cardiovascular complications. The intricate interplay between kidney dysfunction and subsequent metabolic disturbances may provide insights into the underlying mechanisms driving CKD onset and progression. However, there is a lack of comprehensive global metabolomics profiling in CKD, and many key circulating metabolites remain unidentified. Herein, we proposed a widely-targeted metabolomics approach that enables large-scale identification and accurate quantification of thousands of metabolites. We collected plasma samples from 21 healthy controls and 62 CKD patients, categorized into different stages (22 in stages 1-3, 20 in stage 4, and 20 in stage 5). Using LC-MS-based widely-targeted metabolomics approach, we were able to effectively annotate and quantify a total of 1431 metabolites from the plasma samples. Focusing on the 539 endogenous metabolites, we identified 399 significantly altered metabolites and depicted their changing patterns from healthy controls to end-stage CKD. Furthermore, we employed machine learning to identify the optimal combination of metabolites for predicting different stages of CKD. We generated a multiclass classifier consisting of 7 metabolites by machine learning, which exhibited an average AUC of 0.99 for the test set. In general, amino acids, nucleotides, organic acids, and their metabolites emerged as the most significantly altered metabolites. However, their patterns of change varied across different stages of CKD. The 7-metabolite panel demonstrates promising potential as biomarker candidates for CKD. Further exploration of these metabolites can provide valuable insights into their roles in the etiology and progression of CKD.

Keywords: widely-targeted metabolomics; machine learning; chronic kidney disease; biomarker

代谢组 NMR 数据的处理能力和可解释性

陈炜敏，陈洪彪，赵秀举

代谢组NMR 数据具有弱关联的特征高维、共线性、非同分布等，造成处理 能力低下和可解释性差。作者开发了弱关联数据集整合的基础流程，建立了预处 理和分析过程方法特性抉择逻辑，并应用于代谢组NMR 数据处理（积分间隔 0.004 ppm，2106×36）。相对于通常的UV、Par 和Ctr 标度方法，Box-Cox 显著 提高了模型的Q^2；Meta 分析加权提高了模型整合能力；相对于PCA，UMAP 减 小组内差异的同时增大了组建差异，节省计算时间；自适应 LASSO- 岭回归 -Logistic 流程发现了新的生物标志物（r<0.05）。Box-Cox 变换-Meta 归一化-L1 和L2 正则化-特征消除分析提高了模型预测能力和可解释性。

基于图神经网络的代谢组学数据分析方法

张艳慧，苏本哲，林晓惠*
大连理工大学，大连，116024，中国

代谢组学是系统生物学的重要组成部分，基于代谢组学的疾病诊断、预后以及药物研发等是近年精准医疗、个性化治疗和健康管理研究的热点。基于高分辨质谱（HRMS）的非靶向方法是代谢组学研究的主要方法，产生的代谢组数据维数高、结构复杂。因此，研究有效的人工智能算法，从尿液、血液以及组织等样本的高维复杂的代谢组学数据中确定反映机体病变、鲁棒性强的代谢标志物，建立高性能的分类模型，有助于疾病的预警、诊断、预后指导和疗效评价，具有重要意义。

目前代谢组学数据分析通常采用单变量分析、多变量分析、网络分析等技术，从大量的代谢组数据中确定关键的代谢组分，挖掘反映疾病发生发展变化的代谢特征。已知疾病中发生扰动的不仅在单个代谢成分、单个代谢通路，常常涉及多条代谢通路。因此，基于网络的组学数据分析方法日益得到关注。现有的网络分析方法大多基于相关性，确定 HUB 结点为潜在的生物标志物，据此建立的分类模型鲁棒性差，没有体现代谢物之间复杂的关联关系。

本研究分别建立代谢网络和样本网络，开展基于图神经网络技术的代谢组学数据分析方法研究。首先研究代谢成分之间的协同作用，建立差异代谢网络，融合网络拓扑结构信息和代谢组学数据搜索关键信息子网，基于关键信息子网定义单样本属性子图，建立图神经网络分类模型[1]。其次，建立样本网络，通过遗传算法搜索富含信息的特征子集，确定参考样本网络，基于确定的参考样本网络和图神经网络，建立疾病分型模型[2]。将建立的代谢组学数据分析方法用于乳腺癌、糖尿病等疾病代谢组学数据分析，基于确定的标志物所建立的分类模型能很好地区分不同类型的样本。本研究为疾病代谢组学研究提供了新的、有效的数据分析方法。

关键字: 代谢组学，标志物发现，图神经网络.

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Lipid metabolic profiling via quantitative stimulated Raman scattering imaging opens up new avenues for precision medicine

Shuhua Yue[1,*]

1. Key Laboratory of Biomechanics and Mechanobiology, Ministry of Education, Institute of Medical Photonics, Beijing Advanced Innovation Center for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191

ABSTRACT: Dysregulated lipid droplet (LD) biology underlies an increasing number of human diseases. Label-free stimulated Raman scattering (SRS) microscopy enables both LD visualization and compositional analysis in situ. This talk will showcase that lipid metabolic profiling via quantitative SRS imaging opens up new avenues for precision medicine. First, based on newly developed quantitative SRS microscopy, we revealed remarkably dysregulated lipid homeostasis in late-stage compared to early-stage liver fibrosis, i.e. increased unsaturated triglycerides with decreased lipid unsaturation degree. Inspiringly, injured hepatocytes could be rescued by lipid homeostasis remodeling via either supply of unsaturated fatty acids or enhancement of membrane fluidity. Collectively, this work offers new opportunities for treatment of liver fibrosis [1]. Second, we established an SRS-based intelligent molecular cytology (SRMC). In ascites from 80 gastric cancer (GC) patients, we revealed 12 single cell features that are significantly different between peritoneal metastasis (PM) positive and negative specimens, particularly LD amount. Assisted by AI phenotyping algorithm, the SRMC method reached the AUC of 0.85 within 20 minutes per patient. Together, our method shows great potential for accurate and rapid detection of PM from GC [2].

KEY WORDS: Lipid metabolic profiling; Stimulated Raman scattering microscopy; Precision medicine

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脂质异构体串联质谱成像分析方法及应用

张文鹏[1]*

1. 清华大学精密仪器系，北京，100084

脂质是细胞结构的重要组成部分，在信号传导、运输作用以及生物大分子分选过程中扮演着重要角色。对生物系统中的脂质及相关分子进行系统分析和鉴定是深入了解脂质功能的基础，对揭示脂质代谢与细胞、组织乃至机体的生理、病理过程之间的关系具有重要意义。由于脂质分子结构的多样性和复杂性，目前的质谱技术尚不能对脂质精细结构提供确定信息。组织中脂质的质谱成像，是分子病理和空间组学的重要技术手段。实现高质量的脂质组学成像，目前还存在较大挑战，其中一个主要问题是高确定性的组学分析依赖串联质谱来确定具有复杂精细结构的脂质分子，而组织切片成像时单个位点的样品量少，无法支撑多种脂类分子的串联质谱分析的需要。

针对该问题，我们基于小型双离子阱质谱系统，提出了一种多目标离子串联质谱成像（MS^2I）方法。利用离子存储、选择性离子传输及串联质谱分析，实现了单个生物组织切片位点上 10 种脂质分子的结构解析及高灵敏成像，显著提升了生物组织中脂质质谱成像的确定性。将多目标离子串联质谱成像方法与 PB 光化学衍生化方法相结合，利用温控片上衍生化的方法，实现了多目标甘油磷脂分子 C=C 双键位置异构体串联质谱成像分析。以鼠脑样本为模型，在一次成像中同时实现了脂质类型、脂质 C=C 异构体分子的成像分析。将该方法应用于肝细胞肝癌组织切片分析，实现了近 20 种甘油磷脂分子 C=C 异构体的质谱成像。采用降维聚类分析方法对肝细胞肝癌样本的异质性进行分析，利用脂质异构体分类结果对癌变边界区域进行了空间分布重构，建立了病理染色结果与脂质 C=C 异构体分子质谱成像的关联，实现了癌变和癌旁交叠区域的高准确性可视化分析。

关键词: 脂质组学；脂质异构体；质谱成像；疾病标志物.

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MPI-VGAE: protein-metabolite enzymatic reaction link learning by variational graph autoencoders

Cheng Wang[1,2], Chuang Yuan[1,2], Yahui Wang[3,6], Ranran Chen[1,2], Yuying Shi[1,2], Tao Zhang[1,2], Fuzhong Xue[1,2], Gary J. Patti[3,4,5,6,*], Leyi Wei[7,*], Qingzhen Hou[1,2,*]

1.Department of Biostatistics, School of Public Health, Cheeloo College of Medicine, Shandong University, Jinan, 250012, China

2. National Institute of Health Data Science of China, Shandong University, Jinan, 250000, China

3. Department of Chemistry, Washington University in St. Louis, St. Louis, MO, 63130, USA

4. Department of Medicine, Washington University in St. Louis, St. Louis, MO, 63130, USA

5. Siteman Cancer Center, Washington University in St. Louis, St. Louis, MO, 63130, USA

6. Center for Metabolomics and Isotope Tracing, Washington University in St. Louis, St. Louis, MO, 63130, USA

7. School of Software, Shandong University, Jinan, 250100, China

Abstract

Enzymatic reactions are crucial to explore the mechanistic function of metabolites and proteins in cellular processes and to understand the etiology of diseases. The increasing number of interconnected metabolic reactions allows the development of *in silico* deep learning based methods to discover new enzymatic reaction links between metabolites and proteins to further expand the landscape of existing metabolite–protein interactome. Computational approaches to predict the enzymatic reaction link by metabolite–protein interaction (MPI) prediction are still very limited. In this study, we developed a Variational Graph Autoencoders (VGAE)-based framework to predict MPI in genome-scale heterogeneous enzymatic reaction networks across ten organisms. By incorporating molecular features of metabolites and proteins as well as neighboring information in the MPI networks, our MPI-VGAE predictor achieved the best predictive performance compared to other machine learning methods. Moreover, when applying the MPI-VGAE framework to reconstruct hundreds of metabolic pathways, functional enzymatic reaction networks and a metabolite–metabolite interaction network, our method showed the most robust performance among all scenarios. To the best of our knowledge, this is the first MPI predictor by VGAE for enzymatic reaction link prediction. Furthermore, we implemented the MPI-VGAE framework to reconstruct the disease-specific MPI network based on the disrupted metabolites and proteins in Alzheimer's disease and colorectal cancer, respectively. A substantial number of novel enzymatic reaction links were identified. We further validated and explored the interactions of these enzymatic reactions using molecular docking. These results highlight the potential of the MPI-VGAE framework for the discovery of novel disease related enzymatic reactions and facilitate the study of the disrupted metabolisms in diseases.

Keywords: metabolite, metabolite–protein interaction, enzymatic reaction, graph neural network, machine learning

Reference: Wang, Cheng, et al. "MPI-VGAE: protein–metabolite enzymatic reaction link learning by variational graph autoencoders." *Briefings in Bioinformatics* (2023): bbad189.

Transketolase is a critical modulator of inosine metabolism and function in hepatocytes

Lingfeng Tong[1], Zhangbing Chen[1], Xuemei Tong[1*]

1. Shanghai Jiao Tong University School of Medicine, Dept. of Biochemistry and Molecular Cell Biology, Shanghai, China.

ABSTRACT: Nucleosides such as inosine are newly identified carbon sources for glycolysis and TCA cycle in immune cells and tumors. Extracellular inosine is able to promote mitochondrial energy expenditure by activating the cAMP-PKA-UCP1 signaling pathway in adipose tissues. However, how the intracellular level of inosine is determined and whether inosine plays an important role in regulating mitochondrial activity in liver cells remain unknown. Here we employed metabolomic and stable isotope tracing to uncover the pivotal role of the non-oxidative pentose phosphate pathway (PPP) in regulating inosine flux into glycolysis and TCA cycle. The absence of transketolase (TKT), a metabolic enzyme in non-oxidative PPP, promoted de novo inosine biosynthesis from glucose, and prevented the pentose of inosine from entering glycolysis and TCA cycle, resulting in accumulation of ribose-5-phosphate (R5P) and inosine. Moreover, inosine activated the PKA-CREB signaling pathway to enhance mitochondrial function in hepatocytes, in a UCP1-independent way. Lipidomic analysis revealed an inverse correlation between levels of phosphatidylcholine (PC) and TKT protein in the liver. Accordingly, TKT deficiency promoted mitochondrial function in hepatocytes by increasing PC synthesis. We further demonstrated that inosine promoted PC synthesis in hepatocytes by upregulating the CDP-choline pathway. Inhibiting PKA activity or feeding mice with a low-choline diet to suppress PC synthesis eliminated TKT-dependent alterations in hepatic mitochondrial function. In summary, our study has not only identified the critical role of TKT in modulating the intracellular level of inosine, but also revealed the mechanism by which extracellular inosine regulates mitochondrial activity in hepatocytes.

KEY WORDS: transketolase, mitochondrial function, inosine, phosphatidylcholine.

Time-resolved metabolite and lipid profiling depicts macrophage continuum with apoptotic and ferroptotic heterogeneity along foam cell formation

Ling Lin[1,2], Yiwen Wang[1], Liang Qiao[1,*]

1. Department of Chemistry, Fudan University, Shanghai 200441, China

2. Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, China

ABSTRACT:

Atherosclerosis is a chronic inflammatory disease driven by the maladaptive lipid metabolism and inflammatory response of macrophages. In the progression of atherosclerosis, macrophages work as a lipid scavenger and immune regulator by dynamic lipid and metabolic reprogramming in response to the microenvironment. Nevertheless, we are only starting to characterize the function of the heterogeneity in metabolite and lipid compositions. Herein, we developed a four-dimensional micro-manipulation platform for single-cell sample preparation, and observed the macrophage heterogeneity along foam cell formation via a single-cell time-resolved metabolite and lipid profiling. By integrating bulk metabolomic and lipidomic data with single-cell metabolomic and lipidomic data, we were surprised to uncover that macrophages have different fate outcomes during the late foam cell formation process. Some foam cells were more prone to apoptosis, while others are more susceptible to ferroptosis, suggesting heterogeneity in cell fate determination could have important implications for understanding atherosclerosis progression. Single-cell transcriptome sequencing was further performed on late-stage foam cells derived from both THP-1 and peripheral blood mononuclear cells, jointly confirming the divergent cell fates toward apoptosis or ferroptosis. Lastly, we validated the heterogeneity of late-stage foam cell differentiation through a series of in vitro experiments, including caspase activity assay and lipid peroxidation assay. In summary, single-cell multi-omics depict the molecular choreography that dictates the cell death in late atherosclerosis.

KEY WORDS: Single-cell analysis; Metabolomics; Lipidomics; Foam cell; Ferroptosis.

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代谢组学生物样本质量控制及在重大疾病研究中的应用

刘心昱[1]，王晴晴[1], Rainer Lehmann[2]，许国旺[1,*]

1.中国科学院分离分析化学重点实验室 中国科学院大连化学物理研究所，辽宁大连 116023
2. Institute for Clinical Chemistry and Pathobiochemistry Central Laboratory , University Hospital Tuebingen, Germany
*Email: xugw@dicp.ac.cn

代谢组是指生物有机体内源性小分子代谢物的动态整体，基于质谱（MS）的代谢组学是发现生物标志物和解释机制的有力工具。借助快速进步的分析化学技术及不断深入的生物功能解释，代谢组学研究得到了突飞猛进的发展，日趋成为生命科学研究的热点，广泛应用于人类疾病研究等众多领域。

血液等临床代谢组学样本来自不同医院，质量难以评估。样本质量的低劣会导致科学研究的重现性差，甚至是科学研究结果的失真，目前仍缺乏客观可靠的血液样本质量评价方法。本研究针对血液采集过程中普遍存在的室温暴露现象，采用非靶向代谢组学和脂质组学技术，结合多变量、单变量等数据分析方法，发现并验证了评价血液样本质量的标志物，从源头保障了代谢组学数据的可靠性。

在此基础上，通过对肝癌血液代谢组的研究，揭示了肝癌患者的代谢重编程，发现了肝癌诊断和预后标志物。

代谢组学在疫苗免疫作用的评价上也发挥了作用。我们在 SOP 下采集血液样品，在代谢水平探讨了两针疫苗后免疫建立的过程，并用四个代谢物的组合评价免疫效果，进一步提出从代谢视角评价疫苗效果的新理念。

关键词: 代谢组学，生物样本，质量控制，疾病诊断，免疫评价

参比物质构建在临床大队列代谢组学数据中的分析方法及应用

姚瑶[1,2], 张晖[2], 王雪萍[2]，刘万里[2]，栾天罡[1], 黄蓬[2]，胡寓旻[2*]
1. 中山大学生命科学院
2. 中山大学肿瘤防治中心

背景：寻找疾病相关标志物的代谢组学研究日益普遍，因此控制临床大队列数据的系统误差对于结果的可重复性至关重要。受日常设备通量和人员等因素限制，临床大规模样本的收集与处理可能花费数周或数月完成。现有基于质控（Quality control, QC）样品的校正方法中，研究样品信号的校正依赖于 QC 样品的检测批次和进样顺序，因此引入不同批次数量的样品会得到不同的校正值，影响数据分析的可重复性，在大规模样品实际应用中具有一定局限性。为消除多批次的样品前处理和检测可能导致的数据差异，我们提出一种基于参比物质的代谢组学分析策略（Reference Material，Ref-M）。**方法：**本研究中，我们混合 100 例健康人血清池作为参比物质。研究队列包括本院 306 例正常对照、肺部良性结节与肺腺癌的血清样本，拟通过 LC-MS 非靶标代谢组学建立鉴别诊断模型并进一步在本院 104 例以及外院 111 例样本进行内部与外部验证。所有样品分为 31 个前处理批次进行代谢物提取，每个前处理批次均加入参比血清样品和同位素内标（Internal standard, IS），用于后续数据质控与校正。**结果：** **1**）基于 Ref-M 方法校正后，所有样本中 IS 的信号强度 RSD 均小于 15%，PCA 分析中样本的批次效应得到明显消除；+ESI、-ESI 模式下有效特征离子的比例分别从 17.96%、35.93%提高到 65.19%、62.48%。 **2**）代谢组学结果揭示了良性肺结节与肺腺癌之间包括色氨酸、烟酸和烟酰胺、糖酵解、三羧酸循环和嘌呤代谢的主要通路变化，细胞实验与 TCGA 数据库分析进一步阐明肺腺癌细胞吸收色氨酸增加，并通过犬尿氨酸代谢途径产生的 NAD+促进肺腺癌糖酵解活性的机制，从而证实了 Ref-M 血清代谢组分析结果的可靠性。**3**）建立基于 27 种代谢物组合的鉴别诊断模型区分肺部良性结节与恶性肿瘤，其 AUC 达到 0.933，在内部与外部验证集达到 0.915 和 0.945。**结论：**Ref-M 策略有效控制大规模临床样本的批次差异，提高发现代谢标志物准确性与可重复性。结果有效验证了血清代谢组学所发现的标志物在肺癌中的生物学意义。建立了高效的鉴别诊断模型，为目前仅依赖于 CT 影像学扫描的肺癌早筛，提供了一个重要的辅助诊断手段。

关键词: 肺部结节；鉴别诊断；色氨酸代谢；临床代谢组学; 数据校正

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单细胞代谢质谱在肝癌自然杀伤细胞失去抗肿瘤功能研究中的应用

侯壮豪[1,2], 郑小虎[1,3,*], 魏海明[1,3,*], 黄光明[1,2,*]

1. 中国科学技术大学第一附属医院，中国科学技术大学，合肥，安徽 230000
2. 化学与材料科学学院，中国科学技术大学，合肥，安徽 230000
3. 生命与科学学院，中国科学技术大学，合肥，安徽 230000

免疫细胞在肿瘤微环境中起着重要的作用，针对免疫细胞体积小、代谢物成分复杂，代谢变化快等挑战，本研究开发了单个免疫细胞代谢质谱平台，采用免疫分选的方式实现高纯度的 NK 细胞，采用显微取样结合负压抽取的方式，将 NK 细胞的细胞内物质和细胞膜组分同时抽取，采用感应电喷雾进行代谢物与基质的超快分离与离子化，实现了免疫细胞内小分子化合物与脂质等代谢物的同时快速检测。对正常肝组织、正常人外周血和癌患者肝癌肿瘤内的NK细胞进行单细胞代谢质谱分析，发现癌组织中的NK细胞内鞘磷脂的水平显著降低。结合扫描电镜、透射电镜及生物学功能实验，发现癌组织中的 NK 细胞内鞘磷脂的降低与 NK 细胞的突触丢失及抗癌能力减弱有关。对 NK 细胞的单细胞代谢质谱分析，发现肿瘤内 NK 细胞的鞘磷脂降低与细胞内丝氨酸代谢通路有关，抑制肿瘤内 NK 细胞的鞘磷脂的降解能够有效恢复 NK 细胞的抗癌能力。

关键词: 单细胞代谢 质谱分析 自然杀伤细胞 肝癌

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Glutamine synthetase limits -catenin-mutated liver cancer growth by maintaining nitrogen homeostasis and suppressing mTORC1

Weiwei Dai[1,2], Wei-Xing Zong[2*]

1. Department of Biological Science, School of Sciences, Xi'an Jiaotong-Liverpool University, Suzhou 215123, China

2. Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers-The State University of New Jersey, Piscataway, NJ 08854, USA

ABSTRACT: Glutamine synthetase (GS) catalyzes *de novo* synthesis of glutamine that facilitates cancer cell growth. In the liver, GS functions to remove ammonia waste next to the urea cycle. As dysregulated urea cycle is implicated in cancer development, the impact of GS' ammonia clearance function has not been explored in cancer. Here we show that, oncogenic activation of -catenin leads to decreased urea cycle and elevated ammonia waste burden. While -catenin induces the expression of GS, which is thought to be cancer-promoting, surprisingly, genetic ablation of hepatic GS accelerates the onset of liver tumors in several mouse models that involve b-catenin activation. Mechanistically, GS ablation exacerbates hyperammonemia and facilitates the production of glutamate-derived alanine, which subsequently stimulates mTORC1. Pharmacological and genetic inhibition of mTORC1 and glutamic-pyruvic transaminase (alanine transaminase) suppresses tumorigenesis facilitated by GS ablation. While HCC patients, especially those with CTNNB1 mutations, have an overall defective urea cycle and increased expression of GS, there exists a subset of patients with low GS expression that is associated with mTORC1 hyperactivation. Therefore, GS-mediated ammonia clearance serves as a tumor-suppressing mechanism in livers that harbor -catenin activation mutations and a compromised urea cycle[1, 2].

KEY WORDS: Glutamine synthetase; -Catenin; liver cancer; ammonia; mTORC1

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高通量、精准代谢组学方法在免疫代谢调节中的应用研究

栾合密 [1]*
1. 广东工业大学，生物医学工程系

活性代谢物与免疫代谢密切相关，调节免疫系统的功能，对维护机体健康至关重要。高通量、精准代谢组学方法有助于了解发现代谢物与机体免疫之间的相互作用。我们开发并验证了一种快速、灵敏和高通量的方法，通过使用同位素稀释液相色谱-串联质谱准确定量人血清中广泛的代谢物。该方法被进一步应用于大规模的人群队列的研究，临床样本被分为发现集和验证集。利用特征性的代谢物信号构建了基于机器学习的多元分类模型，构建了 RA 的分类模型。该模型随后在验证集上进行了测试，准确率达到 90.2%，灵敏度为 89.7%，特异性为 90.6%。血清阳性和血清阴性患者均可通过该模型识别。共现网络分析发现代谢网络分为六个模块，显示炎症和免疫活动标记物与能量代谢、和氨基酸代谢异常之间存在显著关联。通过高通量、精准代谢组学方法，结合临床样本、动物模型实验等综合信息，能够快速建立基于机器学习的预测模型，为了解免疫代谢调节和机制提供新的方法。

关键词: .代谢组学、免疫代谢、高通量质谱

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Analysis of RNA modifications in breast cancer tissues by hydrophilic interaction liquid chromatography-tandem mass spectrometry

Xiujuan Hong[1], Yiqiu Hu[1], Dongyan Xu[1], Cheng Guo[1,2*]

1. Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education), The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China

2. Cancer Center, Zhejiang University, Hangzhou 310058, China

ABSTRACT: Breast cancer (BC) has become a major disease affecting life due to its high morbidity and mortality[1,2]. RNA methylation modification is recognized to participate in cancer initiation and progression[3]. However, information on the content of RNA modifications in breast cancer tissues is still limited and analysis of different types of RNA modifications is lacking. Herein, we develop a hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) method that can simultaneously analyze 35 RNA methylation modifications. Using this method, we could detect 23 and 7 modifications in total RNA and mRNA, respectively. Compared with normal tissues, we found a total of 13 modifications significantly increased and 2 modifications significantly decreased in total RNA of breast cancer tissues. In mRNA, 1 modification was significantly increased, while 3 modifications were significantly decreased. Our study revealed, for the first time, the changes in total RNA and mRNA modifications in breast cancer tissues and adjacent normal tissues. These significant changes in RNA epigenetic modifications suggest that they may serve as indicators of breast cancer. In particular, changes in modifications on mRNA will help to better study the regulatory roles of RNA epigenetic modifications in breast cancer.

KEY WORDS: HILIC-MS/MS, Breast cancer, RNA modifications, mRNA, Methylation

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Neurodevelopmental Effect of Deltamethrin Exposure on Hypothalamic Neurogenesis in Zebrafish (Danio rerio): A Lipidomics Perspective

Jun Zeng[1,*], Jingwen Hao[1], Longhua Gao[1]
1 College of Ocean Food and Biological Engineering, Jimei University, Xiamen 361021, China
* Correspondence: junzeng@jmu.edu.cn

ABSTRACT: As one of the pyrethroid pesticides, deltamethrin (DM) can accumulate through the food chain and be difficult to degrade. Because of its low toxicity and long-lasting environmental durability, DM has gained popularity as a pest control medication, but this extensive use has also resulted in unexpected environmental issues. Despite an increasing number of findings indicating DM neurotoxicity to organisms, the effects and mechanisms of DM action on the developing brain remain largely unclear, particularly for the potentially susceptible stage of prenatal period, as well as under environmental concentrations. Given that DM-induced estrogenic endocrine-disrupting effects have been reported to affect the neuroendocrine functions and behaviors, and estrogen synthesis and release are regulated by the hypothalamic-pituitary-gonadal (HPG) axis, in this study, zebrafish embryos were studied as the model organism and exposed to DM just before (10-16 hpf), at the onset of (16-24 hpf), at the peak of (24-36 hpf) hypothalamic neurogenesis and across 10-120 hpf (i.e., chronic exposure) with four different dosage levels (0, 1, 100, and 250 nM). To gain a better understanding of the developmental neurotoxicity upon DM exposure, a combined analysis of the neurodevelopmental phenotypes (incl. the total distance traveled, the average movement speeds of zebrafish larvae, and the number of apoptotic cells in the central nervous system) and non-targeted lipidome profiling was carried out. DM exposure at the peak of (24-36 hpf) hypothalamic neurogenesis resulted in considerable changes in both the neurotoxicity phenotypes and lipidome metabolism. The lipidome changes produced by 10-16 hpf exposure are also noticeable, highlighting the early stage's susceptibility to DM exposure. We discovered that developmental neurotoxicity had a dose-dependent pattern and was more pronounced at medium and high doses. As the major lipid categories associated with remodeling by DM treatment were significantly altered in the low-dose group, indicating the sensitivity of metabolic disturbance in response to DM exposure, it is crucial to take into account the disruption caused by low-dose DM at relevant environmental concentrations. The significant disturbance of ceramide, phosphatidylglycerol, cardiolipin, and fatty acid metabolic pathways, is implicated in the link between neurodevelopmental effect of DM exposure and the increased risk of mitochondrial damage.

KEY WORDS: Deltamethrin, neurogenesis, zebrafish embryos, lipidomics

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$PM_{2.5}$组分与新生儿神经行为评分的关联及胎便代谢组的中介效应

张洁

厦门大学公共卫生学院分子疫苗学与分子诊断学国家重点实验室，中国厦门，361102

Emai: jie.zhang@xmu.edu.cn

背景：孕期 $PM_{2.5}$ 暴露会影响新生儿神经行为评分（Neonatal Behavioral Neurological Assessment，NBNA），然而暂无研究指出 $PM_{2.5}$ 组分的具体影响。同时，孕期 $PM_{2.5}$ 暴露影响 NBNA 的分子机制研究仍是空白，胎便代谢组可以间接反映胎儿在母体内的代谢状况，可能为机制研究提供线索。

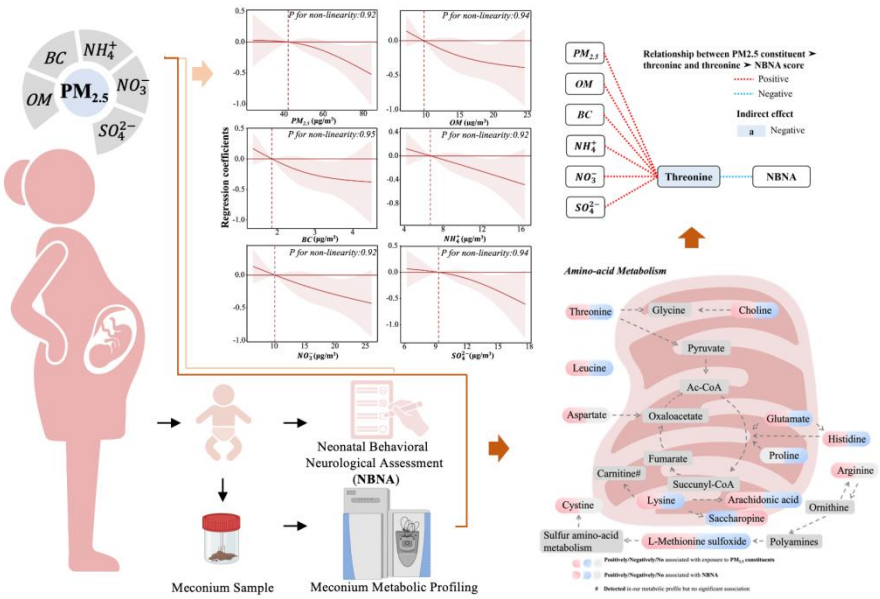
方法：2015 至 2016 年间，孝感出生队列共招募 1760 名孕妇。根据母亲的住所地址，基于卫星估算值和地面测量值的综合评估孕期 $PM_{2.5}$ 的暴露水平。按临床分期将孕期分为全孕期及孕早、中、晚期，利用多数据源模型评估各孕期平均 $PM_{2.5}$ 及组分暴露水平对 NBNA 的影响，并识别易感窗口；结合多元线性回归模型和自然立方样条模型评估剂量-效应关系；利用贝叶斯核机器回归、加权分位数和回归评估各组分影响 NBNA 的贡献度；通过中介分析探讨胎便代谢组在上述关联中的潜在中介作用。

结果：孕中期 $PM_{2.5}$ 及其组分暴露水平均与 NBNA 呈负向关联，其中硫酸盐被确定为主要贡献组分。性别分层后观察到效应只在男婴中存在，提示该影响存在性别特异性。胎便中多种氨基酸（例如苏氨酸、亮氨酸等）均与 $PM_{2.5}$ 成分暴露和 NBNA 评分具有显著性相关，其中苏氨酸具有显著的介导作用。

结论：孕中期 $PM_{2.5}$ 及组分暴露对新生儿神经行为产生不利影响，且存在性别特异性，硫酸盐可能是上述关联的主要贡献组分。氨基酸代谢紊乱可能是 $PM_{2.5}$ 组分暴露对 NBNA 评分产生影响的主要途径。本研究首次阐明了孕期 $PM_{2.5}$ 暴露对新生儿神经行为评分产生影响的组分及其影响机制。

关键词：$PM_{2.5}$ 组分，新生儿神经行为评分，氨基酸代谢，中介效应

摘要图：



衰老小鼠的脑代谢组图谱

丁俊[1,2]，Oliver Fiehn[2,*]

1.中国科学院植物种质创新与特色农业重点实验室，中国科学院武汉植物园，中国湖北武汉 430074;

2.西海岸代谢组学中心，加州大学戴维斯分校，美国加州戴维斯，95616

通讯联系人 E-mail: ofiehn@ucdavis.edu

脑是生命体最复杂的器官之一。其组织结构精细、网络连接多样、细胞种类丰富，由高度复杂的分子网络精准控制着机体的日常生理活动、情绪及高级认知。脑功能的行使离不开基因转录、蛋白质翻译及小分子代谢物的协同调控。目前，脑转录组图谱、蛋白组图谱的绘制为探究脑功能的分子基础提供了重要数据，但小分子代谢物的相关信息仍处于缺失状态。虽然人们对神经递质类代谢物，例如，多巴胺、乙酰胆碱等，在脑结构中的时空分布了解较为清楚，但对修饰核酸、胆汁酸、复杂脂类代谢物如何调节脑的功能、影响脑的衰老仍知之甚少。与 RNA、蛋白质的转录和翻译可通过基因表达预测不同，代谢物往往受到多种负反馈调节机制及调控回路的影响，其种类、分布及动态变化难以直接预测。因此，利用代谢组学技术对脑代谢组图谱进行表征，绘制系统完整的脑图谱显得尤为重要。

代谢组学领域已开始着手挖掘脑代谢的深度信息。大鼠生命周期的脑代谢物变化、老年小鼠脑代谢组的特征陆续得到表征，开启了脑代谢组图谱研究的序幕。然而，现有的研究只实现了 500-700 个脑代谢物的定性，对各代谢通路的覆盖率低；脑的解剖学分区不完整，难以系统反应脑的代谢组学构筑基础及脑衰老的潜在机制。

为完善脑的代谢组图谱并研究脑衰脑引起的代谢组动态变化，我们采用基于高分辨质谱的代谢组学技术，对青少年至成年晚期鼠脑的 10 个脑区进行了深度地代谢组学分析，绘制了衰老小鼠脑代谢组图谱，揭示了 1547 个代谢物在不同脑区、年龄、性别的差异信息 1。通过图谱挖掘，我们发现鞘脂类代谢物与衰老的联系，初步揭示了脑髓鞘在青少年至成年早期仍在不断重构，在成年时期稳定、老年期衰退的新现象。该图谱是目前最全面的脑代谢组图谱，为脑科学的基础研究提供了重要的基础数据。我们进一步建立了衰老鼠脑代谢组图集的可视化工具 https://mouse.atlas.metabolomics.us/，与基因组、转录组及蛋白组学图谱形成了完成的脑系统生物学数据库。

关键词: 代谢组学、图谱、质谱分析、脑代谢、衰老。

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单细胞结构脂质组学及生物医学应用

李自帅[1]，钱耀[1]，曹文波[1]，程思敏[1]，马潇潇[1*]
1. 清华大学精仪系，北京 100084

单细胞分析技术是揭示生物发育代谢动态变化和癌细胞异质性等重要生物学问题的关键手段。然而，由于单个细胞的体积极小，所含代谢物绝对量极低，因此单细胞代谢分析面临代谢物检测灵敏度不足、覆盖率低、定量分析能力差和结构表征能力不足等问题。

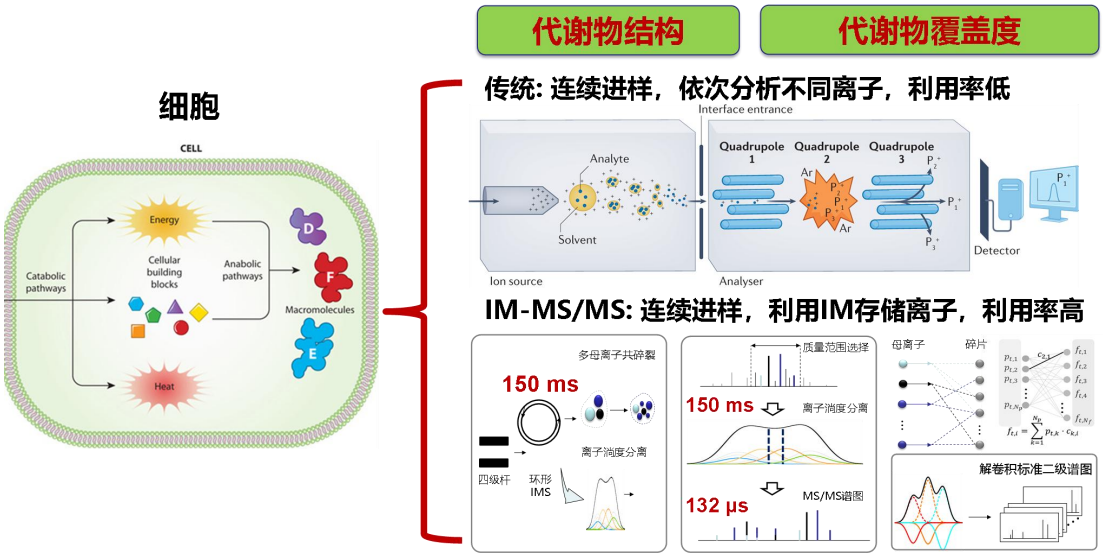

图 1. 离子淌度-多离子同时碎裂技术用于高通量、高覆盖度单细胞代谢质谱分析。

此前，在代谢物结构鉴定方面，我们通过将脂质化学衍生与多级质谱分析相结合[1-5]，发展了单细胞结构脂质组学分析技术，实现了细胞内脂质碳碳双键异构体和 sn 异构体的鉴定及定量，从而提高了所能获取代谢信息的维度，提高了生物医学分析的准确度。在此基础上，为了进一步提高细胞分析的通量和代谢物分析覆盖度，提出将离子淌度（ion mobility）技术引入单细胞分析。在传统单细胞质谱分析中，代谢物只能在时间上依次通过多级质谱碎裂完成结构鉴定，离子利用率很低，导致仅能对少数物质开展分析。通过将离子淌度技术与多离子同时碎裂技术融合，利用谱图解卷积算法，可在显著提升样品利用率的前提下（~100%），大幅提高代谢物分析的覆盖度及结构鉴定能力。实验结果表明，利用该方法可从单细胞中完成结构鉴定的代谢物数量比传统方法提升了一个数量级。技术从技术上而言，细胞中的代谢物经离子化后进入离子淌度池被存储及分离，分离时间 150 ms，经分离的离子经多离子同时碎裂后，对所产生的子离子做质量分析。由于不同淌度分离时刻母离子的组成不同，二级谱图也会随时间变化；利用淌度时间对齐原理建立的谱图解卷积算法可获得代谢物的标准二级谱图（图 1）。该技术有望推动高通量、无标记单细胞有机质谱分析技术在生命科学和医学领域的应用和发展。

关键词: 代谢组学，脂质组学，有机质谱，结构鉴定

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墙报

基于近邻样本的代谢组学数据稀释效应归一化方法

陆可懿 [1]，郭凡靖 [1]，邓伶莉 [2], Daniel Raftery [3],*，董继扬 [1],*

1. 电子科学系，厦门大学，厦门 361005，中国

2. 信息工程学院，东华理工大学，南昌 330013，中国

3. Northwest Metabolomics Research Center, University of Washington, Seattle, WA 98109, USA

稀释效应是代谢组学数据中常见的技术偏差，尤其是以尿液或细胞为样品的代谢组学研究中。为了从下游分析中获得可靠的结论，需要对代谢组学数据进行样本归一化处理。

目前已提出多种样本归一化方法，其中，以概率商标准化(PQN)[1]最为常用，PQN 有效地解决了早期的面积归一化(CSN)方法存在的问题，即，当部分代谢物存在显著改变而无法互相抵消的情况。然而，当超过 50% 的代谢物(变量)发生显著改变时，PQN 这种以变量商中位数作为归一化因子的方法将不再适用，如：基于核磁共振的糖尿病代谢组学数据中[2]，糖尿病组相对于健康对照组有超过 50%的变量发生显著变化，此时，无论是 CSN 还是 PQN 都无法准确归一化，并导致后续统计分析结果不准确。

为了找到准确的归一化因子，我们提出了一种新的基于近邻样本的归一化方法(NSN)。对于每个样本定义最近邻样本集。对于每次迭代，取最近邻样本集的中值谱为参考，计算当前样本与中值谱之间的变量商，迭代计算当前样本的归一化系数，使得当前样本尽可能靠近中值谱。通过近邻样本的选择和迭代归一化，NSN 可以适用于各种代谢组学数据，并在真实世界代谢组学数据集中验证了四种常见方法，CSN，L_2 范数归一化，PQN 和 Quantile [3]。实验验证了 NSN 归一化方法的有效性和鲁棒性，特别地，NSN 方法适用于极端的情况，即，超过 50%的代谢物(变量)发生显著改变的代谢组学数据归一化问题。

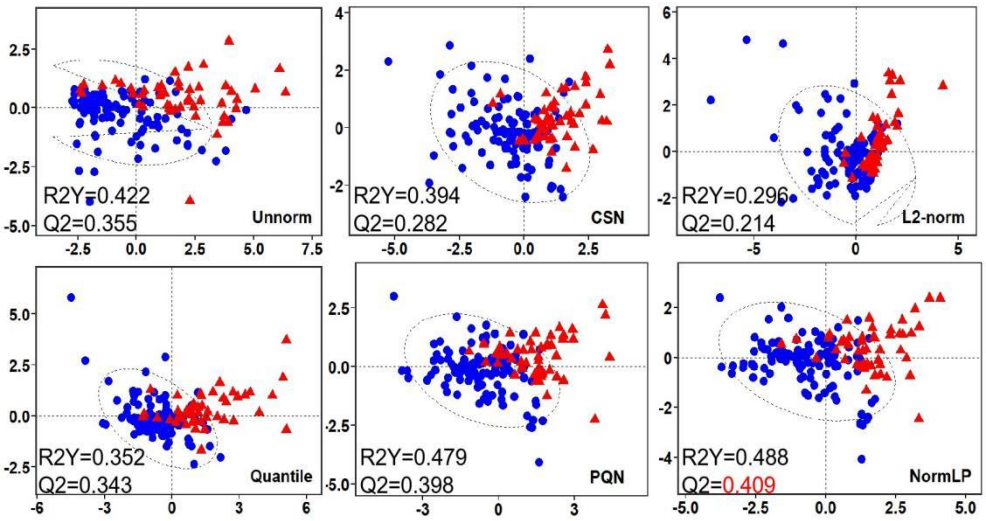

图 1. 不同归一化方法下的 PLS-DA 分析结果.

关键词：代谢组学，稀释效应，局部归一化，数据预处理

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基于局部样本内聚的代谢组学数据归一化方法

郭凡靖 [1]，陆可懿 [1],邓伶莉 [2],*, Daniel Raftery [3]，董继扬 [1],*

1. 电子科学系，厦门大学，厦门 361005，中国

2. 信息工程学院，东华理工大学，南昌 330013，中国

3. Northwest Metabolomics Research Center, University of Washington, Seattle, WA 98109, USA

摘要：代谢组学数据不可避免存在各种偏差，包括生物偏差、技术偏差和随机偏差等。其中，生物样品整体浓度的偏差是一个不可忽略的因素，特别是以尿液或细胞为样品的代谢组学研究中，样品整体浓度偏差校正，即样本归一化(Normalization)，对于后续统计分析结果的可靠性具有重要的意义。

归一化方法通常可分为数据采集前归一化(pre-acquisition)和数据采集后归一化(post-acquisition)两大类[1]。其中，post-acquisition 归一化是代谢组学研究常用的方法，包括：面积归一化(CSN)，概率商归一化(PQN)[2]，分位数归一化(quantile)[3]等等。然而，这些方法大多以某一特定样本(如中值谱)为参考，而将其它样本校正到离参考谱最近的位置上，容易导致样本分布受到改变，影响后续的统计分析结果，特别是，样本集的异质性分析。

本文提出基于局部样本内聚的归一化新方法 LSCN，通过保持最近邻样本集的数据结构，来达到保持全局样本分布的归一化。LSCN 首先为每一个样本定义一个最近邻样本集，然后根据样本距离对待归一样本进行加权归一化。LSCN 方法由于只在最近邻样本集上进行归一，从而减小样本局部结构的破坏，较好地保留了全局样本分布。在模拟数据和真实数据上验证 LSCN 方法的有效性，并与 CSN、L_2-范数归一化、PQN 和 Quantile 等四种常用方法进行比较，结果如图 1 所示。结果表明，LSCN 方法能有效减小各代谢物的变异系数，并更好地保留样本分布。LSCN 方法能有效校正由于样品浓度差异引起的数据偏差，保留更多的生物学信息。

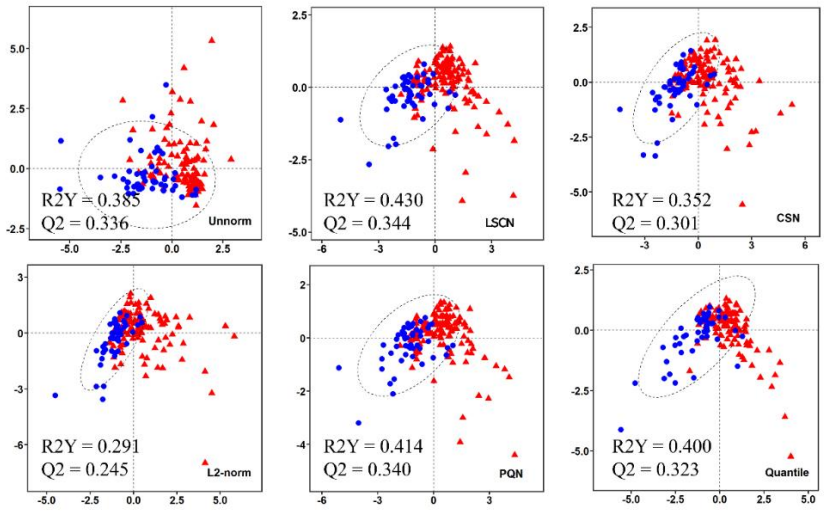

图 1. 不同归一化方法下的 PLS-DA 分析结果.

关键词：代谢组学；样本归一化；样本分布；局部样本内聚；样本局部结构。

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Identification of Metabolite Interference Is Necessary for Accurate LC-MS Metabolomics Analysis

Zhikun Jia[1], Qiongju Qiu[1], Ruiping He[1], Tianyu Zhou[1,2], **Li Chen**[1,2,*]

[1]Shanghai Key Laboratory of Metabolic Remodeling and Health, Institute of Metabolism & Integrative Biology, Fudan University, Shanghai, 200433, China.

[2]Shanghai Qi Zhi Institute, Shanghai, 200030, China

ABSTRACT: Targeted metabolomics has been broadly used for metabolite measurement due to its good quantitative linearity and simple metabolite annotation workflow. However, metabolite interference, the phenomenon that one metabolite generates a peak in another metabolite's MRM setting (Q1/Q3) with close retention time (RT), may lead to inaccurate metabolite annotation and quantification. Besides isomeric metabolites having the same precursor and product ions that may interfere with each other, we found other metabolite interferences as the results of inadequate mass resolution of triple-quadruple mass spectrometry and in-source fragmentation of metabolite ions. Characterizing the targeted metabolomics data using 334 metabolite standards revealed that about 75% of the metabolites generated measurable signal in at least one other metabolite's MRM setting. Different chromatography can resolve 65~85% of these interfering signals among standards. Metabolite interference analysis combined with manual inspection of cell lysate and serum data suggested about 10% out of ~180 annotated metabolites were mis-annotated or mis-quantified. These results highlight a thorough investigation of metabolite interference is necessary for accurate metabolite measurement in targeted metabolomics.

KEY WORDS: Metabolite Interference, LC-MS, Targeted metabolomics

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群体血液代谢组学的长期稳定性

黄庆霞[1]，易小璇[1]，王琪[1]，支海娟[1]，唐惠儒[1*]

1. 复旦大学生命科学学院遗传工程国家重点实验室，人类表型组研究院，张江复旦国际创新中心，上海国际分子表型中心代谢组学与系统生物学实验室，中山医院 上海 200032;

目前基于 NMR 的人血清/血浆样品的代谢组学技术很容易实现 41 个小分子代谢物和 112 个脂蛋白及其亚类的定量分析，但长期稳定性仍不确定。HBV 感染会导致宿主的脂质和葡萄糖代谢等发生变化，但目前还没有关于脂蛋白亚组分组成变化的报道。我们通过测量混合人血清 QC 样品来评估基于 NMR 的代谢组学方法在 24 个月内的长期稳定性，基于此进一步探讨 HBV 感染引起的人体血清脂蛋白亚类及其组分等代谢物的变化。在混合 QC 样品中可准确定量 333 个参数，包括 296 个脂蛋白亚类和 37 个小分子代谢物（如二氢胸腺嘧啶、N-乙酰糖蛋白、氨基酸和脂肪酸比例）。大多数定量参数在 24 个月内可观察到很好的稳定性，其中 329 个参数的 CV<20%，325 个参数的 CV<15%。值得注意的是，VLDL-5 中的游离胆固醇（V5FC）、VLDL-5 中的游离胆固醇百分比（V5FCp）、3-羟基丁酸和丙酮的 CV>20%。同时这几个参数的浓度相对变化率在多个时间点均超过±20%范围（以 0 时间点血清 QC 为参照），平均相对变化率的绝对值>15%。基于以上信息，在 HBV 感染者血清中可准确定量 303 个参数，调整年龄、性别、高血压、糖尿病、冠心病和 AST 后，大多数参数（如 TG、PL、IDL、VLDL 和 HDL 亚类等）仍是 HBV 感染的保护因素，而甲酸是危险因素。这项研究提供的具有长期稳定性的 NMR 可量化参数，可用于大型队列的人类血浆数据的潜在多批次分析（或荟萃分析）。此外，HBV 感染可引起肝脏脂蛋白排泄和循环代谢的显著变化，脂蛋白表型可区分 HBV 感染患者和对照组。

关键词: NMR、脂蛋白、长期稳定性、HBV

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常规600 MHz NMR 谱仪3分钟同时定量nM量级复杂混合物的^{13}C-探针增敏型^{1}H-^{13}C HSQC 2D NMR方法研究

徐莉[1#], 张聪聪[1#],黄庆霞[1], 王玉兰[2], 唐惠儒[1*]
1.复旦大学生命科学学院遗传工程国家重点实验室，人类表型组研究院分子表型组学研究中心代谢组学与系统生物学实验室，中国上海 200438。
2.南洋理工大学 Lee Kong Chian 医学院表型组中心，新加坡 639798

含氨基化合物广泛存在于化学、生物、医药和食品等领域，通常具有重要的功能。例如，含氨基代谢物涉及氨基酸、嘌呤与嘧啶、神经递质、蛋白质的合成与降解等多条重要的代谢通路，在生理病理过程中发挥着重要作用。但是，多数氨基代谢物的功能尚不清楚，其绝对定量分析是功能研究的关键突破口。核磁共振波谱（NMR）因其优异的重现性、无偏向性、结构信息丰富性而成为代谢组学的主流分析技术之一，但其灵敏度是瓶颈。一维氢谱（1D ^{1}H NMR）虽然灵敏度高，但复杂混合物（如生物样本）中数百个信号拥挤在10 ppm谱宽范围，且复杂的耦合裂分会导致谱图中信号严重重叠，难以准确定量低浓度的代谢物。鉴于此，我们建立了一种探针增敏型2D NMR策略，通过在每个氨基上引入磁等价的3个 ^{13}C-甲基标记探针，将NMR检测的 ^{1}H 和 ^{13}C 浓度分别提高倍9和300倍，进而使用基于非均匀采样（NUS）的 ^{1}H-^{13}C HSQC 2D NMR快速定量混合物中的氨基代谢物。与相同采样时间的一维氢谱和常规HSQC相比，我们的方法使用普通600 MHz核磁共振谱仪（3 min采样）可将检测限（LOD）降低到80 nM量级，突破了核磁共振技术数μM的LOD瓶颈。我们以已知浓度的对氨基苯磷酸（PAPP）为化学位移与定量的内标，建立了100多种含氨基化合物的化学位移和定量响应因子数据库，确保该方法具有可靠的定性和定量特性。最后，我们在复杂混合物或多种生物基质（血清、血浆、尿液、组织）中验证了该方法具有良好的定量线性关系、准确度、精密度和适用性。该技术为大批量复杂混合物样本的检测分析提供了一种高灵敏、高通量的定量分析方法。

***通讯联系人：**唐惠儒：电话：021-31246725, E-mail: huiru_tang@fudan.edu.cn.

Functional Metabolomics Reveal the Central Role of Pentose Phosphate Pathway in Resident Thymic Macrophages

Shan-An Chan[2] , Yue Song[1] , Winnie Hunag[2,*]

1. 5-6F, Litong Plaza, No 1350 North Sichuan Road, Shanghai 200080, China

2. No. 36-38 Huanyu 3 Rd, Guangzhou International Bio Island, Guangzhou 510005, China

ABSTRACT: Among of the Multi-omics, the functional metabolomics is a powerful technique for understanding biological systems by measuring the abundance of metabolites, however, data interpretation is often complicated by a lack of dynamic information. The innovation targeted metabolomics combine multi-methods were developed to improve the analytical performance by considering peak shape, separation and metabolite coverage of central carbon metabolism, energy metabolism, nucleic acid metabolism and amino acid to cover over hundreds of metabolites using UHPLC/Q-TOF systems in body fluids, tissue or cultured cell line of biological origin. We apply the solution for the research of the bone marrow-derived macrophages (BMDMs) reveal the pentose phosphate pathway that T cells enter the apoptotic process after failing to pass the selection, which would stress the thymus's crowded environment if the dead cells were not efficiently removed. Thymic-resident macrophages (TMφs), a rare population that only represents 0.1% of total cells in the thymus organ, play an essential role in removing apoptotic thymocytes and maintaining thymus homeostasis. However, it remains unclear how thymic-resident macrophages can efficiently and continuously phagocytose apoptotic thymocytes under hypoxic conditions. We picture the overall metabolic networking of the thymic-resident macrophages and particularly highlight the central role of the pentose phosphate pathway. Many datasets show the good limits of detection with multi-methods and cover whole target metabolites to satisfy the metabolomics and fluxomic research of biologist. The initial result has showed the benefit for the sensitivity and separation for the low-level metabolites from multi-methods that the modifier was added to sample to get the good sensitivity with sugar phosphate metabolites, routinely. The thymic-resident macrophages rely on the pentose phosphate pathway to overcome the oxidative stress brought by phagocytosing apoptotic thymocytes. Our study underlines the importance of the pentose phosphate pathway in the efferocytotic macrophages and sets up a milestone in understanding the rare but essential population.

KEY WORDS: Functional metabolomics; Pentose Phosphate Pathway; Resident Thymic Macrophages (TMφs)

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应用于代谢组学质谱数据分析的工具软件——Met4DX 2.0

尹岩东[1]，朱正江[1,*]

1. 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，200032

质谱技术作为高灵敏度和高覆盖度的检测技术，在代谢组学中有着极为广泛的应用，数据分析技术也逐步迭代更新，越来越多的应用于质谱数据分析的代谢组学软件也逐步被开发出来，如 xcms、openMS、mzMine 和 MS-DIAL 等。这些软件在质谱数据的处理上大多需要复杂的操作，且需要极为专业的知识背景，另外在定量的准确性上，与 skyline 手动积分得到的结果有着较大的差异。由此，我们在实验室累积的数据分析技术的基础上，开发了新一代的代谢组学数据分析软件——Met4DX 2.0，借助图形界面和内置的数据分析流程，极大地简化了数据分析的操作，同时，借助新的统一定量方法，获取高准确度的定量结果，基本达到 skyline 手动积分的水平。

Met4DX 2.0 从仪器的原始数据出发，借助开放的 API 或者 MSConvert 进行自动数据转换，将原始数据转换为统一的标准数据。以峰组装为起点，自下而上建立检测峰信号，经过样品间的保留时间（RT）校正、和峰分组，得到数据采集的特征峰。经过特征峰统一重定量，可以获取准确度极高的样品定量信息。经过二级谱图的指定，能够得到与特征峰对应的二级谱图信息，进而进行谱图匹配，得到代谢物鉴定的结果。对于数据分析的结果，我们提供了数据导出功能，可以用于后续的 MetDNA 进行广泛的代谢物鉴定，和 MetTracer 进行同位素标记实验的数据分析。目前该软件可以应用于液相色谱-质谱联用（LC-MS）和液相色谱-离子淌度质谱（LC-IMMS）采集的数据分析。

关键词: 代谢组学；峰检测；数据分析；代谢物鉴定；

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HM metabolite quantification for Neonatal Inherited Disorders Screening Combined with Whole Genome Sequencing Analysis

Tao Haoran[1,2], Hou Guixue[1,2 *], Diao Zhuo[2], Lin Liang[2, *], Liu Siqi[2, *]
1. University of Chinese Academy of Sciences, Beijing 100049, China
2. BGI Genomics, Shenzhen 518083, China
Email: taohaoran@genomics.cn

ABSTRACT: Background: The application of whole genome sequencing (WGS) has aided in the diagnosis of neonatal inherited diseases, offering a more effective and precise diagnosis based on public databases[1]. However, because of the diverse mutations and complex data interpretation, there were occasional inconsistencies between WGS diagnoses and phenotypes[2], let alone newly discovered mutations classified as variants of uncertain significance (VUS)[3]. To further clarify the various types of clinical mutations, we introduce the HM metabolite quantification method with wide coverage, high sensitivity and high throughput, which is a widely-targeted metabolomics technology, into newborn screening. The HM metabolite quantification kit provides a comprehensive analysis of the primary metabolic pathways in the human body. This study aims to interpret the metabolic disturbances in both positive patients and carriers of neonatal inherited metabolic diseases, as determined by WGS analysis. Additionally, we will explore the comprehensive annotation in the metabolic dimension for suspected mutations found by WGS.
Methods: The dried blood spot (DBS) is a widely-used sample type for newborn screening in China, which is benefit for collecting little sample volume, with easily transported and storage[4]. In this study, we will initially develop a widely targeted metabolomics technology based on DBS samples. During the sample pre-processing, we enhanced quantification sensitivity by optimizing the experiment conditions and modulating standard curves. Our sample pool involved over 2,000 individuals from five different metabolic disease groups, including positive, carrier, and negative groups identified and defined by WGS among 20,000 participants. We analyzed the integration of metabolites and WGS data by mapping global metabolomic pathways.
Results: The HM metabolite quantification method developed in this study can quantify more than 2,000 metabolites in one detection, including more than 700 small molecule metabolites and more than 1,600 lipids, covering the core metabolic pathways of common inherited metabolic diseases. At present, the technique can effectively detect the marker metabolites in the single gene mutation of phenylketonuria (PAH), glucose-6-phosphate dehydrogenase deficiency (G6PD), Fabre's disease (GLA), Wilson's disease (ATP7B), glycogen storage disease type IXd (PHKA1) and glycogen storage disease type II (GAA), etc. When quantifying DBS samples using our technology developed in this study, the coefficient of variation (CV) within or between experiments was less than 30%, while all liquid types were able to disperse uniformly on the blood collection paper. Moreover, we realized a fully automated workflow on the LH-200 Liquid Handler and increased the overall efficiency of sample pre-processing by more than three times with high throughput. Overall, more than 200 positive samples, 400 carrier samples and 1300 negative samples will be detected by automated pre-processing in tandem with LC-MS/MS targeted metabolomics technology, and different mutations of five groups of inherited metabolic diseases will be interpreted.

KEY WORDS: Newborn Screening; Inherited Metabolic Diseases; Widely Targeted Metabolomics; Dried Blood Spot

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一种用于分析不同盐含量发酵红辣酱的深度代谢组学分析方法

李佳[1], 陈曦[1], 詹晶晶[1], 章敏珍[1], 赵爽[1,*], 李治华[2,*]

1.厦门市迈理奥科技有限公司，福建省厦门市翁角西路2052号（厦门生物医药产业园）B2栋703室

2.四川省农业科学院农产品加工研究所（食物与营养健康研究所）

【目的】全面分析不同盐含量的发酵红辣酱，揭示不同食盐含量发酵食品中各种代谢物的差异变化，有利于监测食品发酵过程和进行质量控制，以及优化食品制造过程和开发减盐发酵食品。

【方法】本研究收集经46 天发酵制备的含盐量为 10%与 15%的红辣椒酱，采用包含全球领先的高效化学同位素标记（High Performance Chemical Isotope Labeling, HP-CIL）技术、国际前沿的Agilent 6546超高效液相色谱-串联四极杆飞行时间质谱联用仪器设备、全套优化的具有自主知识产权的IsoMS Pro数据分析系统的DeepMarker MT代谢组学平台对发酵红辣椒酱的代谢物进行高质量的获取和检测分析。其中，HP-CIL结合LC-MS对10%盐含量的发酵红辣椒（FRP_S10）与 15%盐含量的发酵红辣椒（FRP_S15 ）样本进行数据采集，利用IsoMS Pro软件的三层级代谢物数据库对代谢物鉴定，采用主成分分析(principal component analysis，PCA)、t检验、偏最小二乘判别分析(partial least square-discriminant analysis,PLS-DA)、分层聚类分析（hierarchical cluster analysis, HCA）等统计方法分析数据。此外，并使用LC-UV紫外吸收法测量标记的代谢物，确定不同含量的高盐食物之间代谢物的差异变化。

【结果】在发酵红辣椒中总共检测到 6329 个色谱峰对，有 5938 个峰（93.8%）可以被准确鉴定或者是推定得到匹配，代谢峰型较好且有良好的分离效果。通过火山图与PLS-DA等分析筛选发现，FRP_S10中有1037种代谢物的浓度高于FRP_S15，而FRP_S15中有937种代谢物的浓度高于FRP_S10。差异显著代谢物中，脱氨基酪氨酸（根皮酸）在FRP_S10中的浓度是FRP_S15的40倍，鸟氨酸（由精氨酸酶催化）在FRP_S10中的浓度是FRP_S15的近60倍，精氨酸在FRP_S15中的浓度是FRP_S10的50倍，进一步的结合宏基因分析，发现低盐样品主要是由乳酸菌（*Lactobacillus*）驱动，而高盐样品可能更多地依赖于代谢物的物理性质。结果表明，迈理奥DeepMarker MT代谢组学平台可用于轻松区分样品并揭示不同盐度发酵食品的生物学意义。

【结论】本研究首次基于DeepMarker MT代谢组学平台对不同盐含量的高盐发酵红辣酱进行深入的化学成分分析，独有的HP-CIL技术为所有的物质生成同位素内标，有效克服检测时仪器漂移、基质效应等影响，使定量更加精准，提高10-1000倍检测灵敏度，扩大代谢组覆盖率，能有效避免高盐造成对代谢产物鉴定不准确，进而筛选出不同盐含量的高盐发酵红辣酱存在差异的代谢标志物，有利于新产品检测领域的利用与开发。

关键词:高盐发酵食品；发酵红辣椒；代谢组学；DeepMarker MT代谢组学平台；HP-CIL；

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Effects of sample dilution on NMR-derived metabolic profiles of human urine and the epidemiological application

Limiao Jiang[1,*], Mengnan Ma[1], Xiong-Fei Pan[2], and An Pan[1]

1. Department of Epidemiology and Biostatistics, Ministry of Education Key Laboratory of Environment and Health, State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan 430030, China

2. Section of Epidemiology and Population Health, Ministry of Education Key Laboratory of Birth Defects and Related Diseases of Women and Children, West China Second University Hospital & West China Biomedical Big Data Center, West China Hospital, Sichuan University; Shuangliu Institute of Women's and Children's Health, Shuangliu Maternal and Child Health Hospital, Chengdu, Sichuan 610041, China

ABSTRACT: Traditionally, a relatively big urine volume (e.g. 500 μL) is used in NMR-based human metabolomics, which is not feasible for studies with limited/precious samples. Although urine may be diluted before conventional high-throughput metabolomics analysis, the comprehensive effect of urine dilution on metabolic profiles is unknown. Here, for the first time, we systematically investigated the effect of urine dilution on ^{1}H NMR metabolic profiles, by evaluating signal detectability, integration, signal to noise ratio (SNR), chemical shifts (δ) and its variation, and overlaps of 47 metabolites in 10 volunteers. We observed significant linear changes along with increased dilution, including decreased integration and SNR, altered δ, decreased inter-sample variation of δ, and increased separation between overlapped signals, e.g., lactate and threonine, β-D-glucose and an unassigned signal, histidine and 3-methylhistidine. We further tested the 40% dilution level (i.e., employing 300 μL urine) in an epidemiological study containing 1018 pregnant women from the Tongji-Shuangliu Birth Cohort, showing acceptable detectability and chemical shift variability for most of the 47 metabolites profiled. It indicated that mild (e.g., 40%) dilution of human urine can largely preserve the high-abundance metabolites profiled, reduce inter-sample chemical shift variations and increase separations of overlapped signals, which is an improvement of routine sample preparation methods in NMR-based metabolomics and is applicable for studies with limited urine volumes, including large-scale epidemiological studies.

KEY WORDS: urine; dilution; NMR; metabolomics; epidemiology

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全谱二维液相-QTOF 系统用于大鼠抑郁症的非靶向代谢组学研究

周婷[1*]，钟启升[2]，冯洁清[1]
1. 华南理工大学生物科学与工程学院, tingzhou@scut.edu.cn
2. 岛津企业管理（中国）有限公司

本文利用全谱二维液相系统结合四极杆-飞行时间质谱对抑郁症大鼠血浆进行了非靶向代谢组学分析。基于全谱二维液相系统，共找到 8083 个特征峰，其中第一维 2713 个，第二维 5370 个。正交偏最小二乘判别分析（OPLS-DA）表明正常组与模型组有显著差异，共找到 247 种变量投影重要性(VIP)大于 1 的候选差异性代谢物，经数据库比对鉴定出 44 种，主要包括 39 种脂质、2 种氨基酸、3 种生物碱，主要影响的通路包括甘油磷脂代谢、精氨酸和脯氨酸代谢、精氨酸生物合成、谷氨酸代谢等。

关键词: 全谱二维液相 四极杆-飞行时间质谱 抑郁症 非靶向代谢组学.

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Polarity-extended liquid chromatography-triple quadrupole mass spectrometry for simultaneous hydrophilic and hydrophobic metabolite analysis

Rui Guo [1], Qisheng Zhong [2], Jiaqi Liu [2], Peiming Bai [4], Zongpeng Wang [5], Jieling Kou [6], Peijie Chen [4], Jie Zhang [3], Bo Zhang [1]

1. The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China
2. Guangzhou Analytical Center Analytical & Measuring Instruments Division, Shimadzu (China) Co., LTD, Guangzhou, 510656, China
3. State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, School of Public Health, Xiamen University, Xiamen, 361102, China
4. Zhongshan Hospital Affiliated of Xiamen University, Xiamen, 361004, China
5. Jinjiang Jingchun Technology Ltd., Quanzhou, 362200, China
6. ScienceLife (Xiamen) Technology Co., Ltd., Xiamen, 361000, China

ABSTRACT: Although various metabolomic methods have been reported in recent years, simultaneous detection of hydrophilic and hydrophobic metabolites in a single analysis remains a technical challenge. In this study, based on the combination of hydrophilic interaction liquid chromatography (HILIC) and reversed phase liquid chromatography (RPLC), an online two-dimensional liquid chromatography/triple quadrupole mass spectrometry method (2D-LC/TQMS) was developed for the simultaneous analysis of hydrophilic and hydrophobic metabolites of various biological samples. The method can measure 417 biologically important metabolites (e.g., amino acids and peptides, pyrimidines, purines, monosaccharides, fatty acids and conjugates, organic dicarboxylic acids, and others) with logP values ranging from −10.3 to 21.9. The metabolites are involved in a variety of metabolic pathways (e.g., purine metabolism, pyrimidine metabolism, tyrosine metabolism, galactose metabolism, gluconeogenesis, and TCA cycle). The developed method has good intra- and inter-day reproducibility (RSD of retention time <2%, RSD of peak area <30%), good linearity (R^2 > 0.9) and wide linear range (from 0.0025 μg/mL to 5 μg/mL). The applicability of the method was tested using different biological samples (i.e., plasma, serum, urine, fecal, seminal plasma and liver) and it was found that 208 (out of 417) identical metabolites were detected in all biological samples. Furthermore, the metabolomic method was applied to a case/control study of urinary of bladder cancer. Thirty differential metabolites were identified that were involved in carbohydrate and amino acid metabolism.

KEY WORDS: Two-dimensional liquid chromatography; Triple quadrupole mass spectrometry; Targeted metabolomics; Biological sample; Bladder cancer

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HMQuant: A software for quantitative analysis of LC-MRM data in metabolomics study

Haowei Chen[1,2], Guixue Hou[2], Liang Lin[2], Siqi Liu[1,2]
1.University of Chinese Academy of Sciences, Beijing, 100049, PR China
2.BGI-shenzhen, Shenzhen, 102206, PR China

Target metabolomics using liquid chromatography and tandem mass spectrometry (LC MS/MS) at multiple reaction monitoring (MRM) mode enables simultaneous quantification of hundreds metabolites[1]. The MRM data for quantifying over a thousand transitions in samples at large scale is impossible to be manually handling, and a software to automatically deal with the flood data is badly needed. We have developed a software termed as HMQuant, therefore, to treat target metabolomics that functions with automating peak picking, information integration of MS/MS signals, and concentration calculation. Meanwhile, HMQuant can work with a series analysis for data quality control such as correction of peak area bias, retention time shift, and machine stability. Besides, it generates a comprehensive report that includes data analysis graphs, information tables, statistics summaries, and data annotations. Specifically, with concerns of influent factors onto retention times like LC conditions, flow rate variations and temperature fluctuations, HMQuant uses a linear interpolation algorithm for correction of retention time[2]. The treatment of increased drift on retention time makes linear interpolation with smaller calibration errors as compared to linear fitting ($p < 0.05$) and provides higher accuracy in detection window. In addition, this software utilizes KEGG database to generate metabolite ratios in pathways and to offer an overall insight for the quantified metabolites[3]. For instance, HMQuant produced 1,027 new biological features once 700 metabolites derived from HM700 kit were identified and quantified.

KEY WORDS: HMQuant, Target Metabolomics, MRM

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AllCCS2：基于神经网络构建的离子淌度质谱 CCS 值数据库

张浩松[1,2], 罗名都[1,2], 朱正江[1,*]
1. 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，200032
2. 中国科学院大学，北京，100049

离子淌度质谱（IM–MS）的快速发展彻底改变了小分子的多维分离和分析，并应用于代谢组学、脂质组学和暴露组学研究中。在离子淌度分离中，化合物离子在电场的作用下与中性缓冲气体相互作用，导致漂移时间的差异，并以碰撞横截面积（CCS）进行表示。因此，参考 CCS 数据库的建立对于 IM–MS 成功应用于小分子分析起着关键作用。目前的 CCS 数据库建立具有多种策略，其中基于机器学习的训练和预测由于其高精度、高效率和低成本，作为建立参考 CCS 数据库的策略而受到广泛欢迎。例如，我们课题组在 2020 年成功构建了一个名为 AllCCS 的小分子预测 CCS 数据库（*Nature Communications*, **2020**）[1]。随着多种仪器平台的发展，如漂移管离子淌度质谱（DTIMS）、行波离子淌度质谱（TWIMS）、俘获离子淌度质谱（TIMS）等，对支持不同仪器平台的 CCS 数据库的需求不断增加。另一方面，基于机器学习的方法通常依赖于分子表征，例如分子描述符，来建立 CCS 值和小分子之间的关系。因此，深入解析分子并获得全面表征，也是获得预测 CCS 值的重要挑战。而以往的研究多依赖以分子为整体的分子描述符，或只使用简化分子输入线输入系统（SMILES）字符串，这可能会忽略分子的重要拓扑信息，使得表征分子时存在局限性。

针对上述问题，我们进一步开发了 AllCCS2，它是 AllCCS 的增强版本，旨在通用预测小分子的离子淌度 CCS 值，并用于离子淌度质谱的相关研究（*Analytical Chemistry*, **2023**）[2]。AllCCS2 进一步纳入了新获得的实验 CCS 值作为训练数据，其中包括 10384 条 CCS 值记录和 7713 个统一的 CCS 值，并且使用了 1737 个 CCS 值作为外部数据集用于测试模型效果。AllCCS2 利用多种分子表征（包括质谱特征、分子描述符和使用图卷积网络提取的图特征）建立了神经网络预测模型，实现了卓越的预测精度，在训练集、验证集和测试集中分别实现了 0.31%、0.72%和 1.64%的中值相对误差，在准确性和覆盖率方面超越了现有的 CCS 预测工具。此外，AllCCS2 还表现出与不同仪器平台（DTIMS、TWIMS 和 TIMS）的出色兼容性。我们还使用代表结构相似性（RSS）和模型预测变异（MPV）综合研究了 AllCCS2 中来自训练数据和预测模型的预测不确定性。值得注意的是，与训练集结构高度相似且模型预测变化较低的小分子表现出更高的准确性和更低的相对误差，这能够为预测结果的应用提供参考。总之，AllCCS2 是支持 IM–MS 技术应用的宝贵资源，AllCCS2 数据库和预测工具可在 http://allccs.zhulab.cn/免费访问。

关键词: 离子淌度质谱；碰撞横截面积；机器学习；分子表征

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基于稳定同位素示踪的代谢反应网络阐明未知的代谢反应

高扬[1,2]，朱正江[1,*]

1. 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，200032

2. 中国科学院大学，北京，100049

代谢物的合成与降解组成了多种多样的代谢反应，代谢反应发挥着各种功能，如提供能量、构建生物大分子及信号传导。多个代谢反应组成了代谢网络，提供细胞代谢的基本生化结构。尽管 KEGG、REACTOME 等数据库收集了大量已知报道的代谢反应，但是新代谢反应仍然被陆续发现，目前的代谢网络依然不完整。基于质谱的代谢组学技术实现了对生命系统中已知和未知代谢物的解析，例如，GNPS[1]、NetID[2] 和 MetDNA2 [3]。然而，这些方法都无法解释未知代谢物的生物合成过程。 越来越多的研究表明，未表征的代谢物及其代谢反应对于健康和发病机制研究至关重要。因此，识别未知的代谢物及其代谢反应有望补充代谢网络的拓扑结构，进一步的加深代谢对生理病理影响的理解。

目前基于计算或实验的方法被陆续开发去发现未知的代谢反应。计算预测的手段包括：同源性酶功能注释、基于基因组的建模和机器学习等。虽然它的优势在于预测反应的高覆盖率，但其中只有很小一部分可以被实验验证。在基于实验的研究手段中，体外酶活的代谢组学分析是常见的实验方式之一，但这种方式需要纯化蛋白质，以及很多代谢物不容易得到纯品，此外，非酶反应无法通过该方法捕获。稳定同位素示踪的代谢组学技术已日益成为验证新发现的代谢物和代谢反应的首选方法。目前，稳定同位素示踪通常是针对性对感兴趣代谢物进行分析。全局大规模的、无假设约束、数据驱动的未知代谢物及相关代谢反应的发现依然没有实现。

在稳定同位素示踪实验中，生物体发生的代谢反应会导致代谢产物中出现特定的同位素标记模式，称为同位素分布（MID）。 代谢反应的底物和产物通常具有相似的 MID。 因此，通过比较同位素标记的底物和产物的 MID 相似性，可以推断出新的代谢反应。 基于这个推理，我们开发了一种方法，即 MIDnet，通过全局稳定同位素示踪代谢组学来绘制未知代谢反应网络。 这种方法能够在一次实验中发现未知的代谢物以及相关的代谢反应。在 293T 细胞中，我们组建了一个数据驱动的代谢反应网络，包含 841 个标记的已知和未知代谢物、470 个已知和未知代谢反应。其中我们发现了一些与谷胱甘肽和核苷酸相关的新代谢反应，为细胞代谢网络带来了有效补充。

关键词: 稳定同位素示踪；代谢组学；代谢反应网络；质谱；代谢物

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适用于非靶向代谢组学质谱数据的数字图像编码方法 MetImage

王洪淼[1,2](wanghongmiao@sioc.ac.cn)， 尹岩东[1]， 朱正江[1,*]
1. 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，200032
2. 中国科学院大学，北京，100049

摘要：基于液相色谱−质谱联用的非靶向代谢组学技术为疾病临床研究提供了对小分子代谢物的全面定量分析，在临床研究中具有重要作用。传统的临床代谢组学研究往往需要经历复杂且耗时的特征筛选，从而只有少数代谢物可以作为生物标志物进行临床应用。基于全代谢组的疾病诊断研究是一个很有发展前景的策略，但是存在巨大的挑战。基于此，本工作开发了一种将高分辨质谱采集的非靶向代谢组学数据转换为多通道数字图像的方法，名为MetImage （*Anal. Chem.,* **2023**, 95, 6533-6541），以实现基于全代谢组的疾病诊断。MetImage首先将非靶向代谢组学数据转换为全代谢组图像，然后将该图像切分成更小的图块用于后续分析。除此之外，为了最大限度的保留图像中含有的信息，本文使用了基于图块池化信号强度和图像熵的策略进行图块筛选，筛选后的图块所堆叠而成的多通道图像可直接输入基于深度学习的人工智能（AI）模型中进行训练或后续的疾病诊断应用。通过这一方式生成的多通道图像保留了质谱数据的原始形态，因此可以将图像数据与其中的代谢物的信息相关联。

为了验证 MetImage 在临床诊断中的效果，本工作在一个食管鳞状细胞癌（ESCC）人群队列（n=1104）上训练了基于 AI 的 ESCC 筛查模型。该筛查模型取得了优异的性能，模型的敏感性为 85%，特异性为 92%，受试者特征曲线下面积（AUC）为 0.95。进一步的，该筛查模型对不同 ESCC 的进展阶段均展示出良好的判别性能。此外，本文对该 AI 模型的可解释性进行了研究,由于该编码策略保留了原始质谱数据的重要特征，能够轻松对关键图块中的特征代谢物进行鉴定，进而能对模型的诊断原理进行阐明，证明了该 AI 模型并非“黑匣子”。这项研究为利用全代谢组信息进行 AI 辅助的临床应用提供了新的思路，并通过可解释性的深度学习模型提升了其在临床研究中的应用价值。

关键词: 液相色谱−质谱；非靶向代谢组学；卷积神经网络；图像处理；食管鳞状细胞癌

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代谢流组学技术 MetTracer 定量代谢流量水平

王若鸿[1]，尹岩东[2]，蔡玉萍[2]，朱正江[2,*]
1. 中国科学院大学
2. 中国科学院上海有机化学研究所

代谢是生命体的基本特征，代谢紊乱跟多种重大疾病密切相关。目前基于质谱技术的非靶向代谢组学研究大多是“静态分析”，难以测定生物体内代谢物合成和降解等动态变化规律。代谢流分析技术通常采用稳定同位素示踪 (Stable-isotope tracing)实验，通过质谱技术来追踪稳定同位素在代谢物中的流向，进而通过测定代谢网络中代谢物的同位素分布来计算代谢物合成和降解等动态变化规律。为了对生命体的整个代谢组进行全面的代谢流动态分析，我们**结合大规模代谢物结构鉴定方法 MetDNA 和稳定同位素标记代谢物检测技术，发展了基于稳定同位素标记的非靶向代谢流组学技术 MetTracer**。该技术具有以下特点：1，追踪覆盖范围高，能够对上千个代谢物同时进行稳定同位素标记的追踪；2，对稳定同位素标记代谢物的定量准确性高且可重复性好；3，大规模数据分析的准确度高。我们将 MetTracer 技术应用于果蝇衰老的基于高分辨质谱的非靶向代谢流组学分析研究中，在果蝇脑组织和肌肉组织中我们分别鉴定到 390 个和 597 个稳定同位素标记的代谢物。通过多个时间点标记程度的聚类分析进一步系统性地揭示了果蝇衰老过程中代谢反应活性的变化，为衰老相关的代谢调控研究提供了新的思路和潜在干预手段。进一步地，我们在 MetTracer 基础上发展了基于稳定同位素标记的**定量代谢流方法**，定量表征了不同细胞(293T, MEF, 和 Hela 等)代谢反应网络中 92 个代谢反应的代谢流量 flux 水平，包括糖酵解代谢通路，磷酸戊糖途径，和三羧酸循环通路等。

关键词: 质谱技术；稳定同位素标记；非靶向代谢流组学；定量代谢流方法；衰老代谢

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Derivatization of N-Acyl Glycines by 3-Nitrophenylhydrazine for Targeted Metabolomics Analysis and Their Application to the Study of Diabetes Progression in Mice

Li Xiang[1], Yi Ru[1], Jingchun Shi[1], Li Wang[2], Hongzhi Zhao[1], Yu Huang[2,*], Zongwei Cai[1,*]

1. State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong 999077, China

2. Department of Biomedical Sciences, City University of Hong Kong, Hong Kong 999077, China

ABSTRACT: N-Acyl glycines (NAGlys) are an important class of metabolites in the detoxification system of the human body. They have been used in the diagnosis of several metabolic diseases. Liquid chromatography−mass spectrometry (LC−MS) is the most frequently used NAGlys detection platform. Here, we describe a simple and sensitive method of NAGlys detection by LC−MS in plasma and urine samples. This approach is based on the use of a derivatization reagent, 3-nitrophenylhydrazine. The reaction is quick in aqueous solution, and no quenching step is needed. To expand the coverage of NAGlys when standards are not available, NAGlys were first identified based on high-resolution LC−MS. Quantification was subsequently carried out on triple quadrupole LC−MS. This approach allowed a much broader measurement of NAGlys (41 NAGlys in total), especially when authentic standards are unavailable. Comprehensive analysis of NAGlys with this new method was applied in plasma and urine samples of *db/db* diabetic and non-diabetic *db/m*+ control mice. The majority of detected NAGlys were altered with high differentiation ability in plasma and urine samples from diabetic and non-diabetic mice. These identified NAGlys hold the potential to be diagnostic biomarkers for type II diabetes and diabetic complications.

KEY WORDS: N-acyl glycines, LC-MS, 3-nitrophenylhydrazine, *db/db*, plasma, urine

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基于离子淌度质谱的高覆盖四维脂质组学技术

陈曦[1,2], 尹岩东[1], 蔡玉萍[1], 朱正江[1,*]
1. 中国科学院上海有机化学研究所，生物与化学交叉研究中心，上海，200032
2. 中国科学院大学，北京，100049

摘要：脂质在广泛的生物学过程，如信号转导、运输作用以及生物大分子分选过程中扮演着重要角色。由于脂质结构的多样性以及存在数量众多的同分异构体，对复杂生物样本进行高覆盖和高准确的脂质组学分析仍然存在挑战。近年来，离子淌度质谱技术逐步兴起，其分离原理是在电场作用下，不同离子与惰性气体碰撞时具有不同的迁移速度。离子淌度质谱还能够提供碰撞截面积（CCS）用于脂质的鉴定，为脂质结构鉴定提供多一维度的信息。基于此，我们发展了基于液相-捕集离子淌度质谱联用（LC-TIMS-MS）的四维非靶向脂质组学方法。捕集离子淌度质谱具有较高的淌度分辨率，同时配备双捕集离子淌度，更有利于提高占空比和灵敏度。基于捕集离子淌度质谱的四维脂质组分析显著提升了同质物与同分异构体的分离，在 MS1 层面提高了同位素保真度，在 MS2 层面降低二级谱图复杂性，提高了母离子分离的纯度以及二级谱图的质量。捕集离子淌度质谱与平行累积连续碎裂技术（PASEF）的联用，显著提升了二级谱图的覆盖度。我们进一步证明了使用捕集离子淌度质谱获得的脂质的碰撞截面积与漂移管离子淌度质谱的结果高度一致。结合 Lipid4DAnalyzer 中四维数据库匹配与规则精简的联合策略，该方法在多种生物样本中展现出高覆盖度与高准确的脂质鉴定。标准生物样本 NIST SRM 1950 human plasma 的脂质鉴定和定量结果也通过实验室间结果进行了验证。最后，我们应用该方法来表征小鼠大脑中 1,397 种不同脂质的空间分布，并证明大脑区域之间不同的脂质分布和成分有助于大脑区域的不同功能。

关键词: 离子淌度质谱，四维脂质组学，碰撞截面积，脂质鉴定

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Adipocyte Senescence Induces Microvascular Endothelial Progeria and Functional Disorder

Qianhui Tai[1], Jing Zhang[2], Baibing Mi[3], Jing Shi[1], Xiaoli Hui[1], Huan Chen[1], Jing Xu[1], Wei Cui[1,*]

1. Department of Elderly Endocrinology, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an City 710061, People's Republic of China;
2. Department of Kidney Transplantation, First Affiliated Hospital of Xi'an Jiaotong University, Xi' an City 710061, People's Republic of China;
3. Department of Epidemiology and Biostatistic School of Public Health& Global Health Institute, Health Science Center of Xi'an Jiaotong University, Xi'an City 710061, People's Republic of China.

ABSTRACT: Diabetic kidney disease (DKD) is a world-wide health burden. Obesity and dyslipidemia are risk factors for DKD, but the specific mechanisms are not fully understood. 3T3-L1 cells were induced to differentiate to young and senescent adipocytes. HMEC-1 cells were cultured in the conditioned medium from young, senescent adipocytes and control medium. The senescent state was tested by immunofluorescence detection of γ H2AX and SA- β -Galactosidase staining. The expression level of senescence-associated secretory phenotype (SASP), ICAM-1, IRS1, JUN, H3K4me2 and H3K4me3 were detected by qPCR and western blot. Lipid peroxidation indices were also detected. Differential gene expression (DGE) of ageing kidney and early diabetic nephropathy on Gene Expression Omnibus (GEO) were analyzed bioinformatically. Among SASP, SCT1 was upregulated in induced senescent HMEC-1 cells, while most inflammatory factors were downregulated. However, induced young HMEC-1 cells exhibited the contrary. IRS1 was unchanged in induced senescent HMEC-1 cells while downregulated in induced young cells. JUN and H3K4me3 were higher in induced young HMEC-1 cells compared with induced senescent ones. PPI network and enrichment analyses indicated IL6-SOCS3-IRS1 to be the core signaling pathway that mediated the senescent state and early metabolic changes. Our data provides direct evidence that the SASP of aging adipocytes can transmit aging and lead to dysfunction of microcirculation endothelial cells, which may be related to epigenetic regulation.

KEY WORDS: senescence, 3T3-L1 cell, SASP, diabetic kidney disease

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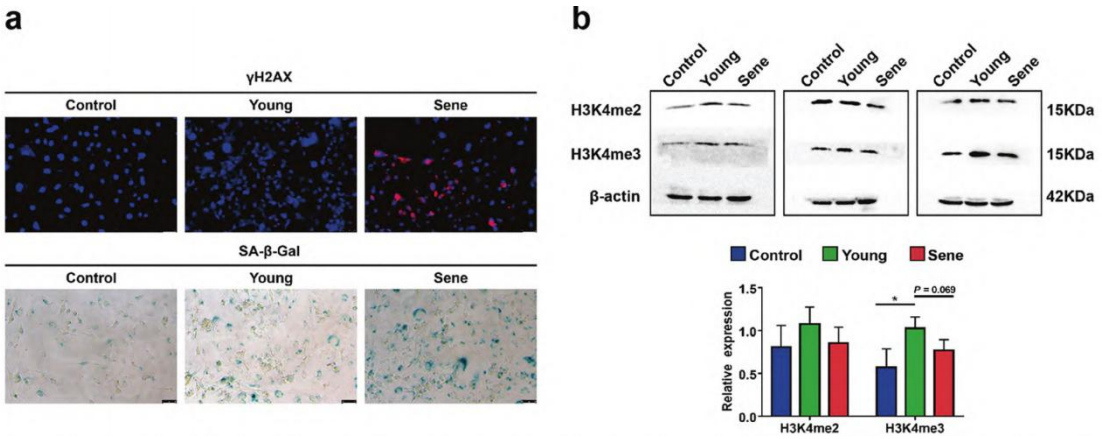
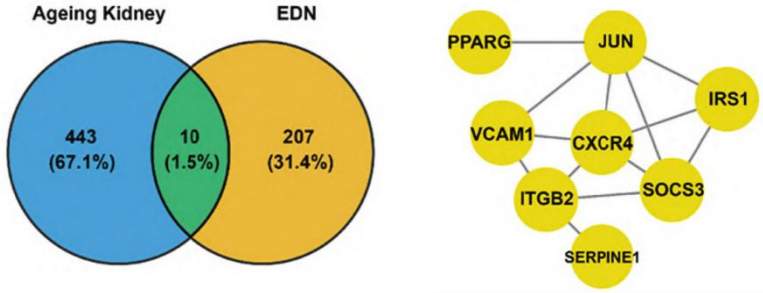
Fig.1



a HMEC-1 cells treated with young AC-CM, senescent AC-CM and control medium. Sene, senescent. b Immunoblotting for H3K4me2 and H3K4me3 in HMEC-1 cells. Quantitative analysis was also shown. *$P<0.05$.

Fig.2



Venn diagram of 10 intersection genes from upregulated DEGs of ageing kidney and downregulated DEGs of EDN (early diabetic nephropathy). Key genes in PPI network.

NMR-based Metabolomic Analysis of the Protection of Taurine Against Ferroptosis-induced Damage in C2C12 myoblasts

Xi Liu[1], Yu Zhou[1], Zhen Qi[1], Caihua Huang[2], Donghai Lin[1,*]

[1] Key Laboratory for Chemical Biology of Fujian Province, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China. Email address: Donghai Lin [*]: dhlin@xmu.edu.cn, Xi Liu: lancyliu2584@163.com

[2] Research and Communication Center of Exercise and Health, Xiamen University of Technology, Xiamen, 361024, China.

Abstract: Ferroptosis is a non-apoptotic form of programmed cell death caused by the accumulation of lipid peroxides and reactive oxygen species in an iron-dependent manner. Previous studies have shown that ferroptosis can decrease the viability of C2C12 myoblasts and their capacity to differentiate into myogenic cells, leading to damage on skeletal muscle health. Compared to molecular biology, fewer changes in the endogenous environment caused by ferroptosis have been identified. Adequate knowledge of the endogenous changes in ferroptosis-injured myoblasts is helpful to understand the biological implications and mechanisms of ferroptosis at metabolic level, as well as to investigate the potential of endogenous small molecules on anti-ferroptosis. Our research showed that ferroptosis-injured myoblasts had decreased levels of partial REDOX metabolites including taurine. Supplementation with exogenous taurine could restore the viability of damaged cells, increase GSH levels, reduce MDA and ROS, and improve myogenic differentiation capacity. NMR-based metabolomic analysis revealed that taurine could protect myoblasts from ferroptosis by regulating histidine metabolism, glutathione metabolism, and D-glutamine and D-glutamate metabolism. We found that taurine could reduce the abnormal levels of HO-1 in ferroptosis-injured myoblasts, which was on duty of catabolizing heme and releasing Fe^{2+}. Consistently, our observation that intracellular Fe^{2+} accumulation was significantly reduced after taurine supplementation. The results showed that taurine can increase REDOX substrate levels by regulating cellular metabolism and reduce intracellular iron overload by controlling HO-1 levels, thereby alleviating damages caused by ferroptosis in myoblasts. Our research suggests that taurine might be a potential inhibitor of ferroptosis on protecting skeletal muscle from ferroptosis-induced damage. This study sheds lights on the molecular mechanisms underlying the protective effect of taurine against ferroptosis-induced damage in C2C12 myoblasts.

KEY WORDS: Taurine; Ferroptosis; Metabolomics; Iron Accumulation (Fe^{2+}); C2C12 myoblasts.

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3-羟基丁酸减缓恶病质骨骼肌萎缩的双重作用机制探究

邱旭[1]，黄彩华[2]，吴文芳[1]，林东海[1,*]
1. 厦门大学化学化工学院化学生物学系，福建 厦门，361005
2. 厦门理工学院运动健康研究与传播中心，福建 厦门，361021

骨骼肌占机体重量的40%以上，是机体最大的代谢器官，与能量代谢及信号调控密切相关。然而，癌症恶病质的发生会诱导骨骼肌萎缩，严重影响患者生活质量，缩短患者生存时间。探寻减缓恶病质骨骼肌萎缩的新方法具有重大的科学意义和临床应用价值。近年来，酮体已被证明可以抑制肿瘤细胞增殖，减轻患者肿瘤负担，延缓癌症患者死亡。但是，目前有关癌症恶病质骨骼肌的酮体代谢研究还很少，酮体减缓癌症恶病质骨骼肌萎缩的作用机制仍不清楚。本研究选取小鼠成肌细胞 C2C12 和肠癌细胞 CT26 细胞系，以酮体的主要成分 3-羟基丁酸（3-HB）为研究对象，采用分子生物学和基于 NMR 技术的代谢组学方法，阐明 3-HB 减缓恶病质骨骼肌萎缩及抑制肿瘤生长的双重作用机制，以期为探寻治疗恶病质的新靶点提供科学依据。我们获得主要的研究结果：（1）3-HB 作为信号调控分子，作用于 C2C12 细胞表面 G 蛋白偶联受体 GPR109a，调控钙离子通道的开启，引起 Ca^{2+}内流；（2）3-HB 介导 Ca^{2+}-NFAT 通路，调控基因转录，促进 C2C12 细胞增殖；（3）3-HB 调控 AMPK 蛋白磷酸化，调控 C2C12 细胞的能量代谢稳态；（4）3-HB 上调 C2C12 细胞和 CT26 细胞的单羧酸转运体（MCTs）的表达；（5）3-HB 进入 CT26 细胞后与胞内 lactate 竞争转运体 MCTs，使得胞内 lactate 堆积，造成细胞酸中毒；（6）3-HB 调控 CT26 细胞的凋亡蛋白表达，诱导细胞凋亡。我们的结果表明：3-HB 可作用于恶病质骨骼肌直接减缓其萎缩，也可通过抑制肿瘤生长间接减缓骨骼肌萎缩。3-HB 不仅可作为能量代谢的底物，而且也是一种重要的信号调控分子，在减缓恶病质骨骼肌萎缩的过程中发挥双重作用。本研究阐明了酮体减缓癌症恶病质骨骼肌萎缩的代谢机制，有利于开发酮体在治疗癌症恶病质的临床应用价值。

关键词: 骨骼肌；恶病质；3-羟基丁酸；NMR 代谢组学；信号分子.

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Facile synthesis of spherical covalent organic frameworks for enrichment and quantification of aryl organophosphate esters in mouse serum and tissues

Wen Xie [1], Canrong Chen [1], Linyan Yang [1], Yijing Wu [1], Yanhui Zhong [1,*], Zian Lin [1,*], and Zongwei Cai [2,*]

1. Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350108, China.

2. State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, 999077, Hong Kong, SAR, P. R. China

ABSTRACT: Here, an imine-linked-based spherical covalent organic framework was prepared at room temperature. The as-synthesized spherical covalent organic framework served as an adsorbent in dispersive solid phase extraction, by its virtue of great surface area (1542.68 m^2 g^{-1}), regular distribution of pore size (2.95 nm), and excellent stability. Therefore, a simple and high-efficiency dispersive solid phase extraction method based on a spherical covalent organic framework coupled with HPLC-MS/MS was established to determine aryl organophosphate esters in biological samples. This approach displayed favorable linearity in the range of 10.0-1000.0 ng L^{-1} ($r > 0.9989$), high enrichment factors (13.4-25.7 folds) with low limits of detection (0.2-5.0 ng L^{-1}). Moreover, it could effectively eliminate complex matrix interference to accurately extract seven aryl organophosphate esters from mouse serum and tissue samples with spiked recoveries of 82.0-117.4%. The as-synthesized spherical covalent organic framework has been successfully applied in sample preparation. The dispersive solid phase extraction-HPLC-MS/MS method based on a spherical covalent organic framework has potential application to study the pollutant's metabolism in vivo.

KEY WORDS: Aryl organophosphate esters; Covalent organic frameworks; Dispersive solid phase extraction; Biological samples; Quantification

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Metabolic changes of glycerophospholipids during the reparative phase after myocardial infarction injury

Jin Wang[1], Xinyi Yu[1], Liu Yao[*]
[1]Tianjin Medical University, Tianjin, 300070, China
[*] Corresponding author: yaoliu@tmu.edu.cn

ABSTRACT: Myocardial infarction (MI) is a fatal manifestation of coronary heart disease, and its underlying mechanism is still largely unknown. Lipid levels and composition alterations predict the risk of MI complications. Glycerophospholipids (GPLs) are important bioactive lipids and play a crucial role in the development of cardiovascular diseases. However, the metabolic changes in the GPLs profile during post-MI injury remain unknown.

In the current study, we constructed a classic MI model by ligating the left anterior descending branch and assessed the alterations in both plasma and myocardial GPLs profiles during the reparative phase post-MI by liquid chromatography–tandem mass spectrometry analysis.

We found that myocardial GPLs, but not plasma GPLs, were markedly changed after MI injury. Importantly, MI injury is associated with decreased phosphatidylserine (PS) levels. Consistently, the expression of phosphatidylserine synthase 1 (PSS1), which catalyzes the formation of PS from its substrate phosphatidylcholine, was significantly reduced in heart tissues after MI injury.

Furthermore, oxygen-glucose deprivation (OGD) inhibited PSS1 expression and reduced PS levels in primary neonatal rat cardiomyocytes, while overexpression of PSS1 restored the inhibition of PSS1 and the reduction in PS levels caused by OGD. Moreover, overexpression of PSS1 abrogated, whereas knockdown of PSS1 aggravated, OGD-induced cardiomyocyte apoptosis. Our findings revealed that GPLs metabolism was involved in the reparative phase post-MI, and cardiac decreased PS levels, resulting from inhibition of PSS1, are important contributor to the reparative phase post-MI. PSS1 overexpression represents a promising therapeutic strategy to attenuate MI injury.

KEY WORDS: myocardial infarction, metabonomics, glycerophospholipids, phosphatidylserine, phosphatidylserine synthase 1

Lactate induces skeletal muscle fiber-type switch and metabolism remodeling via Ca^{2+}-NAFTC1 pathway

Yu Zhou[1], Xi Liu[1], Zhen Qi[1], Caihua Huang[2], Donghai Lin[1*]

1.Key Laboratory for Chemical Biology of Fujian Province, MOE Key Laboratory of Spectrochemical Analysis and Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China; zhouyu_921@163.com(Yu Zhou); dhlin@xmu.edc.cn(Donghai Lin)

2.Research and Communication Center of Exercise and Health, Xiamen University of Technology, Xiamen 361024, China.

ABSTRACT: Lactate is not only the metabolite of lactobacillus in intestinal tract, but only the production of glycolysis in skeletal muscle. Various benefits of lactate on skeletal muscle were demonstrated over the years, however it's still not clear the mechanism of lactate in switching skeletal muscle fiber-type. In this study, we observed that lactate administration could improve the ability of anti-fatigue, especially in trained mice. We further found that lactate elevated the expression of MYHC I, MYHC IIa and Myoglobin while reduced the level of MYHC IIb in vivo and in vitro, indicating lactate could induce the transformation of skeletal muscle fiber-type from glycolytic to oxidative. Besides, lactate could also enhance the activities of HK, LDHB, IDH, SDM and MDH and regulate the transcription of metabolic enzymes in skeletal muscle. NMR-based metabonomics study shown lactate changed the metabolic pattern of C2C12 myotubes obviously. And we found that lactate administration facilitated the utilization of itself in myotubes by promoted the expression of MCT1 and CD147 which were regarded as the lactate metabolic related proteins. Interestingly, we noticed lactate increased the cellular content of Ca^{2+} and the nuclear translocation of NFATC1, while BAPTA-AM inhibited the effects of lactate on the translocation of NFATC1 and expression of myosin heavy chain, but with no influence on the lactate metabolic related proteins. Our study showed that lactate can remodel skeletal muscle metabolism through facilitating the utilization of itself and induce the transformation of skeletal muscle fiber-type via the Ca^{2+}-NFATC1 pathway. This finding reveals that lactate possesses the potential acting as a kind of sports supplements and provided new insight on the effects of lactate in exercise.

KEY WORDS: lactate; fiber-types switch; Ca^{2+}-NAFTC1 pathway; metabolism reprogram.

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Hydrogen Sulfide associated persulfidation orchestrates homocystine metabolism and ferroptosis in NSCLC

Hualei Zheng, Huidi Chen, Xilin Li, Min Shen, Junjia Liu, Yongyou Zhang*
(School of Life Sciences, Xiamen University, Xiamen, Fujian 361102, China.
Email (Dr. Yongyou Zhang): yongyouzhang@xmu.edu.cn)

Abstract: Ferroptosis is a new form of regulated cell death triggered by the iron-dependent peroxidation of phospholipids, associated with various redox reaction and cellular metabolism, and shows great potential for cancer therapy. Hydrogen sulfide (H_2S), an endogenous metabolite of the transsulfuration pathway, is closely associated with ferroptosis. However, the molecular mechanisms by which H_2S regulates ferroptosis remain largely elusive. In this study, we defined exogenous H_2S as a regulator for ferroptosis and systematically characterized its regulatory role in non-small cell lung cancer (NSCLC). We demonstrate that H_2S increases the sensitivity of NSCLC to ferroptosis both in vitro and in vivo. Mechanistically, we found that H_2S persulfidated cysteine residues of S-adenosyl homocysteine hydrolase (SAHH), thereby inhibiting the activity of SAHH and decreasing homocysteine level. The decreased homocysteine inhibits the transsulfuration pathway and decreases levels of cysteine and glutathione under the condition of cysteine depletion, which ultimately contributed to ferroptosis. Thus, our findings reveal that H_2S directly regulates homocystine metabolism and dedicates the response of NSCLC to ferroptosis, and highlights a candidate therapeutic target to improve the ferroptosis-based antitumor therapies.

犬尿酸-GPR35 信号轴调控骨髓来源单核/巨噬细胞炎症激活及心肌细胞线粒体能量代谢减轻糖尿病射血分数保留心衰的作用及机制研究

王蕾 [1,3*]，生文慧 [2]
1. 首都医科大学
2. 首都医科大学公共卫生学院
3. 首都医科大学公共卫生学院营养与食品卫生学系

随着人口老龄化及生活方式的改变，糖尿病的发病率显著升高，其中心血管并发症是导致糖尿病患者死亡的首要原因。糖尿病会导致心肌细胞代谢紊乱及心脏组织重塑，早期表现为射血分数保留型舒张功能障碍，晚期出现心脏收缩功能下降，最终发展为心力衰竭。糖尿病合并心血管并发症已成为威胁公众健康并给社会造成沉重经济负担的重要公共卫生问题。因此，糖尿病心衰的早期干预迫在眉睫。然而，目前糖尿病早期合并射血分数保留心衰的病理生理机制仍未完全阐明，临床亦无有效的诊疗措施，亟需明确其发病机制及诊疗靶点。

犬尿酸是色氨酸代谢的分支产物，既往研究已证实，在调控机体能量代谢及免疫反应中均发挥关键作用。然而，犬尿酸-GPR35 信号轴在糖尿病射血分数保留心衰发生发展中的作用及其相关分子机制目前仍不清楚。因此，本研究提出研究假设：犬尿酸通过激活 GPR35 信号通路调控骨髓来源单核/巨噬细胞炎症反应及心肌细胞线粒体能量代谢减轻糖尿病射血分数保留心衰。

研究发现，糖尿病射血分数保留型心力衰竭患者及小鼠的血浆及心脏组织中犬尿酸水平均显著下降，且血浆犬尿酸水平降低与心脏舒张功能下降密切相关。补充犬尿酸可明显改善糖尿病射血分数保留心衰小鼠糖脂代谢紊乱、心肌细胞线粒体能量代谢、心脏组织免疫炎症反应、组织重塑及舒张功能异常；然而，利用犬尿酸处理 GPR35 全敲鼠、骨髓移植 GPR35 敲除嵌合体鼠、髓系特异性 GPR35 敲除鼠及心肌细胞特异性 GPR35 敲除鼠糖尿病射血分数保留型心衰模型，犬尿酸减轻糖尿病射血分数保留型心衰的作用丧失，证实犬尿酸通过激活骨髓来源单核/巨噬细胞及心肌细胞 GPR35 信号通路介导糖尿病射血分数保留型心衰的保护作用。体外实验进一步证实，犬尿酸通过 GPR35 信号通路调控骨髓来源单核/巨噬细胞免疫激活及心肌细胞线粒体能量代谢。运动及补充维生素 B6 可明显提高血浆犬尿酸水平，改善糖尿病射血分数保留型心衰。

本研究通过人群队列 、基因编辑鼠及细胞模型深入研究犬尿酸-GPR35 信号轴调控糖尿病射血分数保留型心衰的作用及机制，为糖尿病心肌病的早期防治提供理论依据及营养干预靶点。

关键字：犬尿酸，GPR35，糖尿病合并射血分数保留型心力衰竭，骨髓来源单核巨噬细胞，心肌细胞

The purine metabolite inosine monophosphate accelerates myelopoiesis and acute pancreatitis progression

Jiwei Zhao[1,3], Yuan Dong[1,2,3], CenYan[1], Yuejie Zhang[1], Xuanyi Yang[1], Yalan Deng[1], Yingmei Feng[1,2,*]

1. Department of Science and Development, Beijing Youan hospital, Capital Medical University, Beijing 100069, China;
2. Laboratory of Clinical Medicine, Capital Medica University, Beijing 100069, China;
3. Beijing Institute of Hepatology, Beijing Youan Hospital, Capital Medica University, Beijing 100069, China

ABSTRACT:Type 2 diabetes mellitus (T2DM is not only an independent risk factor for acute pancreatitis (AP) but also correlates with AP severity. We previously reported that diabetic db/db mice at the age of 18 weeks old were featured with excessive myeloid cell expansion. In an established murine AP model induced by caerulein, injection of diabetic db/db bone marrow cells (BMCs) aggravated disease severity compared with injectin of wild-type BMCs. In the study, we interrogated the role of metabolites that result from the altered metabolic state in T2DM, and investigated whether the identified metabolites could also modulate the proliferation of myeloid progenitor cells, resulting in myelopoiesis and strengthened inflammation in AP. By FACS analysis, we identified the increased frequency and proliferation of granulocyte/monocyte progenitors (GMPs) in BM of 24-week aged db/db mice than those at 8 weeks old. Using targeted metabolomics, we identifyied an increase in inosine monophosphate in FACS-sorted GMP cells of diabetic db/db mice. We demonstrated that IMP treatment stimulated cKit expression, ribosomal S6 activation, GMPs proliferation, and Gr-1^+ granulocyte production *in vitro*. IMP also activated pAkt in non-GMP cells. *In vivo*, administration of IMP-treated BMCs accelerated the severity of AP. This effect was abolished in the presence of a pAkt inhibitor. Accordingly, the results of targeted metabolomics revealed that plasma levels of guanosine monophosphate were significantly higher in diabetic patients with AP. Taken together, these findings provide a potential therapeutic target for the control of vascular complications in diabetes.

KEY WORDS: inosine monophosphate, granulocyte/monocyte progenitors, acute pancreatitis, type 2 diabetes mellitus, targeted metabolomics

TNNI3 and MYBPC3 variants promote metabolic disorders in HCM

Wu Hao [1,2], Li Huimin[1], Yang Zhulin[1], Xia Zilin[1], Zhang Hong[*2], Yi Lunzhao[1*]

1. Faculty of Food Science and Engineering, Kunming University of Science and Technology, Kunming, 650500, China

2. Department of Cardiology, First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming, 650000, China

ABSTRACT:

Background Hypertrophic cardiomyopathy (HCM) is a common inherited cardiovascular disease with heterogeneous clinical presentations. Important insights into the genetic landscape of HCM have enhanced our understanding of the molecular pathogenesis, empowered gene-based diagnostic testing to identify at-risk individuals. However, the metabolic alterations and their function in HCM are unknown.

Methods The experimental group consisted of 45 patients with hypertrophic cardiomyopathy from the First People's Hospital of Yunnan Province, of which 8 were from two families, and the control group consisted of 137 patients from the Health Management Center. Among them, 23 were from individuals with normal phenotypes in both families. The clinical information of HCM patients and plasma samples from all populations mentioned above was collected and analyzed. The whole-exon sequencing (WES) was applied for screening variant in all HCM patients and two family members and the results were validated by Sanger sequencing technology. Non-targeted metabolomics of plasma metabolites in all populations using ultra high-performance liquid chromatography high-resolution mass spectrometry (UPLC-HRMS).

Results Age, gender, and BMI were adjusted through propensity score matching (PSM), resulting in a total of 26 control group members and 26 patients with HCM being matched. Non-targeted metabolomics reveled the metabolic alteration and the increased free fatty acid concentration in HCM. The similar results were also observed in comparisons between familial hypertrophic cardiomyopathy (FHCM) cases and normal individuals within the family. Mutation variants were identified in a total of 13 samples, including 4 sporadic cases, 8 FHCM cases, and 1 individual with normal phenotype in family A. To further evaluate the relationship between gene mutations and metabolites, 22 controls and 22 patients with sporadic hypertrophic cardiomyopathy (SHCM) were matched. 13, 30 and 15 differential expression metabolites were analyzed between the no variant group and the Control group, the no variant group and HCM with TNNI3, and the no variant group and HCM with MYBPC3 group, respectively. Compared with no mutation, TNNI3 and MYBPC3 mutations presented similar amino acid and lipid metabolism characteristics, but some metabolites showed differences.

Conclusion The significant metabolic disorder, especially lipid metabolism, occur in hypertrophic cardiomyopathy, and there are also differences in metabolites among different mutated genes. The results of this study also improve our understanding of HCM, which can provide valuable suggestions for medical diagnosis and intervention.

KEYWORDS: Hypertrophic cardiomyopathy, whole-exon sequencing, ultra high-performance liquid chromatography high-resolution mass spectrometry, metabolomics

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基于质谱的代谢组学分析冠状动脉狭窄和钙化对循环代谢物的影响

李慧敏[1]，吴昊[2,3]，王瑞云[3]，顾颖[1]，杨柱林[1]，付光辉[4]，赵燕[3]， 壮可[3]，任达兵[1]，张宏[3,*]，易伦朝[1,2,*]

1.昆明理工大学食品科学与工程学院，昆明 650500

2.昆明理工大学化学工程学院，昆明 650500

3.昆明理工大学附属医院/云南省第一人民医院心血管内科，昆明 650032

4.昆明理工大学理学院，昆明 650500

*E-mail: yilunzhao@kust.edu.cn

摘 要 冠心病是一种常见的心血管疾病，严重威胁人类健康。冠状动脉钙化在冠心病患者中非常普遍，钙化的进展与不良心血管疾病预后息息相关。本研究采用超高效液相色谱-高分辨质谱联用（UPLC-HRMS）技术，分析非冠心病（29 例）和冠心病（58 例）以及冠心病中血管钙化（46 例）和无钙化患者（12 例）的血浆代谢特征，探究冠状动脉狭窄和钙化对循环代谢物的影响。本研究对血浆中的 101 个内源性代谢物进行了定性定量分析，在此基础上，基于稀疏正则化的子抽样-支持向量机（SRS-SVM），并结合 t 检验筛选组间差异性特征代谢物，并进行富集和代谢通路分析。研究结果表明，冠状动脉狭窄是影响循环代谢的主要因素，主要影响丙氨酸、天冬氨酸和谷氨酸代谢为主的氨基酸代谢、亚麻酸代谢和甘油磷脂代谢；冠状动脉狭窄基础上的血管钙化，不仅影响氨基酸代谢、脂质代谢和三羧酸循环等代谢路径，还对肠道微生物的循环代谢产生影响。

关键词: 代谢组学；超高效液相色谱-高分辨质谱联用技术；支持向量机；冠心病；冠状动脉钙化

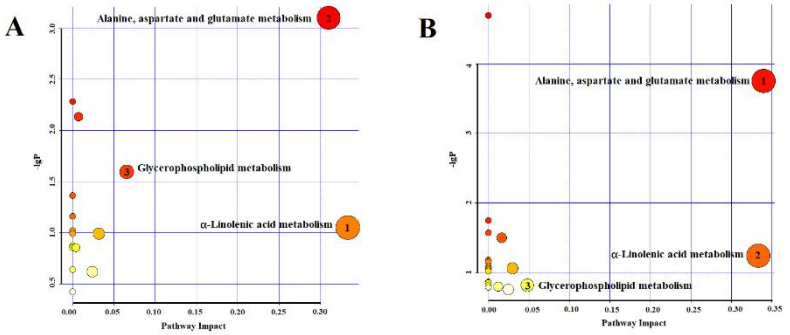

图 A：非冠心病组和冠心病组的代谢通路分析结果；图 B：非钙化组和钙化组的代谢通路分析结果。

Figure A: metabolic pathways analysis result in Non-CHD and CHD; Figure B: metabolic pathways analysis result in Non-CAC and CAC.

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基于血液代谢组学分析缺血性心脏病患者疾病进程的代谢紊乱特征

杨柱林[1],田林云[2]，吴昊[2,3]，李慧敏[1]，顾颖[1]，任达兵[1]，壮可[2]，赵燕[2]，张宏[2,4*]，易伦朝[1,3*]

1.昆明理工大学食品科学与工程学院，云南 昆明 650500;

2.昆明理工大学附属医院，云南省第一人民医院心血管内科，云南 昆明 650032;

3.昆明理工大学化学工程学院，云南 昆明 650500;

4.昆明理工大学生命科学学院，云南 昆明 650500;

缺血性心脏病是目前临床医学中常见且较为严重的心血管系统疾病，因其发病率死亡率逐年升高，已成为全球死亡和疾病负担的主要原因之一，是重要的公共卫生问题。本研究以47 例心绞痛患者、51 例心肌梗死患者和 80 例心力衰竭患者血浆为样本，结合超高效液相色谱-高分辨质谱联用技术和化学计量学方法，分析缺血性心脏病患者疾病进程的代谢紊乱特征。本研究共定性定量出 97 种内源性代谢物，在此基础上，基于 t 检验、主成分分析、偏最小二乘-判别分析、变量重要性投影等方法，分别筛选出 28 种和 32 种可区分心绞痛与心肌梗死患者，心肌梗死与心力衰竭患者的差异性特征代谢物。代谢通路分析结果表明，从心绞痛到心梗，以及心梗到心衰的疾病进程中，氨基酸代谢和三羧酸循环等能量代谢均发生了紊乱。从心梗到心衰的疾病进程中，以甘油磷脂代谢和脂肪酸生物合成为代表的脂质代谢紊乱显著。ROC 分析结果表明，联合糖胆酸、富马酸、棕榈酸、肌钙蛋白、高密度脂蛋白、谷丙转氨酶、谷草转氨酶等指标，可提高心肌梗死诊断精度，AUC 值达到 1.0000；联合瓜氨酸、柠檬酸、硬脂酸、甘油磷酸胆碱、NT-proBNP 可提高心力衰竭诊断精度，AUC 值达到 0.9570。本研究发现可为缺血性心脏病患者的精准诊断和疾病发展过程中的药物和营养干预提供重要的代谢靶标。

关键词：超高效液相色谱-高分辨质谱联用技术；代谢组学；心绞痛；心肌梗死；心力衰竭；化学计量学

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Noninvasive fecal metabolomic analysis of primary Sjögren's Syndrome and disease activity implementing concepts of predictive, preventive, and personalized medical approach

Ting Cui[1], Nan Wang[2], Lili Shang[2], Rui Zhang[1], Zhixing Fu[1], Liyuan Nie[1], Jing Luo[2*], Zhenyu Li[1*]

1. Modern Research Center for Traditional Chinese Medicine of Shanxi University, Taiyuan 030000, China;
2. Division of Rheumatology, Department of Medicine, The Second Hospital of Shanxi Medical University, Taiyuan 030000, China

ABSTRACT:

Objectives: To systemically profile metabolic alterations and establish diagnosis model for primary Sjögren's Syndrome (pSS), and discover potential biomarker metabolites relating with disease activity.

Methods: Fecal samples of 135 participants, including 93 pSS and 42 healthy controls (HCs), were analyzed by high-resolution chromatography-mass spectrometry- based metabolomics. LASSO was used for variable selection, and then the diagnosis model was established by logistic regression (LR) analysis. The support vector machine (SVM) and random forest (RF) were performed to evaluate the diagnostic model. Moreover, the diagnostic model was validated by an independent cohort (30 pSS vs. 10 HCs). The partial correlation analysis was performed to reveal the relationships between metabolites and clinical indexes in pSS.

Results: 156 fecal metabolites were found significantly altered in pSS patients when compared with HCs. A panel consisting of MG (0:0/14:0/0:0), LysoPE (14:0/0:0), sphinganine and octadecanamide could differentiate pSS from HCs, with an AUC of 0.990 for the discovery cohort and 0.997 for the test cohort. Moreover, the combination of arachidoyl ethanolamide, indole-3-propionic acid, N-acetylglucosamine, and coprostanol showed satisfactory diagnostic performance for distinguishing high-activity group from low-activity group with the AUC value of 0.818 in the discovery cohort and 0.781 in the test cohort. The partial correlation differed greatly both in the number and strength of the correlations for the high-activity and low-activity groups.

Conclusion: Fecal metabolic signatures in the pSS and those relating with different disease activity may provide useful information in understanding the molecular mechanisms and deliver valuable biomarkers for the clinical diagnosis of pSS.

KEY WORDS: primary Sjögren's Syndrome, Metabolomics, Diagnostic Marker, Disease activity, ESSDAI score

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High-fat diet responsive gut microbiota impairs social behavior via gut-to-brain metabolic signaling

Youying Zhang, Xiao Zheng*, Haiping Hao*
Department of Pharmacology, School of Pharmacy, China Pharmaceutical University, 210009 Nanjing, Jiangsu, China.

ABSTRACT: The field of nutritional psychiatry has generated enormous data supporting the role of dietary patterns in depression and other mental illnesses. Chronic exposure to a high-fat diet (HFD) has been regarded as a common risk factor for depressive behavior, but the underlying mechanism is still unclear. Here, we show that HFD bidirectionally modulates social behavior in mice via gut microbial metabolites. Short-term HFD exposure (3 weeks) protects against repeated social defeat (RSD) stress-induced social avoidance, which switches to an exacerbating effect in the long term (15 weeks). The gut microbiota temporally shaped by the HFD is causally linked to the differential social avoidance behavior via fecal microbiota transplantation. *Alloprevotella, Bacteroides,* and *Alistipes* were identified as potential functional bacteria, which may act on hypothalamic oxytocin. To mediate the behavioral outcome. Mechanistically, bacteria-triggered metabolomic changes involving histidine and bile acid metabolites in the hippocampal, mPFC, and hypothalamus correlate with social behavior. Overall, this study reveals that the gut microbiota modulates host metabolism and social behavior susceptibility in mice.

KEYWORDS: high-fat diet; depression; metabolic signal; gut microbiota; gut-brain axis

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基于深度学习模型整合多组学数据挖掘疾病关键分子和通路

赵金慧[1,3]，周言彦[1,2]，王鑫欣[1,3]，赵莹[1]，路鑫[1,3,*]，许国旺[1,3,*]

1. 中国科学院分离分析化学重点实验室 中国科学院大连化学物理研究所，辽宁 大连 116023；
2. 大连医科大学附属第一医院，大连，116111；
3. 中国科学院大学，北京 100049；

多组学数据整合分析需求日益增强，随着高通量技术的快速发展。目前，传统多组学数据整合方法主要分为两种。首先，以单组学研究结果为基础，提取关键通路或调控信号，然后利用其他组学手段进行验证。其次，分别提取不同组学结果后映射到通路中取交集作为最后的研究目标通路。然而，传统整合方式仍然涉及数据拼接，可能导致信息丢失，未真正将不同调控层次从生物功能角度联系起来。此外，由于生物数据集中存在噪声、高维度和稀疏性，传统统计方法在从这些数据中获取生物学见解方面面临极大困难。因此，如何整合高维多模态多组学数据以筛选关键分子和锁定关键通路仍然是组学数据下游处理分析的瓶颈问题，亟需发展多组学数据整合方法。

MODA 与当前多组学数据整合方法不同之处在于，首先利用基因规模化代谢网络结合前列腺癌转录组数据进行代谢流通量分析，提高了数据利用率，同时增强了代谢组学与其他组学之间的联系。然后，通过多种机器学习方法计算指标，综合评价不同分子判别前列腺癌不同分期样本的能力。此外，整合多个开源知识图谱构建生物分子相互作用背景网络，并根据机器学习结果提取种子节点，提取子网以降低计算冗余度。以机器学习指标作为节点属性输入，以分子打分作为输出训练网络，模型训练过程中无过拟合现象，训练集和测试集的损失函数均降至 0.8 左右。利用训练好的模型预测 4427 个无属性节点以挖掘潜在关键分子。使用社区发现算法划分网络模块，去除无用模块可大大降低候选分子（98.87%），并挖掘到 25 个疾病单元。最后，使用随机森林和 ROC 分析评估每个疾病模块的诊断能力，最终获得 8 个关键社区及 6 个前列腺癌关键分子。通过人群组织样本和细胞分子生物学实验成功验证了关键分子具有判别不同分期前列腺癌患者的能力，表明 MODA 挖掘到的潜在分子和关键通路具有生物学可解释性。

本研究开发了 MODA 作为一种多组学数据整合方法，有效地克服了样本量不足、数据标签不平衡、生物数据高维度和生物可解释性等问题。MODA 巧妙地利用知识图谱挖掘隐藏分子和提取关键模块，有助于发现新的生物标志物，并通过人群实验和细胞实验进行了验证。MODA 将在生命科学领域发现新的标志物和关键通路方面发挥重要作用。；

关键词：多组学，生物网络，深度学习，关键分子，靶标通路；

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A framework of analysis of metabolic networks based on metabolic flux analysis method

Yonghong Hao[1], Ziwei Dai[1,*]

1. School of Life and Science, Southern University of Technology and Science, 518055, China

* Correspondence: daizw@sustech.edu.cn

ABSTRACT: Metabolic flux (the rate of the metabolic reaction) is indispensable to reflect the dynamics of metabolic networks, which can tell researchers changes in the metabolic fluxes after metabolic network is disturbed. On the one hand, limited by technology, only part of the extracellular fluxes can be measured, such as extracellular acidification rate (ECAR) and oxygen consumption rate (OCR). On the other hand, there are many computational methods to solve metabolic fluxes, including metabolic flux analysis (MFA) method which is a powerful method. Based on MFA method, we propose a framework, in which we constructed the metabolic network and models to simulate mass isotopomer distribution (MID) at the steady state or the non-steady state. By minimizing the difference between simulated MID and experimental MID which obtained from published literatures, we can solve metabolic fluxes in the metabolic network. These calculations of fluxes will provide insight into metabolic mechanism behind the changes in physiological function. And the framework to explore mechanism of metabolic pathways is very efficient for the analysis of metabolite isotope tracer data.

KEY WORDS: metabolic flux analysis, metabolic network, mass isotopomer distribution

APP/PS1 转基因小鼠中枢与外周代谢组学分析揭示嘌呤代谢通路的双重重要作用

周金月[1]，陈淑淑[1]，刘鹏举[1]，王钦文[1,*]，唐春兰[1,*]
1. 宁波大学医学部公共卫生学院，浙江，315211, 中国

目的：多项研究表明阿尔茨海默病（AD）与多个外周系统存在关联。本研究旨在通过 APP/PS1 小鼠和其野生型小鼠（WT 组）的脑部和外周样本的代谢组学分析，以探索 AD 中枢和外周之间的关系，为 AD 的诊断和治疗提供新的方向。**方法：**采用 UPLC-Q-TOF-MS 代谢组学技术，筛选 APP/PS1 小鼠与 WT 小鼠在不同脑部区域和外周样本中的差异代谢物，挖掘关键代谢通路。**结果：**AD 小鼠外周系统和不同脑区的代谢物发生扰动，其中脑区筛选到 70 个差异代谢物，涉及 27 条代谢通路，外周筛选到 111 个差异代谢物，涉及 38 条代谢通路。其中，嘌呤代谢通路在外周和脑区均参与了调控。**结论：**嘌呤代谢通路在 AD 的中枢和外周发挥重要作用，为进一步理解 AD 发病机制以及开发相关的诊断和治疗策略提供了线索。

关键词：阿尔茨海默病，中枢，外周，嘌呤代谢

Role of eicosanoid related metabolites in heart failure

Weijian Hang[1], Chengcheng Zhao[1], Liyuan Peng[1], Dao Wen Wang[1], Junfang Wu[1,*]
Division of Cardiology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
Email: *Junfang.wu@tjh.tjmu.edu.cn*

ABSTRACT: Heart failure (HF) is a growing health problem worldwide, with nearly 50% of 5-year mortality and hospital readmission rates. Identifying novel factors common to individuals at a higher risk of adverse outcomes may reveal potential targets for interventions and improve life quality. The eicosanoid related epoxyeicosatrienoic acids (EETs), which exert multiple endogenous protective effects, are hydrolyzed into less active dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH). However, commercial drugs related to sEH or she are not yet in clinical use. Here we enrolled 500 patients with different subtypes of HF (HFpEF/HFrEF) and 500 healthy controls between February 2010 and March 2016. Eight types of sEH-related eicosanoids were measured according to target metabolomics, and their correlation with clinical endpoints was also analyzed. The primary endpoint was cardiac mortality, and the secondary endpoint was a composite of cardiac events, including heart failure (HF) readmission, cardiogenic hospitalization, and all-cause mortality. Furthermore, the effect of sEH inhibitors on cardiac diastolic function in different subtypes of HF was investigated in vivo and in vitro. Our study found different mechanism involved in the disease progression of two types of HF. We also demonstrated two novel sEH inhibitors that were identified as promising therapeutic strategy to improve cardiac function.

KEY WORDS: Heart Failure; eicosanoid; epoxyeicosatrienoic acids; translational research.

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Integration of Metabolomics and Machine Learning Accurately Distinguish Different Phases after 2/3 Partial Hepatectomy

Runbin Sun[1, 2] *

1. Phase I Clinical Trials Unit, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, 210008, China;
2. Phase I Clinical Trials Unit, China Pharmaceutical University Nanjing Drum Tower Hospital, Nanjing, 210008, China

ABSTRACT: In mammals, the liver is the only organ that can regenerate itself, yet the exact mechanism has not been fully elucidated. To reveal the metabolic change at different phases in liver regeneration, we analyze the endogenous metabolites in the plasma of C57BL/6J mice at various periods (initiation, progression, and termination phase) following a 2/3 partial hepatectomy (PHx) using an LC-QTOF/MS-based metabolomics approach. Compound identification, multivariate and univariate data analysis and pathway analysis were performed subsequently. The residual liver proliferated in the first three days and had about 90% of its initial weight by the seventh day. Total bile acids in the serum increased considerably at 36 h and decreased to normal after seven days. The results of PCA and PLS-DA showed that PHx caused a significant metabolic shift at 36h after 2/3 PHx that was reversed during the liver regeneration phase. Further pathway analysis found arginine metabolism, cysteine and methionine metabolism, tryptophan metabolism, steroid hormone biosynthesis, purine and pyrimidine metabolism were the most influenced pathways. Machine learning algorithms including "glm", "lda", "knn", "logitboost","rpart", "rf", "svmRadial", "nb", and "nnet" were used for classification between different phases. In summary, several amino acid metabolic pathways and steroid metabolism underwent dynamic changes during liver regeneration. A random forest method consisting of five metabolites can accurately distinguish different phases during liver regeneration.

KEY WORDS: Metabolomics; Random forest; Liver regeneration; Partial hepatectomy

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基于代谢组学技术的蒙药京大戟成分炮制转化机理研究

刘冰冰，刘璐，郁红礼，吴皓*
南京中医药大学，江苏省南京市栖霞区仙林大道 138 号，邮编 210023，中国

关键词： 蒙药；京大戟；诃子；炮制；代谢组学

摘要：目的 蒙药巴格-塔日努（京大戟，*Euphorbia pekinensis*）为大戟科（Euphorbia）植物京大戟的干燥根，具有“泄水逐饮、消肿散结”等功效，临床上常用于治疗“水肿胀满，胸腹积水，痰饮积聚，气逆喘咳，二便不利”等症状[1]。诃子为植物诃子（*Terminalia chebula* Retz.）或绒毛诃子（*Terminalia chebula* Retz. var. tomentella）的干燥成熟果实。在蒙药民族药体系中，“诃子制”是特色炮制方法。诃子制能降低京大戟毒性，缓和其泻下作用，但对于诃子制解毒的机制，尚未有明确报道。通过基于质谱的代谢组学技术，对京大戟生品及不同诃子用量炮制品的化学组成进行分析，探究不同炮制条件对京大戟化学成分的影响，从而阐明民族药特色炮制方法解毒的机制。**方法** 采用超高效液相色谱－四极杆－飞行时间质谱联用技术（UPLC-Q-TOF-MS），在正、负离子模式检测，根据文献、自建库和公共数据库，利用 Peakview 软件注释样品中的成分，通过多元统计分析筛选京大戟炮制前后的差异成分。**结果** 正、负离子模式下几组样品共匹配出化合物 71 种，主要分为萜类、酯类、黄酮类、有机酸、甾体和鞣质等成分。对于差异最大的生品组和炮制品 S4 组，筛选出 65 个特征差异离子（其中正离子 30 个，负离子 35 个），进一步鉴定得到 10 个差异成分，其中有 7 个来源于京大戟，3 个来源于诃子，其余未知成分有可能为京大戟成分的转化产物。对于 2 个来源于京大戟的萜类成分 daphnodorin B analogue 和 euphol analogue，推测了可能的炮制反应机理。**结论** 炮制对于京大戟的化学组成有显著影响，降低了京大戟的几种毒性萜类成分含量，增加了具有抗氧化、抗炎活性的诃子成分[2-5]。且随着诃子用量增加，炮制品与生品的化学组成相差越大。通过代谢组学技术可以准确分析京大戟炮制品质量，优化炮制工艺。

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作者简介：刘冰冰（1992—），女，博士，南京中医药大学讲师，研究方向为中药炮制机理、代谢组学、空间代谢组学。Tel.: 18252067670。 E-mail: bbliu@njucm.edu.cn
*通讯作者：吴皓（1957—），女，博士，南京中医药大学教授，研究方向为中药炮制解毒增效的科学内涵和饮片质量研究。E-mail: whao5795@njucm.edu.cn

Integrated lipidomics, network pharmacology and pharmacokinetics strategies to reveal the Eight Zhes Decoction's regulation of lipid dysfunction of nonalcoholic fatty liver disease

Chunlan Tang[1,*], Yuping Zhou[2], Jinyue Zhou[1], Ze Dai[1]

1. School of Public Health, Health Science Center, Ningbo University, Ningbo, Zhengjiang, 315211, China

2. The First Affiliated Hospital of Ningbo University, Ningbo, Zhengjiang, 315020, China

ABSTRACT: Nonalcoholic fatty liver disease (NAFLD) has developed into the most common chronic liver disease and can lead to liver cancer. Currently, the diagnosis and therapy of NAFLD is minimal. Traditional Chinese medicines (TCMs) have remarkable therapeutic effects on NAFLD [1,2]. Our laboratory developed a novel prescription denoted "Eight Zhes Decoction" (EZD) based on many years of practice in TCM clinical treatment of NAFLD, and this decoction has achieved good clinical efficacy against NAFLD. The pharmacodynamic material basis and mechanism were investigated using a strategy integrating lipidomics, network pharmacology and pharmacokinetics. EZD could attenuate the degrees of collagen deposition and steatosis in the livers of nonalcoholic steatofibrosis model mice. Glycerophospholipid metabolism, arachidonic acid metabolism, glycerolipid metabolism and linoleic acid metabolism with PLA2G4A and CYP450 as the core targets and 12,13-EpOME, 12(S)-HETE, LTB4, PGE2, PCs and TGs as the main lipids were found to be involved in the treatment of NAFLD by EZD. Importantly, naringenin, artemetin, canadine, and bicuculline were identified as the active ingredients of EZD against NAFLD; in particular, naringenin reduces PC consumption by inhibiting the expression of PLA2G4A and thus promotes sufficient synthesis of very-low-density lipoprotein to transport excess TGs in the liver. This research provides valuable data and theoretical support for applying EZD against NAFLD.

KEYWORDS: Eight Zhes Decoction; nonalcoholic fatty liver disease; lipidomics; network pharmacology; pharmacokinetics.

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A simple, rapid and sensitive HILIC LC-MS/MS method for simultaneous determination of 16 purine metabolites in plasma and urine and is application in AKI hospitalized patients

Xiaona Li[1,2 #], Zhini Liu[1,3 #], Libo Zhao[1,2, *]

# Xiaona Li and Zhini Liu contributed equally.

* Libo Zhao were the corresponding authors.

E-mail address and Tel:

Libo Zhao, libozhao2011@163.com, 010-82266673

1 Department of Pharmacy, Peking University Third Hospital, Beijing, 100191, China

2 Therapeutic Drug Monitoring and Clinical Toxicology Center of Peking University, Beijing, 100191, China

3 School of Basic Medical and Clinical Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu Province 211198, China

ABSTRACT: Purine intermediates play important roles in physiological function[1–3] and participate in the kidney disorders[4,5], while a targeted quantification of the metabolic alterations in the purine metabolism in acute kidney injury (AKI) individuals has not been conducted. In the study, a novel, rapid and sensitive LC-MS method for simultaneous quantification of 16 purine metabolites was developed using hydrophilic interaction separation mode in human plasma and urine. The developed method was validated as selective, sensitive, stable and precise by using charcoal-stripped plasma and urine as blank matrix. The method provided a wider coverage of purine metabolites and completed good separation of interfering compounds of nucleosides, deoxynucleosides and their corresponding nucleobases without derivatization, which was time-saving and labor-saving for the large-scale analysis. Furthermore, the method was successfully applied to plasma and urine samples of hospitalized patients without and with AKI. The results revealed that purine metabolism was greatly disturbed in the AKI patients, with a dramatic increase of nucleosides (adenosine, guanosine and inosine) and deoxynucleosides (2'-deoxyadenosine) in plasma and decreased levels of nucleobases (guanine, xanthine and hypoxanthine), nucleosides (adenosine and guanosine), deoxynucleosides (2'-deoxyadenosine and 2'-deoxyinosine) and nucleotides (cAMP) in the urine, which might associate with body inflammatory response and energy metabolism disturbance[6–11]. Besides, compared to urinary purine metabolites, metabolites in plasma showed a stronger correlation with kidney function, which correlated positively with Scr and negatively with eGFR. Among them, deoxyadenosine, adenosine and guanosine represented the strongest correlation. Additionally, the ROC analysis demonstrated general prediction accuracy of purine metabolites, except for deoxyadenosine in plasma (AUC = 0.904). The novel method can facilitate the quantitative analysis of purine metabolites in biological fluids, and exhibit great prospects in providing more information on how purine metabolic alterations in plasma and urine participate in the occurrence of AKI, which helped to understand the complex pathogenesis of AKI.

KEY WORDS: Purine metabolites; HILIC; LC-MS/MS; Acute kidney injury (AKI)

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Se@Albumin Nanoparticles Attenuates DSS-Induced Colitis via Modulation of Intestinal Barrier Function, Microbiome and Adenosine Metabolism

Ruirui Wang[1], Meiting Yang[1], Ming Shi[1], Yongbo Peng[2], Zhi Tang[1,*]

1. School of Public Health, Guangdong Medical University, Dongguan 523808, China;

2. College of Pharmacy, Chongqing Medical University, Chongqing 400016, China.

([*] Zhi Tang, 唐志，ztang@gdmu.edu.cn, 13713627069)

ABSTRACT: Ulcerative colitis (UC) is a chronic inflammatory bowel disease closely related to the imbalance of gut microbiota and intestinal homeostasis.[1] However, the progress in exploring its pathogenesis and finding effective prevention and treatments is still not ideal. A new form of Se@Albumin complex nanoparticles (Se@HS) with significant antioxidant properties and anti-inflammatory activity was developed, [2] but its mechanism of action on UC has not been clarified. In this work, the intervention effect of Se@HS on UC, and its potential mechanism were investigated.

Our results showed that Se@HS can obviously alleviate the symptoms of UC in mouse model: improving the weight loss of mice, alleviating the degree of diarrhea and hematochezia in mice, and reducing the inflammatory damage of colon tissue. Compared to the dextran sodium sulfate (DSS) induced colitis group, The expression of cytokine TNF- α, and claudin-2 decreased, while the expression of IL-10 and claudin-1 increased in Se@HS treated group ($P<0.05$); The 16S rRNA sequencing of gut microbiome showed that the diversity of intestinal microbiota in the model group mice decreased, and supplementation of Se@HS could significantly enhance the diversity of intestinal microbiota ($P<0.05$); In the DSS-induced colitis group, the abundance of Verrucomicrobia at the phylum level was reduced, and the abundance of Dubosiella newyorkensis, Akkermansia muciniphila (AKK), Lactobacillus and Vibrio butyricus at the genus level were reduced. After Se@HS intervention, the relative abundance of the above bacterial communities were increased, with the increase in AKK bacteria being more significant in the high-dose group ($P<0.01$). Colonic tissue metabolomics suggest that significant changes of arginine and proline metabolism, purine metabolism and unsaturated fatty acid biosynthesis were occurred after Se@HS intervention. Furthermore, there was a certain correlation with changes in gut microbiota. It is worth noting that our study found that AKK bacteria were significantly positively correlated with purine metabolites and unsaturated fatty acids.

In summary, Se@HS can significantly alleviate DSS-induced UC in mice, and intestinal microbiota and metabolic remodeling may plays an important role in Se@HS ameliorating acute colitis in mice. Specifically, it is of note that AKK bacteria were critically involved in intestinal metabolic reactions, thereby improving the intestinal mucosal barrier function after Se@HS intervention.

KEY WORDS: Se@Albumin nanoparticles, Colitis, Akkermansia muciniphila, Metabolomics, Inosine

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多组学联合 PRM 技术探讨栀子炒炭凉血止血作用机制研究

王云[1]，高雅[2]，宋亚南[1]，张村[1*]
1.中国中医科学院 中药研究所，北京 100700
2.中国中医科学院 医学实验中心，北京 100700

目的：栀子饮片味苦性寒，炒炭后止血作用增强，是通过炒炭增强凉血止血的代表性饮片。但其发挥凉血止血的作用机制尚不清楚。通过考察栀子炒炭前后的主要化学成分变化及其对干酵母联合无水乙醇致血热复合出血模型大鼠的凉血止血作用，采用定量蛋白质组学联合非靶向代谢组学技术探索栀子炭凉血止血的作用机制，为栀子炭的临床使用提供理论依据。

方法：运用 HPLC-MS/MS 检测栀子饮片炒炭前后主要化学成分含量变化情况。建立干酵母联合无水乙醇致血热出血大鼠模型，以 4.5 mg/kg 栀子炭灌胃给药，以体温差、病理组织和 IL-6、NO 含量相结合，评价栀子炭发挥凉血止血的药效作用；采用 TMT 同位素标记的蛋白质组学和非靶点代谢组学技术分析栀子炭的胃组织样品，将差异代谢物和差异蛋白进行关联分析，构建与血热出血疾病相关联的蛋白-代谢物整体调控网络，应用平行多反应监测技术对聚焦的通路涉及的生物分子进行定量验证。

结果：栀子炭在 4.5g/kg 剂量下，可降低模型大鼠胃黏膜出血损伤。提示栀子炭对胃黏膜有一定的保护作用。血清中 NO 含量结果显示，栀子炭对异常升高的 NO 有回调作用，可降低 IL-6 的表达。共鉴定胃组织蛋白 6190 种，定量蛋白 5241 种，与正常组相比，模型组上调蛋白 86 种，下调蛋白 91 种；栀子炭给药组上调蛋白 8 种，下调蛋白 46 种。正离子模式下，与正常组相比，鉴定到胃组织差异代谢物 269 个，其中上调 137 个，下调 132 个；与模型组相比，栀子炭给药组差异代谢物 126 个，其中上调 100 个，下调 26 个。负离子模式下，与正常组相比，鉴定到差异代谢物 252 个，其中上调 171 个，下调 81 个；与模型组相比，栀子炭给药组差异代谢物 409 个，其中上调 291 个，下调 118 个。将蛋白质组学和代谢组学分析得到的显著差异蛋白质与代谢物进行皮尔森相关性分析，结果表明，glycine, serine and threonine metabolism, nitrogen metabolism, glycolysis/gluconeogenesis, neuroactive ligand-receptor interaction, pentose phosphate pathway, metabolic pathways, tyrosine metabolism and drug metabolism-cytochrome 等 8 条通路显著富集。

结论：本研究将不同层次的生物分子间进行关联，为深入研究栀子炭凉血止血的作用机制提供更全面、有针对性的线索，同时也对同类中药炮制机理的研究提供技术参考。

关键词：栀子炭，凉血止血，蛋白组学，代谢组学，关联分析

孕期黑碳纳米颗粒暴露对孕鼠及胎鼠肺组织代谢谱的影响

高碧玲[1],申河清[1,*]

1. 厦门大学公共卫生学院分子疫苗学与分子诊断学国家重点实验室，中国厦门，361102

Email：hqshen@xmu.edu.cn

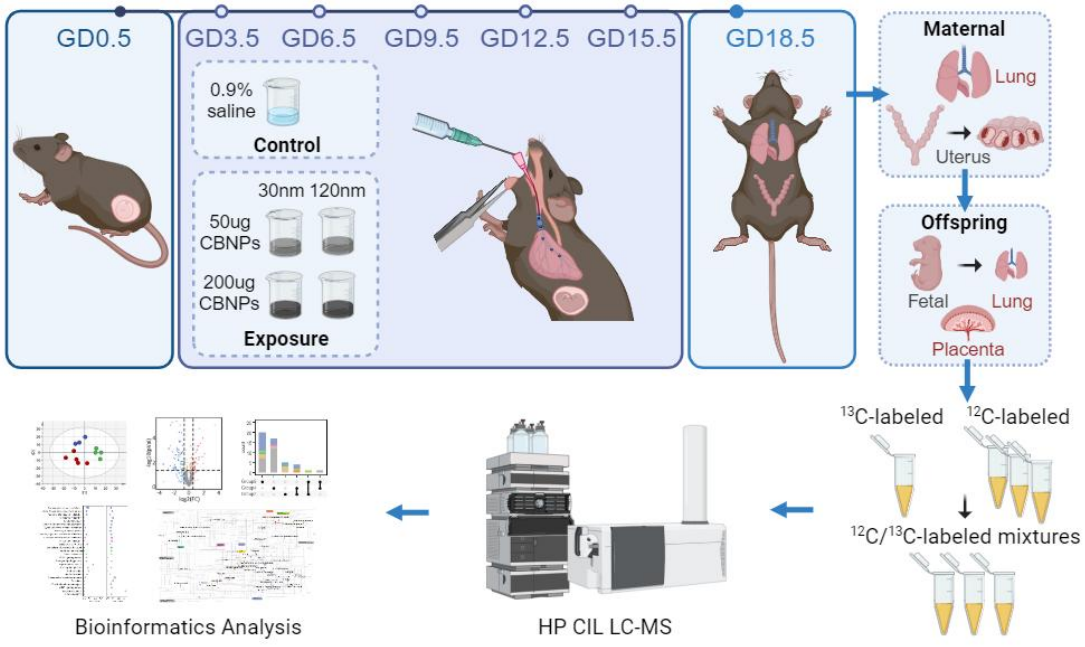
背景与目的：黑碳纳米颗粒（CBNP）可以通过在体外和体内诱导炎症、氧化应激和遗传毒性来诱导毒性，流行病学研究表明，黑碳纳米颗粒暴露与母婴健康密切相关，孕期暴露可引起子代肺组织损伤，其健康效应可能是通过胎盘介导的。然而，目前缺乏黑碳粒径大小和暴露剂量对损伤程度的影响，且仅研究宫内暴露的极少，结果尚不一致。

研究方法：9 周龄的 C57BL/6 雌性小鼠交配成功后，随机分为对照组和不同暴露剂量和粒径大小的 4 个处理组，每 3 天进行 1 次气管滴注暴露，所有孕鼠于妊娠 18.5 天处死，分离获得孕鼠肺组织、胎鼠肺组织和胎盘为实验样品，分别进行高效化学同位素标记代谢组学分析。

研究结果：本研究发现在大粒径暴露条件下，不同暴露剂量的孕鼠肺组织代谢物扰动较明显；在低剂量暴露条件下，不同粒径的孕鼠肺组织代谢物扰动较明显；在大粒径暴露条件下，不同暴露剂量的胎鼠肺组织代谢物扰动较明显；在高剂量暴露条件下，不同粒径的胎鼠肺组织代谢物扰动较明显；主要影响氨基酸代谢、碳水化合物代谢、脂质代谢。

研究结论：暴露粒径和暴露剂量都对孕鼠和胎鼠的肺组织代谢物产生了影响，且较大粒径和较高剂量对代谢物扰动更大，特别是在氨基酸、碳水化合物和脂质的代谢方面。

关键词: 黑碳纳米颗粒，孕期暴露，子代，代谢组学



急性颗粒物暴露重塑代谢分子图谱

常昊[1]，申河清[1,*]

1. 厦门大学公共卫生学院分子疫苗学与分子诊断学国家重点实验室，中国厦门，361102

*通讯作者

Email：hqshen@xmu.edu.cn

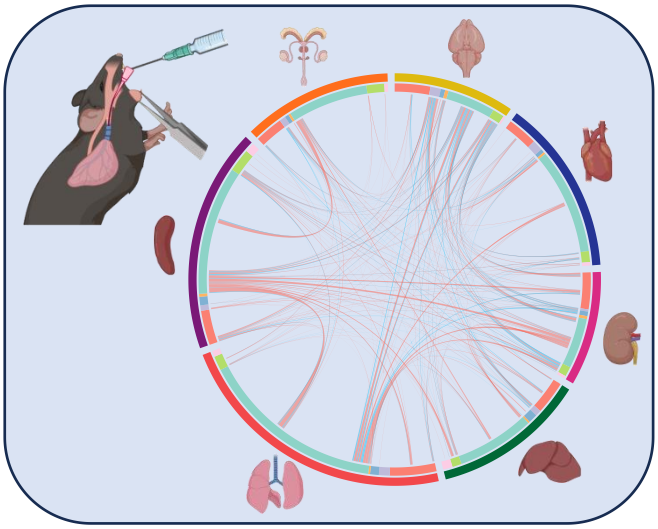
研究目的与意义：随着空气污染对全球公众健康的威胁日益凸显，其中环境颗粒物（PM）作为一个主要的污染源，已引起了广泛的关注。这些颗粒物不仅粒径不一，还包含了众多的化学成分。尽管早期的研究已针对 $PM_{2.5}$ 对全局血清或某些特定组织的潜在毒性机制进行了深入探索，但关于其在不同组织间以及组织与全身循环系统间的相互作用的研究仍然非常有限。本研究运用代谢组学技术，系统地研究急性 $PM_{2.5}$ 暴露对小鼠各组织及系统的毒性影响，为进一步深入理解 $PM_{2.5}$ 的毒性机制提供新的维度。

研究方法：将 7 周龄的 C57BL/6J 雄性小鼠随机分为对照组、低剂量组（25 μg）和高剂量组（150 μg）进行气管滴注暴露实验。两天后，取出小鼠的大脑、心脏、肾脏、肝脏、肺脏、脾脏和睾丸组织，同时收集血清样本。使用 LC-MS 系统对样本进行非靶向代谢组学分析，旨在深入探索 $PM_{2.5}$ 暴露如何干扰各组织及血清的代谢，从而构建一个全面的多组织代谢组学分析模型。

研究结果：$PM_{2.5}$ 的暴露对各组织及血清产生了明显的代谢扰动，且呈现出剂量依赖性。不同组织中的代谢扰动模式有所不同。值得注意的是，$PM_{2.5}$ 暴露加强了各组织间的代谢协同作用，特别是在肺与心和肺与脾组织之中。在这些组织里，氨基酸代谢、脂质代谢、核酸代谢和糖代谢受到了明显的扰动，并通过血清实现了代谢物的协同转运。

研究结论：$PM_{2.5}$ 的暴露导致各组织及血清的代谢产生剂量依赖性变化，尤其在在肺与心和肺与脾组织，其氨基酸、脂质、核酸和糖的代谢受到显著的影响。此外，本研究提供了一个新颖的视角和系统化的方法，用于探讨环境污染的毒性机制。

关键词: 细颗粒物；急性暴露；代谢组学；多组织分析



A novel integrated strategy based on metabolomics and network pharmacology for mechanism study of natural medicine: a case study of CDD-2101 against constipation

Jingchun Shi [a,b], Li Xiang [c], Lin Zhu [a,b], Zhaoxiang Bian [a,b]

a Centre for Chinese Herbal Medicine Drug Development, Hong Kong Baptist University, Hong Kong 999077, China

b School of Chinese Medicine, Hong Kong Baptist University, Hong Kong 999077, China

c State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong 999077, China

Abstract:

Constipation affects more than 14% of the population worldwide. The related pathogenesis is complex. Single component/formula medicines currently dominate the market; however, they normally have side effects (abdominal pain, diarrhea, etc.). Compound Traditional Chinese Medicine (TCM) is promising for constipation due to its multiple-component-multiple-target properties. Given the complexity of both TCM and constipation, it is likely that no single analytical method can reveal the relationships among the components, efficacy and mechanisms. Therefore, we developed an integrated analytical strategy based on metabolomics and network pharmacology to decipher the mechanisms of compound CDD-2101, a manufactured version of a time-tested Chinese herbal formula for constipation.

We found that CDD-2101 alleviated constipation in a loperamide-induced acute constipation mice model without adverse effects. Metabolomics identified 33 metabolites related to bile acid secretion, steroid hormone biosynthesis, glycerophospholipid metabolism, and linoleic acid metabolism. Fecal phytochemistry identified 17 prototype and four metabolite-type ingredients. We used network pharmacology to predict the pathways by which these metabolites might interact. Four key targets (RXRA, CYP1A1, CYP1A2, and PLA2G4) were found, that corresponded to the four main active components (rhein, hesperetin, albiflorin, and magnolol) of the formula. Molecular docking showed high affinities between targets and active components. Comparison with Senna, the first-line Western treatment for constipation and a single-formula natural medicine, revealed similarities (rhein, RXRA, etc.) but suggested its single-component nature compromised its efficacy.

In conclusion, this study reveals the "multi-component-multi-target-multi-pathway" mechanisms of CDD-2101 against constipation. Our work provides a novel method to explore active compounds and pharmacological mechanisms in natural medicine development.

Keywords: Metabolomics, network pharmacology, constipation, natural medicine

Specific triacylglycerol, diacylglycerol,and lyso-phosphatidylcholine species for the prediction of type 2 diabetes

Xiuli Su[1], Chloe YY Cheung[2,3], Junda Zhong[2,3], Yi Ru[1], Carol HY Fong[2,3], Chi-Ho Lee[2,3], Yan Liu[2,3], Cynthia KY Cheung[2,3], Karen SL Lam[2,3,*], Aimin Xu[2,3,*], Zongwei Cai[1,*]

1. State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong 999077, China;

2. Department of Medicine, The University of Hong Kong, Hong Kong 999077, China;

3. State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong 999077, China

ABSTRACT: The pathophysiology of type 2 diabetes (T2D) involves bioactive lipids that affect insulin secretion and sensitivity[1, 2]. To identify new lipid species correlated with incident T2D, we conducted a nested case-control study within a long-term prospective Chinese community-based cohort (median follow-up: 16 years). Untargeted lipidomics analysis was performed on plasma samples obtained from 196 incident T2D cases and 196 age- and sex-matched non-T2D controls, who were selected from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS), followed by targeted lipidomics to verify potential predictive lipid species identified by Boruta analysis. The study found that after adjusting for triacylglycerol/high-density lipoprotein (TG/HDL) ratio, body mass index (BMI) and prediabetes, four triacylglycerol (TG) species (TG 12:0_18:2_22:6, TG 49:0, TG 16:0_11:1_18:2 and TG 51:1) and one diacylglycerol (DG) species (DG 18:2_22:6) were independently associated with increased risk of T2D, while four lyso-phosphatidylcholine (LPC) species (LPC O-16:0, LPC P-16:0, LPC O-18:0 and LPC 18:1) were independently correlated with decreased risk of T2D. Adding the selected lipid species to the clinical prediction model (TG/HDL ratio, BMI and prediabetes) improved the area under the receiver operating characteristics curve (AUROC) by 3.8%. Functional studies revealed that two of the LPC species (LPC O-18:0 and LPC O-16:0) noticeably increased glucose-induced insulin secretion, while neither of the DG 18:2_22:6 or TG 12:0_18:2_22:6 had any impact. Therefore, decreased levels of LPC O-16:0 and LPC O-18:0 may contribute to the development of T2D by reducing insulin secretion.

KEY WORDS: Type 2 diabetes; metabolomics; insulin resistance; prediction.

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Study on the Catabolism of Neophaseic Acid in 9'-hydroxylation Pathway of Abscisic Acid based on Chemical Labeling-Assisted UHPLC-HRMS

Yin Xiaoming[1,#], Bai Ya-Li[1,#], **Ye Tiantian**[1,*], Feng Yu-Qi[1,*]
[1] Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China

ABSTRACT: Abscisic acid (ABA) is a phytohormone with a sesquiterpenoid structure that plays critical roles in numerous plant physiological processes1. ABA catabolism is an important pathway for ABA inactivation and homeostasis maintenance. However, the current understanding of the ABA catabolic pathway is limited. In this study, a screening and identification strategy for ABA catabolites was established based on ultrahigh-performance liquid chromatography coupled with high-resolution mass spectrometry assisted by chemical isotope labeling (CIL-UPLC-HRMS). In this strategy, a pair of isotope reagents N,N-2-dimethylaminoethylamine (DMED) and d4-DMED were used as labeling reagents to label the carboxyl groups in ABA and its catabolites. Furthermore, using our strategy, ABA, t-ABA, and 18 ABA catabolites were identified from seven plant samples. Of the identified catabolites, a new downstream catabolite of neophaseic acid (neoPA) in ABA 9′-hydroxyl pathway was identified as epi-neodihydrophaseic acid (epi-neoDPA) by comparing its accurate mass, retention time, and MS^n spectra with those of our chemically synthesized epi-neoDPA. In addition, combining CIL-UPLC-HRMS and RNA-Seq techniques, a new reductase responsible for converting neoPA into epi-neoDPA in Arabidopsis was identified and named as neoPA reductase 1 (NeoPAR1). Site-directed mutation experiment suggested that Tyr163 in the conserved motif of NeoPAR1 was crucial for the reductase activity of NeoPAR1. Finally, phenotypic analysis revealed that NeoPAR1 may play an important role in seed germination. Our findings contribute to ABA catabolic network improvement. The CIL-UPLC-HRMS method proposed in this study would provide a new strategy for the screening and identification of unknown catabolites of ABA and other plant hormones.

KEY WORDS: abscisic acid catabolism, epi-neoDPA, neoPA reductase, chemical isotope labeling, liquid chromatography-mass spectrometry

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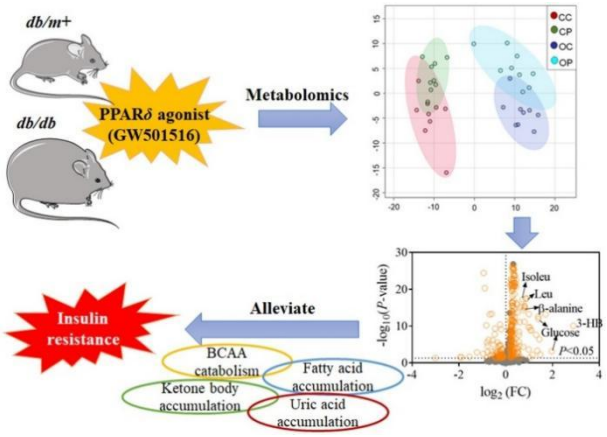
Integrated metabolomics analysis of the effect of PPARδ agonist GW501516 on catabolism of BCAAs and carboxylic acids in diabetic mice

Li Xiang, Juan Nie, Jingchun Shi, Zongwei Cai*, Yu Huang*

Abstract: The peroxisome proliferator-activated receptor (PPARδ) agonists are reported to improve insulin sensitivity, reduce glucose levels, and alleviate dysfunctional lipid metabolism in animal models of type 2 diabetes mellitus. However, the underlying mechanisms remain incompletely understood. Metabolism plays an essential role in the biological system. Monitoring of metabolic changes in response to disease conditions or drug treatment is critical for better understanding of the pathophysiological mechanisms. In this study, metabolic profiling analysis by gas chromatography-mass spectrometry integrated with targeted analysis by liquid chromatography-mass spectrometry was carried out in plasma samples of *db/db* diabetic mice after six-week treatment of PPARδ agonist GW501516. GW501516 treatment significantly altered levels of metabolites, such as branched-chain amino acids (BCAAs), BCAA metabolites (3-hydroxyisobutyric acid and 3-hydroxyisovaleric acid), long-chain fatty acids, uric acid and ketone bodies (3-hydroxybutyric acid and 2-hydroxybutyric acid) which are all associated with the impaired systemic insulin sensitivity. The present results indicate the beneficial effect of PPARδ agonist in alleviating insulin resistance of diabetic mice by favorably modulating metabolic profile, thus providing valuable information in understanding the therapeutic potential of PPARδ agonists in correcting metabolic dysfunction in diabetes.



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Physiochemical responses of C.elegans under exposure to lanthanum and cerium affected by bacterial metabolism

Jingjing Nian [1#],Wenning Lan [1,2,3,4#], Fangrong Zhang [1*]

[1] Key Laboratory of Gastrointestinal Cancer, Ministry of Education, School of Basic Medical Sciences, Fujian Medical University, Fuzhou 350122, China

[2] Institute of Material and Chemistry, Ganjiang Innovation Academy, Chinese Academy of Sciences, Ganzhou, 341000, China

[3] School of Rare Earths, University of Science and Technology of China, Hefei, 230026, China

[4] Xiamen Key Laboratory of Rare Earth Photoelectric Functional Materials, Xiamen Institute of Rare Earth Materials, Haixi Institute, Chinese Academy of Sciences, Xiamen 361021, China

[#] These authors contributed equally to this work and should be considered co-first authors

The increasing demand for rare earth elements (REEs) in modern applications has drawn significant attention. REEs can be introduced into the environment through REE-containing fertilizers, abandoned REE-rich equipment, and mining, persisting and impacting soil quality, nutrient cycles, and plant growth. Scientists have raised concerns about REEs entering the food chain from the environment and eventually accumulating in organisms. Decades of experimental evidence have shown that these effects include inhibited growth, impaired liver function, and alterations in children's intelligence quotients. However, there exists a paucity of research that has elucidated the metabolic-level biological impacts of REEs. In our study, Caenorhabditis elegans (C. elegans) was used as a model organism to investigate physiological and inherent metabolic changes under exposure to different concentrations of REEs. The diet bacteria of nematodes play a key role in their life and development. Therefore, we investigated the influence of bacterial activity on the nematodes' response to REE exposure. We observed a concentration-dependent accumulation of REEs in nematodes, which consequently led to a reduction in lifespan and alterations in body length. Exposure to a mixed solution of REEs, in comparison to a single REE solution, resulted in greater toxicity toward nematodes. The metabolic results showed that the above changes were closely related to REE-induced amino acid metabolism disorder, membrane disturbance, DNA damage, and oxidative stress. Of note, the presence of living bacteria elicits REE effects in C. elegans. These findings highlight the potential intrinsic metabolic changes occurring in nematodes under REE exposure. Our study raises awareness of the exposure risks associated with REEs, provides valuable insight into the metabolic-level biological impacts of REEs and contributes to the development of effective mitigation strategies to reduce potential risks to human health.

Keywords: Rare earth element; NMR spectroscopy; Metabolomics; C. elegans; Bacterial activity

秀丽隐杆线虫模型中诱导加速衰老

王紫然[1]，蓝文宁[1]，张芳榕[1*]
1. 福建医科大学基础医学院消化道恶性肿瘤教育部重点实验室

摘要：衰老是一个普遍的生理过程，伴随着代谢和信号传导通路改变。目前，衰老模型主要通过促衰药物实现，但大都不能很好地复制人类衰老过程或衰老相关疾病发展的模型。本研究对秀丽隐杆线虫的不同时期进行了自然衰老的代谢组学分析。同时，利用鱼藤酮、抗霉素A和多柔比星研究促衰老模型下的代谢组学。研究表明，促衰组和自然衰老组均共同调控谷氨酰胺和肌酸磷酸盐等代谢通路，表明其在线虫衰老过程中发挥重要作用，而不同促衰组的代谢指纹存在差异，这表明它们的促衰靶点有差异性。

关键词：衰老模型；秀丽隐杆线虫；促衰；代谢组学；

Comprehensive metabolomic and lipidomic alterations in response to heat stress during seed germination and seedling growth of Arabidopsis

Wenjuan Qian[1], Yuxuan Zhu[1], Qinsheng Chen[1], Shuiyao Wang[1], Longlong Chen[1], Ting Liu[2], Huiru Tang[1,*], Hongyan Yao[1,*]

1. State Key Laboratory of Genetic Engineering, School of Life Sciences, Human Phenome Institute, Metabonomics and Systems Biology Laboratory at Shanghai International Centre for Molecular Phenomics, Zhongshan Hospital, Fudan University, Shanghai, China
2. SCIEX, Analytical Instrument Trading Co., Ltd, Shanghai, China

ABSTRACT: Temperature affects seed germination and seedling growth, which is a critical and complex stage in plant life cycle. However, comprehensive metabolic basis on temperature implicating seed germination and seedling growth remains less known. Here, we applied the high-throughput untargeted metabolomic and advanced shotgun lipidomic approaches to profile the Arabidopsis 182 metabolites and 149 lipids under moderate (22°C, 28°C) and extreme high (34°C, 40°C) temperatures. Our results showed that a typical feature of the metabolism related to organic acids/derivates and amines was obviously enriched at the moderate temperature, which was implicated in many cellular responses towards tricarboxylic acid cycle (TCA), carbohydrates and amino acids metabolism, peptide biosynthesis, phenylpropanoid biosynthesis and indole 3-acetate (IAA) biosynthetic pathway. Whereas, under extreme high temperatures, there was no seed germination, but 148 out of total 182 metabolites were highly enriched, involving in the galactose metabolism, fatty acid degradation, tryptophan/phenylalanine metabolism, and shikimic acid-mediated pathways especially including alkaloids metabolism and glucosinolate/flavone/flavonol biosynthesis. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) also exhibited the gradually increased tendency from moderate temperatures to extreme high temperatures; whereas phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylglycerol (PG), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) were contrary to decrease. Another typical feature of the distinguished metabolites between 22°C and 28°C, the TCA, disaccharides, nucleotides, polypeptides, SQDG and the biosynthesis of fatty acids and glucobrassicin-mediated IAA were obviously decreased at 28°C, while amino acids, trisaccharides, PE, PC, PA, PS, MGDG, DGDG and diacylglycerol (DAG) preferred to enrich at 28°C, which characterized the alteration of metabolites and lipids during fast seedling growth. Taking together, our results provided the comprehensive metabolites phenotyping, revealed the characteristics of metabolites necessary for seed germination and/or seedling growth under different temperatures, and provided insights into the different metabolic regulation of metabolites and lipid homeostasis for seed germination and seedling growth.

KEY WORDS: seed germination, seedling growth, heat stress, Arabidopsis, phenotyping.

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Fisetin Alleviates Aging of C2C12 Myoblasts through Regulating Redox Metabolism and Energy Metabolism

Yue Zhang[1], Donghai Lin[1,*]

Key Laboratory for Chemical Biology of Fujian Province, MOE Key Laboratory of Spectrochemical Analysis and Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

ABSTRACT: Fisetin (3,3',4',7-tetrahydroxyflavone), a natural flavanol compound abundantly present in fruits and vegetables, exhibits a range of biological activities, including anti-tumor, anti-inflammatory, and antioxidant effects. This study investigated the potential of fisetin to prevent D-galactose-induced cellular senescence in C2C12 myoblasts. The results showed that fisetin treatment significantly decreased the expression of senescence markers such as p16, p53, and SA-β-gal, and profoundly increased the proliferation and differentiation abilities of myoblasts. NMR-based metabolomic analysis revealed significant changes in metabolites involved in redox metabolism and energy metabolism, including glutathione, taurine, arginine, glucose, lactate, and pyruvate. Transcriptomic analysis also showed similar results. These findings suggest that fisetin may protect against myoblast ageing by regulating redox metabolism and energy metabolism. This study is useful for exploring the potential of fisetin application against cellular senescence in myoblasts.

KEY WORDS: Fisetin; C2C12 Myoblasts; Cellular senescence; NMR-based metabonomics.

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氯代多环芳烃诱导 THP-1 巨噬细胞免疫抑制—以氨基酸代谢紊乱为特征

李心砚[1]，马梅[2]，赵碧琳[1]，李娜[2]，方玲[3]，王东红[2]，栾天罡[1#]

[1]广东工业大学生态环境与资源学院，广州，510006;[2]中国科学院生态环境研究中心，北京，100085;[3]中山大学分析测试中心，广州，510275

摘要

氯代多环芳烃（Cl-PAHs）是一种新兴的持久性有机污染物，其主要来源于不完全燃烧过程中产生的副产物，并在地表水、空气、食物等环境介质中广泛检出。此外，最新研究证实了 Cl-PAHs 存在于人体血清的暴露证据。因此，对 Cl-PAHs 的健康风险和毒性研究已成为迫切需要。研究表明，部分 Cl-PAHs 可表现出类似二噁英的特性，这意味着其具有免疫毒性潜力，但缺乏直接证据，且具体机制不明。考虑到免疫与代谢之间紧密的相互作用，代谢物既是调节免疫细胞功能的重要驱动因素，也是免疫应答过程的生物标志物。因此，本研究通过使用高内涵筛选系统（HCS）和超高分辨率质谱，探究了 THP-1 巨噬细胞中 Cl-PAHs 及其母体 PAHs（PPAHs）诱导的免疫功能障碍和代谢紊乱。通过建立免疫表型和代谢组学结果的相关性分析，判定出氨基酸代谢重编程的 Cl-PAH/PAH 诱导巨噬细胞免疫毒性的潜在原因；并鉴定植物鞘氨醇和 L-犬尿氨酸为 Cl-PAH 暴露的潜在免疫抑制生物标志物。本文首次提供了 Cl-PAHs 在不激活 AhR 的情况下引发免疫毒性的直接证据，并讨论了代谢物对巨噬细胞 Cl-PAH/PPAH 诱导免疫毒性的贡献，强调了开发基于免疫代谢机制的环境化学品毒性风险评估新方法具有相当潜力。

关键词: 多环芳烃、代谢组学、高内涵筛选、免疫毒性

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基于代谢组学探讨养阴清肺汤调控花生四烯酸代谢通路治疗小鼠肺损伤的活性成分

刘鹏举[1], 周金月[1], 洪航[1], 陈正君[2], 徐瑾妍[2], 唐春兰[1,*]
1. 宁波大学医学部公共卫生学院，浙江，315122，中国
2. 宁波大学第一附属医院，浙江，315020，中国

目的：基于代谢组学研究养阴清肺汤调控花生四烯酸代谢通路治疗小鼠肺损伤的活性成分，为养阴清肺汤的临床应用及新药研发提供科学依据。

方法：在养阴清肺汤治疗 $PM_{2.5}$ 诱导的小鼠肺损伤的药效试验基础上，通过 UPLC-Q-TOF-MS 代谢组学技术，靶向分析花生四烯酸代谢通路及入肺成分，结合多元数据统计分析筛选养阴清肺汤调控花生四烯酸代谢通路治疗小鼠肺损伤的活性成分。

结果：在肺组织中共检测到桃叶珊瑚苷、贝母辛等 13 个活性成分，其中浙贝丙素和桃叶珊瑚苷与花生四烯酸代谢通路中的代谢物 20-HETE、花生四烯酸、PGE1、PGE2、PGF2α、LA、γ-LA、EPA、DPA、PGH2 高度相关。

结论：浙贝丙素和桃叶珊瑚苷可能是养阴清肺汤调控花生四烯酸代谢通路治疗小鼠肺损伤的活性成分，为中药新药的研发提供科学依据。

关键词：代谢组学，养阴清肺汤，代谢相关性，药效物质基础

Multi-omics reveals changes in astrocyte fatty acid metabolism during early stages of Alzheimer's disease

Jie Zhong [1], Jie Pan [2], Ziwei Dai [3]*, Jun Wan [3]*

1. Shenzhen Key Laboratory for Neuronal Structural Biology, Biomedical Research Institute, Shenzhen Peking University - The Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong Province, PRC;

2. Department of Pathology and Neuropathology, Stanford University School of Medicine, CA 94305, USA;

3. Department of Biology, School of Life Sciences, Southern University of Science and Technology, Shenzhen, Guangdong Province, PRC

*Correspondence: daizw@sustech.edu.cn wanj@ust.hk

Alzheimer's disease (AD) is a devastating neurodegenerative disorder originating from the overproduction and spreading of amyloid-beta peptides followed by the emergence of hyperphosphorylated tau oligomers. An increasing body of evidence highlights the role of astrocytes in the initiation, progression, and pathology of AD. The dynamics of cell-type-specific gene expression and metabolic features during various stages of AD pathological progression remain to be fully elucidated. To better understand the factors associated with AD, we conducted RNA-seq analyses on astrocytes freshly isolated from both wild-type and APP-PS1 mouse brains at five time points that represent different stages of AD progression. Singular value decomposition analysis indicated that the critical onset of AD is at six months of age, during which abnormal changes in fatty acid metabolism are evident at both the transcriptomic and proteomic levels. To delve deeper into the relationship between dysregulated fatty acid metabolism in astrocytes and early stages of AD progression, we employed spatially resolved metabolomics using MALDI 2 Tims-Tof flex imaging mass spectrometry, identifying specific metabolites that change during the early phases of AD. Additionally, our single-cell data analysis from human subjects at the Allen Brain Institute revealed a significant downregulation in fatty acid metabolism during the early stages of AD. These findings underscore that the reprogramming of fatty acid metabolism in astrocytes is a key characteristic of early Alzheimer's disease progression.

Key words: Alzheimer’s disease, astrocyte, transcriptomics, proteomics, metabolism

Serum Metabolome Profiling of Endurance and Resistance Exercise In Healthy Young Men Without Regular Exercise

Pan Xinliang[1*], Yan Yi[1,2]

1 School of Sport Science, Beijing Sport University, Beijing 100084;

2 Laboratory of Sports Stress and Adaptation of General Administration of Sport, Beijing 100084

Purpose: The alterations in the metabolome following exercise at specific intensity can serve as molecular indicators of exercise-induced changes and provide insights into metabolic characteristics and exercise performance assessment. However, there is a lack of research investigating the molecular responses in untrained individuals exercising at the recommended intensities and how these molecular changes may translate into practical applications. Hence, we focused on untrained individuals and implemented a time-resolved sampling approach to comprehensively depict the metabolic profiles following endurance exercise (EE) and resistance exercise (RE), aiming to provide practical recommendations for exercise prescription.

Method: Fifteen male participants with no prior exercise experience were recruited. Prior to the exercise interventions, the participants underwent tests to determine their VO_{2max} and their one-repetition maximum (1-RM) for the squat and bench press. Following the guidelines provided by the American College of Sports Medicine, the treadmill running intensity was set at 75% of their VO_{2max}, and the RE intensity was set at 65% of their 1-RM. The study employed a crossover experimental design, where each participant underwent both endurance exercise and resistance exercise in a randomized order, with a minimum of 7 days between the two sessions. All exercise sessions were conducted in the morning, with the participants in a fasted state. Throughout the study, a total of 150 venous blood samples were collected from each participant at various time points, including pre-exercise, immediate post-exercise, 15th minute, 30th minute, and 60th minute post-exercise. The collected samples were subjected to non-targeted metabolomics analysis using LC-MS/MS.

Results: The study demonstrated that both EE and RE significantly activated and enhanced energy metabolism process, particularly during exercise. Metabolites associated with purine metabolism, including uric acid, xanthine, and 5'-phosphoribosylamine, exhibited a significant increase immediately after exercise and remained elevated up to 60 minutes post-exercise. A significant increase till 60 minutes in oleic was observed immediately after EE, indicating the utilization of lipids as the energy substrates. Our study revealed a sustained decrease in 2-hydroxybutyrate levels after 15 minutes of EE, while in contrast, it exhibited a persistent increase after RE. We also observed a significant increase in cysteine levels following RE.

Conclusion: Multi-time point sampling of metabolomics revealed distinct features in energy metabolism reactions in response to EE and RE. EE is inclined to enhance fatty acid oxidation and lipolysis, while RE predominantly relies on non-fat energy sources, resulting in a relatively minor impact on lipid metabolism.

Key Words: Serum Metabolome; Endurance Exercise; Resistance Exercise; Men

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急性耐力运动后血清代谢组变化及能量消耗相关标志物发现

严翊[1,2], 潘新亮[1]

[1] 北京体育大学运动人体科学学院，北京 100084；

[2] 国家体育总局运动应激适应重点实验室，北京 100084

目的：随着代谢组学在运动领域的应用，为描绘运动促进健康背后的生物网络提供了技术支撑。然而由于运动的特异性和普遍性，机体如何适应运动并获得健康益处的生物学机制尚未完全了解。近些年来，越来越多的证据表明耐力运动能够增加机体能量消耗进而达到肥胖防控的目的，然而在此过程中起调控作用的关键分子还不明了。因此，本研究通过探讨无运动习惯人群急性耐力运动后机体血清代谢组的变化，以期发现耐力运动调节机体代谢的途径、揭示潜在的代谢机制，为运动调控机体代谢提供理论依据；并采用机器学习模型，识别与能量消耗相关的标志性代谢物。

方法：招募无运动习惯、健康男性青年 15 名（身高：177.71±4.43 cm, 体重：71.13±4.11 kg，BMI：22.53±1.14 kg/m^2；平均值±标准差），使用运动心肺功能测试系统（CORTEX MetaLyzer 3B）、采用 Bruce 跑台方案测量受试者最大摄氧量（VO_{2max}）。耐力运动方案采取 75% VO_{2max} 强度在跑步机上连续跑步 1 小时，受试者在至少禁食 8 h 后于每日上午 6:30-9:00 完成跑步，运动过程中，受试者全程佩戴呼吸面罩，测定安静状态和运动中消耗的 O_2 和生成的 CO_2，通过查询呼吸商对应的氧热价，计算运动中的净能量消耗（Energy Expenditure, EE）。分别于安静时、运动后即刻采集静脉血，4℃、3500 r/min 离心取得血清后（n=2×15，组×样本量），采用 LC-MS/MS 进行非靶向代谢组测试。差异代谢物满足条件：①配对样本 t 检验比较组间差异，P＜0.05；②运动后于运动前比较差异倍数（Fold Change, FC），FC＞1.5 或＜0.6。采用 MetaboAnalyst 软件对差异代谢物进行 KEGG 代谢通路富集分析；使用最小绝对值收敛和选择算子算法（Least Absolute Shrinkage and Selection Operator, LASSO），采用留一法进行机器学习模型训练，以系数绝对值筛选 EE 的生物标志物。

结果：(1) 15 名受试者最大摄氧量为 44.8±4.81 ml/kg/min，60 min 连续跑步中氧气消耗量为 31.65±5.23 ml/kg/min，二氧化碳消耗量为 31.64±4.17 ml/kg/min，平均呼吸商为 1.01±0.07，净能量消耗为 579.33±89.65 kcal。(2) LC-MS/MS 平台共检测到 829 个代谢物，差异代谢物 154 个。运动后参与三羧酸循环反应的柠檬酸（P=5.31×10^{-8}, FC=1.7）水平显著增加；进一步对差异代谢物富集分析，不饱和脂肪酸的生物合成（油酸、γ-亚麻酸、二十碳五烯酸）、鞘脂代谢（鞘氨醇-1-磷酸、鞘氨醇）、色氨酸代谢（甲酰苯胺酸，N-甲基色胺）和嘌呤代谢（肌苷、黄嘌呤、鸟嘌呤）等 KEGG 代谢通路被显著富集。(3) 基于 LASSO 分析的机器学习发现，耐力运动中 EE 生物标志物为油酸、牛磺酸脱氧胆酸、鞘氨醇和鹅去氧胆酸。与运动前相比，油酸（P=1.09×10^{-5}, FC=1.6）、鞘氨醇（P=1.85×10^{-4}, FC=2.5）水平显著增加，牛磺脱氧胆酸（P=6.28×10^{-4}, FC=0.3）、鹅去氧胆酸（P=3.63×10^{-4}, FC=0.4）水平显著降低。

结论：（1） 运动后机体代谢组的变化说明，运动中机体不断增加的能量需要主要从三羧酸循环和脂肪酸代谢反应中获取，柠檬酸、油酸和γ-亚麻酸，鞘氨醇以及肌苷、黄嘌呤等代谢物参与机体代谢过程的调节；（2） 机器学习模型识别了能耗相关标志物，油酸和鞘氨醇的氧化增加确定了他们在能量供应中的作用，牛磺脱氧胆酸则表征了随着能量消耗增加血管平滑肌收缩加强。

关键词：血清代谢组; 急性耐力运动; 机器学习; 能量消耗

Developing a synthesis and quantification method for the analysis of α-keto-δ-(NG,NG-dimethylguanidino) valeric acid by using UPLC-MS/MS

Yueyuan Han[1,2], Qianling Xiong[3], Mu Wang[1,2], Yi Li[1,2], Yan Liu[3], Ken Cheng[1,2]

1. Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu, 215123, China

2. University of Liverpool, Liverpool, L69 7ZB, UK

3. Guangdong Provincial Key Laboratory of Food, Nutrition and Health and Department of Nutrition, School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong, 510275, China

∗E-mail: Ken.Cheng@xjtlu.edu.cn

ABSTRACT: Dimethylguanidino valeric acid (DMGV) is a product of the metabolism of asymmetric dimethylarginine (ADMA) by the liver enzyme alanine-glyoxylate aminotransferase 2 (AGXT2)[1]. Untargeted metabolomics-based epidemiological studies have shown that DMGV is an independent biomarker of several metabolic diseases, such as non-alcoholic fatty liver disease, cardiovascular disease and type 2 diabetes[2,3]. In addition, the levels of DMGV in circulation were found to be closely related to lifestyle including dietary intake and physical activity[4]. However, due to the lack of commercial standards for DMGV, current studies are based on relative quantification of DMGV levels by untargeted metabolomics, which lacks precision and absolute quantification. Moreover, previous reports regarding DMGV were conducted in European and American populations, due to the huge difference in genetic backgrounds and lifestyles, its distribution in Chinese remain largely unknown.

So far, we have successfully synthesized the DMGV standard by optimizing the method developed by Poirier et al.[5]. The synthesized DMGV (purity>90%) has been verified using high-resolution mass spectrometry, ^{1}H nuclear magnetic resonance spectroscopy, and HPLC (Figure 1A-D). We have established a quantitative method using liquid chromatography-tandem mass spectrometry (LC-MS) with excellent linearity ($R^2 > 0.99$) within the range of 1.6-200nM, high sensitivity (LOD: 0.4nM, LOQ: 1.6nM), precision (1.1% at LOQ), and accuracy (10% at LOQ). This analytical method has been applied to measure the levels of DMGV in plasma samples from a Chinese cohort (n=626). Females were found to have lower concentration of DMGV than males, suggesting that there is a gender difference in DMGV metabolism (Figure 1E). We will further apply this method to explore the correlations between DMGV levels and the risk of diabetes in Chinese population and investigate its potential molecular mechanism underlying disease progression.

KEY WORDS: DMGV, LC-MS/MS, absolute quantification, disorder of metabolism.

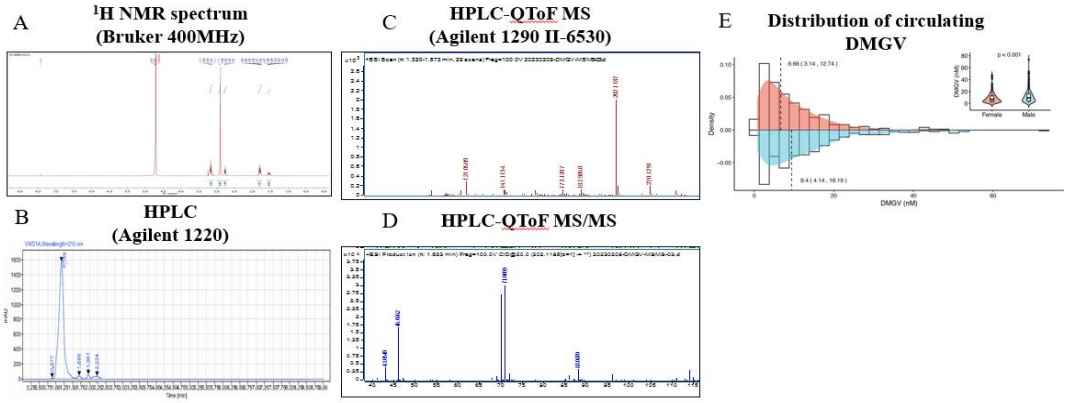

Figure 1. A. ^{1}H nuclear magnetic resonance spectroscopy of DMGV; B. Liquid phase chromatography of DMGV; C. Positive ESI-MS spectrum of DMGV; D. ESI-MS^{2} spectrum of DMGV; E. Distribution of circulating DMGV from a Chinese cohort

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From Integrin Structures to Explore: What Does the Future of Structural Biology Look Like?

Chun-ying Gu[1], Ting-ting Wang[1], Jia-fu Liu[1*]

[1] School of Basic Medical Sciences, Harbin Medical University-Daqing, Daqing, 163319, China

ABSTRACT: Integrins transmit signals between their extracellular ligands and the intracellular cytoskeleton through bidirectional signaling across cell membrane. At the ligand-binding βI domain, the α1/α1'-helix changes from a bent to a straightened α-helical conformation upon integrin headpiece opening. The binding of the cytosolic protein ligands to both the membrane-proximal (MP) and membrane-distal (MD) regions of the integrin cytoplasmic tail (CT) triggers the inside-out signaling. How conformational signals initiated from one end of the integrin are transmitted to the other end remains elusive for structural biology to explore.

We demonstrated that a conserved glycine at the α1/α1' junction is critical for maintaining the bent conformation of the α1/α1'-helix in the resting state. Mutations that facilitate α1/α1'-helix unbending rendered integrin constitutively active. However, mutations that block the α1/α1'-helix unbending abolished soluble ligand binding upon either outside or inside stimuli. Studies also showed that β-integrin CTs have common binding sites for regulatory proteins like talin and kindlin. In contrast, α-integrin CTs are only highly conserved at the MP regions, whereas the MD regions vary substantially both in sequence and length. We examined the role of αIIb, αV and αL integrin CT MD regions in the regulation of integrin activation. We report that the α-integrin CT MD region helps maintain integrin in the resting state and is indispensable in talin- and kindlin-induced integrin conformational change and ligand binding. The proper length and proper amino acids are important for the α-integrin CT MD region to exert its effect on integrin activation.

X-ray, NMR, and EM are the traditional methods of structural biology. However, a deep-learning approach, AF2, brings a serious shock to this field recently. We will have to think about: What Does the Future of Structural Biology Look Like?

Key Words: integrin, structural biology, NMR, X-ray, EM, Af2

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HS−SPME−GC−MS Untargeted Analysis of Normal Rat Organs Ex Vivo: Differential VOC Discrimination and Fingerprint VOC Identification

Yue Liu[1,2], Dianlong Ge[1,3], Yannan Chu[1,3*]

1. Anhui Province Key Laboratory of Medical Physics and Technology, Institute of Health and Medical Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, Anhui, China

2. University of Science and Technology of China, Hefei 230026, Anhui, China

3. Hefei Cancer Hospital, Chinese Academy of Sciences, Hefei 230031, Anhui, China

ABSTRACT: The investigation of volatile organic compounds (VOCs) in human metabolites has been a topic of interest as it holds the potential for the development of non-invasive technologies to screen for organ lesions *in vivo*. However, it remains unclear whether VOCs differ among healthy organs. Consequently, a study was conducted to analyze VOCs in *ex vivo* organ tissues obtained from 16 Wistar rats, comprising twelve different organs. The VOCs released from each organ tissue were detected by headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technique. In the untargeted analysis of 147 chromatographic peaks, the differential volatiles of rat organs were explored based on the Mann-Whitney U test and fold change (FC>2.0) compared with other organs. It was found that there were differential VOCs in seven organs. A discussion on the possible metabolic pathways and related biomarkers of organ differential VOCs was conducted. Based on the orthogonal partial least squares discriminant analysis (OPLS-DA) and receiver operating characteristic curve (ROC), we found that differential VOCs in the liver, cecum, spleen and kidney can be used as the unique identification of the corresponding organ. In this study, differential VOCs of organs in rats were systematically reported for the first time. Profiles of VOCs produced by healthy organs can serve as a reference or baseline that may indicate the presence of disease or abnormalities in the organ's function. Differential VOCs can be used as the fingerprint of organs, and future integration with metabolic research may contribute to the development of healthcare.

KEY WORDS: Rat organ tissue • Volatolomics • Volatile organic compounds • Untargeted analysis • Headspace solid-phase microextraction-gas chromatography-mass spectrometry

GC-MS 非靶向分析方法研究不同培养基条件下肺癌细胞共同的差异性 VOCs

周吉娟
中国科学院合肥物质科学研究院健康所

其他报告

长期运动对血浆代谢组的调控研究及运动效果预测模型的建立

孙昱[1,2]，李晨曦[1,2]，李燕春[3]，梅涛[3]，魏慧慧[1,2]，何子红[4]，陈艳华[1,2]*，再帕尔·阿不力孜[1]*

1.中国民族大学质谱成像与代谢组学国家民委重点实验室，北京 100081

2.中央民族大学生命与环境科学学院生物成像与系统生物学研究中心，北京，100081

3.北京体育大学，北京 100091

4.国家体育总局体育科学研究所，北京 100061

摘要：长期运动可以对机体健康产生持久的影响。代谢组学能反映生物体受刺激或扰动前后所有内源性代谢物的整体及其变化规律[1]，是研究运动对机体代谢调控作用最直接有效的手段[2]，且有望建立基于代谢物表达的运动效果预测模型[3]。本研究建立了基于非靶向和拟靶向的代谢组学分析方法，应用到长期有氧运动血浆代谢组学研究中，并通过代谢物与体成分及最大摄氧量效应量之间的相关性分析，发现与运动效果密切相关的代谢物，建立基于运动前代谢物表达的有氧运动和抗阻运动效果定量预测模型。结果表明，长期有氧运动主要调控谷氨酰胺、谷氨酸代谢通路、三羧酸循环以及与脂肪酸氧化相关的代谢通路等，在发现集和验证集中共有 40 个生物标志物在运动前后发生显著性变化，其中 LPE 18:2、AC10:2、AC18:1 和 3-HIBA 运动前后的变化与 BMI 的改变密切相关；运动前血浆中次黄嘌呤的含量与有氧运动最大耗氧量效应值密切相关。针对其进行定量研究，建立了基于运动前血浆中次黄嘌呤含量、运动前个体的 BFM%和 WHFR 的有氧运动定量预测模型。

综上，本研究旨在通过有氧和抗阻运动对机体血浆的代谢调控作用，发现并验证与运动健康促进相关的代谢类生物标志物，构建有氧运动调控人体代谢的生物网络，并以此建立其运动效果定量预测模型，从而为运动促进健康个性化精准指导提供基础。

关键词：长期有氧运动；代谢组学；身体成分；运动效果

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Integration of microbiomics and metabolomics for screening and validation of antidepressant active ingredients of Atractylodes macrocephala Koidz. based on Chinese medicine “jianpi” theory

Xiaoyi Chen[1,2], Yunhao Zhao[1,2], Zhenning Wu[1,2], Xuemei Qin[1,2,3], Junsheng Tian[1,2,3*]

1. Modern Research Center for Traditional Chinese Medicine of Shanxi University, Taiyuan 030006;

2. Key Laboratory of Effective Substances Research and Utilization in TCM of Shanxi Province, Taiyuan, 030006;

3. Shanxi Key Laboratory of Redevelopment of Famous Local Traditional Chinese Medicines, Taiyuan 030006

Background *Atractylodes macrocephala Koidz.* (AM) is regarded as the first effective medicine for “*jian pi*”, and it can enhance the effect of “*jian pi*” after being stir-fried in the soil. The theory of “*jian pi*” in traditional Chinese medicine is mainly related to gastrointestinal disorders. However, the traditional Chinese medicine theory has not been scientifically verified. **Purpose** Metabolomics technology was utilized to screen for substances exerting "*jian pi*" effects after AM soil frying. Combining microbiomics and fecal metabolomics to elucidate the antidepressant mechanism of AM based on the traditional Chinese medicine "*Jian pi*" theory from the perspective of host-gut flora interaction. **Methods** Differential metabolite analyses of AM, AM fried with wheat bran and AM fried with earth were performed using LC-MS to screen for substances that enhance the spleen-enhancing effects of AM after earth-frying**.** Depressed mouse models were established by fecal transplants and intervened with *atractylenolide II* and paroxetine, and the antidepressant effects of the drugs were evaluated using behavioral tests. freshly expelled mouse feces were collected for 16S rRNA sequencing and metabolomics analysis. **Results** *Atractylenolide II* content was significantly increased after earth frying of AM. Depression-like behavior after CSDS fecal transplantation in mice is significantly improved by 20 $mg \cdot kg^{-1}$ *Atractylenolide II* and paroxetine. Sequencing analysis of the intestinal flora showed that the intestinal flora richness was significantly improved by 20 $mg \cdot kg^{-1}$ *Atractylenolide II*, and the metabolic pathways regulated by the differential flora were mainly enriched in amino acid metabolism. Metabolomics results showed that the metabolic disorders in fecal transplant depressed mice were significantly modulated by 20 $mg \cdot kg^{-1}$ *Atractylenolide II*, and enrichment analysis revealed that it was mainly related to amino acid metabolism. **Conclusion** *Atractylenolide II* is a key substance in the “*jian pi*” effects of AM. The substance exerts its “*jian pi*” effect by regulating the disruption of amino acid metabolism after the interaction between the bacteria and the host, thus producing an antidepressant effect.

Key words: *Atractylodes macrocephala Koidz.*, *Atractylenolide II*, Metabolomics, Microbiome, Host-gut microbiota interactions

High resolution mass spectrometry-based lipidomics reveals the lipid metabolism biomarkers of coronary heart disease and its comorbidities

Zilin Xia[1], Hao Wu [1,2], Zhulin Yang[1], Huiming Li[1], Dabing Ren [1], Hong Zhang [2], Lunzhao Yi [1*]
1. Kunming University of Science and Technology, Kunming, China,650500
2. First People's Hospital of Yunnan Province, Kunming, China,650000
*E-mail: yilunzhao@kust.edu.cn

ABSTRACT: Introduction: Coronary heart disease (CHD) and its comorbidities seriously threaten human health. Disorders of metabolism, especially lipid metabolism, are a common cause of CHD and its comorbidities. However, insufficiency of metabolite annotation and issues in quantitation accuracy occur in conventional tandem mass spectrometry because of the many types of lipid metabolites in biological matrices, large concentration differences, and diversification of isomers. Objectives: (1) To solve the problem of inaccurate annotation in mass spectrometry (MS)-based lipidomics caused by insufficient secondary mass spectra obtained by conventional mass spectrometry. (2) To solve the problem of inaccurate quantification of MS-based lipidomics, which causes difficulty in differentiating lipid isomers by means of extracted ion chromatogram. Methods: Ultra-performance liquid chromatography–high resolution mass spectrometry (UPLC–HRMS) in data-independent acquisition (DIA) mode was applied to collect abundant MS/MS data, which provided valuable information for the annotation of lipid metabolites. For the lipid isomers which could not be completely separated by chromatography, parallel reaction monitoring (PRM) mode was used to separate them from MS/MS. Through multivariate statistical analysis, 80 plasma samples were divided into 4 groups according to CHD and its comorbidities. Results: A total of 223 plasma lipid metabolites were annotated, and 116 of them were identified for their fatty acyl chain composition and location. In addition, 152 lipid metabolites in the plasma of patients with CHD comorbidities were quantitatively analyzed. Multivariate statistical analysis results showed that the metabolism of sphingolipids and glycerophospholipids in patients with related diseases was disordered. Conclusion: In our study, the data obtained through DIA mode significantly promoted the quality and quantity of MS/MS, and PRM scanning mode can accurately quantify the lipid isomers. This study provides a novel insight that helps in high-throughput characterization of the lipid metabolites of plasma. The results of this study also improve our understanding of CHD and its comorbidities, which can provide valuable suggestions for medical intervention.

KEY WORDS: Data-Independent Acquisition, Parallel Response Monitoring, Coronary Heart Disease, Lipidomics, UPLC-HRMS

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The purine metabolite inosine monophosphate accelerates myelopoiesis and acute pancreatitis progression

Yuan Dong[1,2,3], CenYan[1], Yuejie Zhang[1], Yingmei Feng[1,2,*]

1. Department of Science and Development, Beijing Youan hospital, Capital Medical University, Beijing 100069, China;
2. Laboratory of Clinical Medicine, Capital Medica University, Beijing 100069, China;
3. Beijing Institute of Hepatology, Beijing Youan Hospital, Capital Medica University, Beijing 100069, China

ABSTRACT:
Type 2 diabetes mellitus (T2DM) is not only an independent risk factor for acute pancreatitis (AP) but also correlates with AP severity. We previously reported that diabetic db/db mice at the age of 18 weeks old were featured with excessive myeloid cell expansion. In an established murine AP model induced by caerulein, injection of diabetic db/db bone marrow cells (BMCs) aggravated disease severity compared with injectin of wild-type BMCs. In the study, we interrogated the role of metabolites that result from the altered metabolic state in T2DM, and investigated whether the identified metabolites could also modulate the proliferation of myeloid progenitor cells, resulting in myelopoiesis and strengthened inflammation in AP. By FACS analysis, we identified the increased frequency and proliferation of granulocyte/monocyte progenitors (GMPs) in BM of 24-week aged db/db mice than those at 8 weeks old. Using targeted metabolomics, we identifyied an increase in inosine monophosphate (Bird, #125) in FACS-sorted GMP cells of diabetic db/db mice. We demonstrated that IMP treatment stimulated cKit expression, ribosomal S6 activation, GMPs proliferation, and Gr- 1+ granulocyte production *in vitro*. IMP also activated pAkt in non-GMP cells. *In vivo*, administration of IMP-treated BMCs accelerated the severity of AP. This effect was abolished in the presence of a pAkt inhibitor. Accordingly, the results of targeted metabolomics revealed that plasma levels of guanosine monophosphate were significantly higher in diabetic patients with AP. Taken together, these findings provide a potential therapeutic target for the control of vascular complications in diabetes.

KEY WORDS: inosine monophosphate, granulocyte/monocyte progenitors, acute pancreatitis, type 2 diabetes mellitus, targeted metabolomics

Urinary metabolomics identified metabolic disturbance associated with polycystic ovary syndrome

Zhandong Yang[1, 2], Xuzi Cai[3], Zhiyong Xie[1,*], Xuefeng Wang[3,*]

1. School of Pharmaceutical Sciences (Shenzhen), Sun Yat-Sen University, Guangzhou, 510006, China

2. Guangzhou National Laboratory，Guangzhou, 510005, China

3. Department of Obstetrics and Gynecology, The Third Affiliated Hospital of Southern Medical University, Guangzhou, 510000, China

ABSTRACT: Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder. Nevertheless, its accurate mechanisms remain unclear [1, 2]. Metabolomics is a powerful technique to identify small molecules that could be used to discover pathogenesis and therapeutical targets of disease [3,4]. In the present study, a urinary untargeted metabolomics combined with targeted quantification analysis was performed to uncover metabolic disturbance associated with PCOS. A total of thirty-eight metabolites were obtained between PCOS patients and healthy controls, which were mainly involved in lipids (39.5%), organic acids and derivatives (23.7%), and organic oxygen compounds (18.4%). Based on enrichment analysis, fourteen metabolic pathways were found to be perturbed in PCOS, particularly glycerophospholipid metabolism and tryptophan metabolism. Targeted quantification profiling of tryptophan metabolism demonstrated that seven compounds (tryptophan, kynurenine, kynurenic acid, quinolinic acid, xanthurenic acid, 3-hydroxyanthranilic acid and 3-hydroxykynurenine) were up-regulated in PCOS. And these tryptophan-kynurenine metabolites showed significant correlations with PCOS clinical features, such as positively associated with testosterone, free androgen index, and the ratio of luteinizing hormone to follicle stimulating hormone. Thus, this study disclosed urinary metabolome changes associated with PCOS, and might provide new insights into PCOS pathogenesis elucidation and therapeutical target development.

KEY WORDS: Polycystic ovary syndrome; Untargeted metabolomics; Targeted quantification analysis; Glycerophospholipid metabolism; Tryptophan-kynurenine metabolism

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Plasma desmosterol level is positively associated with risk factors of MAFLD in T2DM patients

Shao Hua[1], Yan Huan[1], Wang Yanan[1,2,*]

1.Center for Immunological and Metabolic Diseases, Med-X Institute, The First Affiliated Hospital of
Xi'an Jiaotong University, Xi'an 712000, China;

2.Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China
Giant Structure No.21, Western China Innovation Harbor

*Corresponding author.

E-mail: y_wang@xjtufh.edu.cn

ABSTRACT:

Background

Metabolic-associated fatty liver disease (MAFLD) is one of the most common chronic liver diseases worldwide. MAFLD is a spectrum of liver diseases ranging from fatty liver to non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis and hepatocellular carcinoma. Dysregulation of cholesterol synthesis pathway results in excess cholesterol accumulation in the liver, subsequently leading to hepatic inflammation and injury. In contrast, desmosterol, one of the most important precursor molecules for cholesterol synthesis, has been shown to prevent hepatic steatosis and inflammation. In this study, we aimed to investigate the correlation of plasma cholesterol and desmosterol levels with risk factors of NASH, and search for potential biomarkers for diagnosing NASH.

Methods

The cross-sectional study included 539 T2DM patients and 103 healthy controls, of which 144 subjects were selected for determining plasma cholesterol and desmosterol levels using the method of gas chromatography mass spectrometry. The t-test, Mann–Whitney U-test, and the chi-square test were used to compare differences in continuous variables, or categorical variables, respectively. Logistic regression analysis was performed to determine the independent protective or hazardous factors of T2DM with MAFLD. The receiver operating characteristic curve (ROC) was used to evaluate the ability of plasma desmosterol levels to predict MAFLD.

Results

MAFLD was detected in 278 of 539 patients with T2DM. The results of the demographic and clinical characteristics of the patients showed that significant differences in ALT, AST, FBG, HbA1c, TC, TG, HDL-C, LDL-C between T2DM with or without MAFLD groups. In an in-depth analysis, univariate logistic regression analysis showed that BMI, waist circumference, TG, LDL-C were associated with MAFLD in the subgroups. The results of the ROC analysis showed plasma desmosterol was a relatively better predictor of MAFLD than plasma cholesterol.

Conclusions

Plasma desmosterol level was positively correlated with risk factors of MAFLD in T2DM patients. Our data indicate that desmosterol may serve as a plasma biomarker for MAFLD development in patients with T2DM.

KEY WORDS: desmosterol, MAFLD, T2DM

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应用 ^{1}H NMR 代谢组学筛选乳腺癌转移代谢标志物

黄馨萍[1] 陈婉婷[2] 张咏莉[1] 杨永霞[3*]

(1 广东药科大学基础医学院，2 广东药科大学药学院，3 广东药科大学医药信息工程学院，广东 广州， 510006）

乳腺癌是中国女性中最常见的癌症，而转移是乳腺癌患者死亡的主要原因。乳腺癌倾向于淋巴和血液扩散，导致远端转移和预后不良。有数据显示，转移性乳腺癌患者的 5 年生存率只有 38%。目前，临床已有多种方法应用于乳腺癌转移的诊断，包括：超声，钼靶 X 线检查和磁共振 MRI 等，但这些检查都是基于出现组织形态学上的改变。研究发现，在组织发生明显形态学改变之前，代谢水平上的变化已经发生了，这为乳腺癌的早期转移的预测提供了可能[1,2]。

在本研究中，我们利用 5ng/ml TGF-β1 和 TNF-α诱导乳腺癌 HCC1806 细胞建立细胞转移模型(EMT) [3]。实验发现，诱导的 HCC1806 细胞的转移能力明显增强(图 1 A)。PCA 分析两组细胞的代谢图谱，结果显示经 5ng/ml TGF-β1 和 TNF-α处理前后的 HCC1806 在细胞代谢水平上存在明显差异(图 1 B)。通过筛选二者差异代谢物，发现牛磺酸 VIP 值最高，且具有显著统计变化(图 1 C)。进一步我们收集非转移性和远端转移性乳腺癌患者血清样本，对血清中牛磺酸含量进行 NMR 及 HPLC 定量测定(图 1 D)，发现转移的乳腺癌患者血清中牛磺酸显著降低。因此，以上实验初步表明：牛磺酸与乳腺癌转移密切相关。因此，牛磺酸可能是转移性乳腺癌的重要代谢标志物。

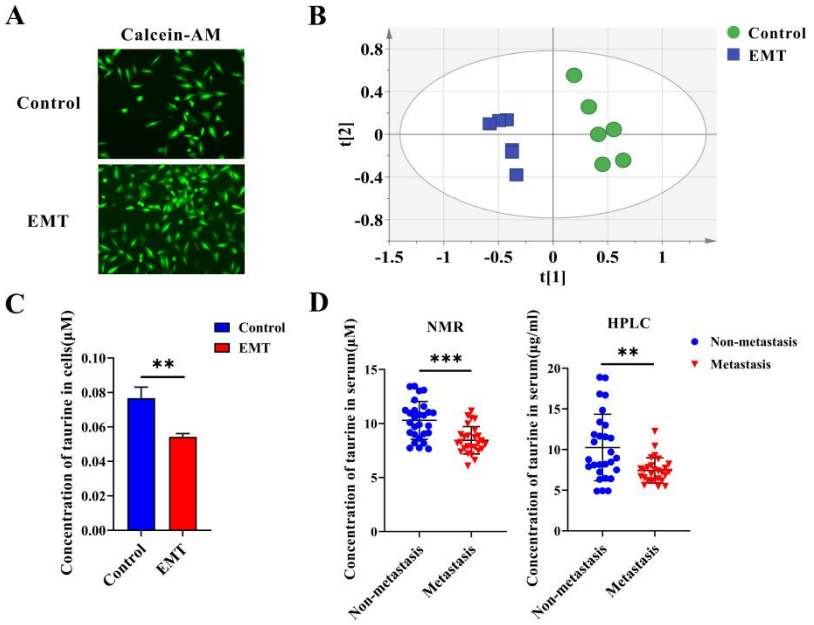

图 1 A：经 5ng/ml TGF-β1 和 TNF-α诱导前后的 HCC1806 细胞的钙黄绿素染色。B：经 5ng/ml TGF-β1 和 TNF-α诱导前后的 HCC1806 细胞 ^{1}H 代谢谱数据的 PCA 得分图 (R^2X=0.958，Q^2=0.787)。C：经 5ng/ml TGF-β1 和 TNF-α诱导前后的两组细胞中牛磺酸的变化。D：非转移性乳腺癌患者和远端转移性乳腺癌患者血清中牛磺酸的变化。(**P＜0.01，***P＜0.001)

Figure 1A: Calcein staining of cells before and after 5ng/ml TGF-β1 and TNF-α induction. B: PCA scores of ^{1}H metabolic profile data of HCC1806 cells before and after 5ng/ml TGF-β1 and TNF-α induction. (R^2X=0.958, Q^2=0.787). C: Changes of taurine in HCC1806 cells before and after 5ng/ml TGF-β1 and TNF-α induction. D: Serum taurine changes in patients with non-metastatic and metastatic breast cancer. (**P < 0.01, ***P < 0.001)

关键词：乳腺癌；转移；牛磺酸；NMR；代谢标志物

***通讯联系人：**杨永霞，电话：020-39352197，E-mail:yangyongxia@gdpu.edu.cn

基于 ^{1}H NMR 代谢组学研究高糖对乳腺癌细胞代谢的调控作用及对线粒体功能的影响

李倩[1] 侯冉冉[2] 黄馨萍[1] 徐满[1] 杨永霞[3*]

(1 广东药科大学基础医学院， 2 广东药科大学药学院，3 广东药科大学医药信息工程学院，广东 广州， 510006）

糖尿病和乳腺癌是两种常见的代谢相关疾病， 不少研究证明两者之间存在一定的关联[1-2]，糖尿病和乳腺癌之间的关联可能是由于多种因素共同作用的结果， 高血糖和胰岛素抵抗是糖尿病的主要特征， 这些因素可能通过促进乳腺癌细胞的增殖和侵袭来促进肿瘤的发展。

研究认为肿瘤和糖尿病均能够引起代谢重编程。那么，糖尿病是否引起乳腺癌的代谢改变，从而促进癌细胞的生长和迁移？在本研究中，我们利用代谢组学技术研究不同浓度葡萄 糖培养的乳腺癌细胞代谢特征， 阐明高糖对癌细胞代谢的影响。图 1A PCA 分析显示无糖、 低糖和高糖环境培养的MDA-MB-231 细胞代谢区分明显，热图及聚类分析（图 1B）给出三 组的代谢差异物。我们发现随着培养基糖浓度的升高，MDA-MB-231 细胞内的糖原含量显 著变化，其在糖处理的两组中 VIP 值均为最高。 因此，糖原可能是糖尿病合并乳腺癌与单 纯乳腺癌之间非常关键的差异代谢物。另外我们还发现这三组不同糖浓度的细胞之间，能量代谢的相关代谢物琥珀酸、柠檬酸、乳酸及丙酮酸也存在差异， 表明高糖引起癌细胞能量代谢发生 变化。进一步对不同糖浓 度的 MDA-MB-231 细胞 进行了线粒体膜电位的检 测（图 1C），结果发现随 着糖浓度的升高，线粒体 膜电位增加明显，说明细 胞外糖浓度可能影响细胞 内线粒体的活性，从而导 致能量代谢发生改变。

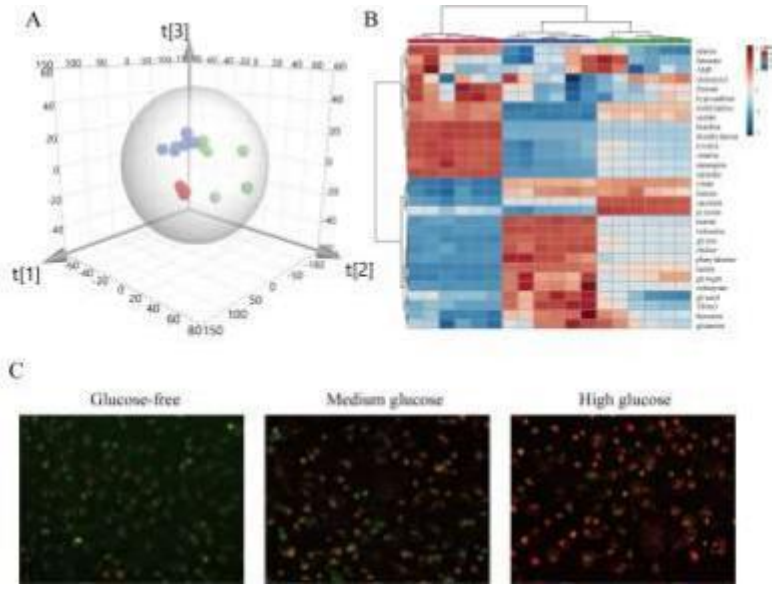

图 1A 三种糖浓度培养的 MDA-MB-231 细胞的 PCA 分析模型图。 ●0.0g/L ●2.25g/L● 4.5g/L 。B:三组细胞代谢物热图及聚类分析图。 C:三组细胞的线粒体膜电位检测。

关键词： 糖尿病合并乳腺癌、 1H-NMR、代谢

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*通讯联系人： 杨永霞，电话： 020-39352197，E-mail:yangyongxia@gdpu.edu.cn

基于 ^{1}H NMR 代谢组学筛选乳腺癌分子分型相关代谢标志物

徐满[1] 林宇凤[2] 李伟萍[2] 侯冉冉[3] 杨永霞[4*]

(1 广东药科大学基础医学院，2 广东药科大学附属第一医院乳腺科，3 广东药科大学药学院，4 广东药科大学医药信息工程学院，广东 广州， 510006）

乳腺癌是最常见的恶性肿瘤之一，其作为一种异质性疾病，四种分子分型具有不同的分子特征，临床上治疗方案、疗效及预后也与分子分型有关。肿瘤的发生和发展伴随代谢重编程[1]，乳腺癌不同分子分型是否具有不同的代谢特征？本文采用基于 ^{1}H NMR 代谢组学方法研究乳腺癌不同分子分型的代谢特征，筛选表征不同分子分型的代谢标志物。

本研究纳入 55 例健康体检者为对照组，81 例未进行任何治疗的乳腺癌患者为病例组，其中 Luminal A 型 14 例，Luminal B 型 46 例，Her2 过表达型 11 例，三阴性型 10 例。收集各组空腹血清样本，应用 ^{1}H NMR 技术采集每组样本的血清代谢谱，运用模式识别分析方法对其代谢数据进行分析。

OPLS-DA 分析发现四种分子分型乳腺癌患者组与健康对照组区分明显，结合代谢物 VIP 值及 T 检验统计分析结果，筛选出四种分子分型对应的四组血清代谢标志物。图 1A 表明四种分子分型中共同变化的代谢物有胆碱、磷脂酰胆碱/甘油磷脂酰胆碱。内分泌型即：Luminal A 型和 Luminal B 型共同变化的代谢物有胆碱、磷脂酰胆碱/甘油磷脂酰胆碱、柠檬酸、肌酸、磷酸肌酸、1-甲基组氨酸。其中 Luminal A 型独有的代谢物变化有甲硫氨酸、甘氨酸、谷氨酸、谷氨酰胺、苏氨酸；Luminal B 型独有的变化是氧化三甲胺\牛磺酸、醋酸盐、乳酸、葡萄糖、牛磺酸。非内分泌型即 Her2 过表达型和三阴性型共同变化的代谢物有胆碱、磷脂酰胆碱/甘油磷脂酰胆碱、乳酸。其中 Her2 过表达型独有的差异代谢物有磷酸肌酸、柠檬酸、1-甲基组氨酸、肌酸；三阴性型独有的变化是谷氨酰胺、苏氨酸、葡萄糖、牛磺酸。聚类分析直观展示了这四种分子分型间的代谢物差异（图 1B）。对以上四种分子分型代谢标志物进行 ROC 曲线分析（图 1C），发现四组分子分型相关的血清代谢标志物对分子分型分类预测效果良好。以上结果表明，乳腺癌不同分子分型血清代谢特征存在明显差异，其差异代谢物对四种分子分型具有良好的区分预测能力。

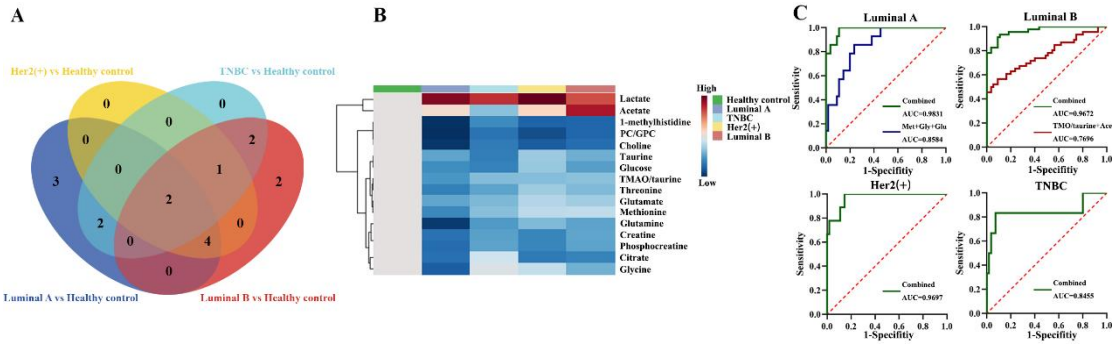

图 1A：四种分子分型代谢标志物 Venn 图；B：差异代谢物的聚类热图；C：四种分子分型代谢标志物 ROC 曲线分析。

Figure 1A: Venn maps of molecular subtypes biomarkers; B: Cluster heat map of differential metabolites; C: ROC curves for combined biomarkers.

关键词：乳腺癌；分子分型；代谢标志物；NMR

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***通讯联系人：**杨永霞，电话：020-39352197，E-mail:yangyongxia@gdpu.edu.cn

柴胡疏肝散（CSS）改善围绝经期症状的药效物质探寻及其作用机制研究

李婷[1]，陈学勤[*2]，吴彩胜[*1]

1 厦门大学药学院，厦门市翔安区翔安南路，361102；2 厦门大学附属第一医院，厦门市中华街道 361003

围绝经期是女性的生理过渡期，主要表现为激素波动水平大，容易出现潮热、夜汗、骨质疏松等症状，严重情况下会有心血管以及糖尿病、抑郁症变化等出现。根据柴胡疏肝散（CSS）记载的“疏肝解郁”功效以及中医“肝郁气滞”的理论，CSS 在临床上可以用于慢性肝炎、胃炎、肋间神经痛等。本研究通过围绝经期 SD 大鼠造模实验，用 CSS 提取液进行灌胃干预，结果证明 CSS 可明显逆转模型组大鼠 TMAO 升高、糖皮质激素水平升高和雌激素水平下降的趋势。进一步药理学研究表明 CSS 还能够有效调节 HPA 轴激素水平，改善血浆和肝脏甘油三酯和总胆固醇的异常升高，上调大鼠体内 IL-10 表达，通过网络药理学发现 CSS 高暴露成分（甘草素、异樱花素、橙皮素）均与 FMO3 酶具有良好的亲和力。研究结果表明 CSS 有可能是通过下调 TMAO 的表达，逆转围绝经期大鼠雌激素的下降和糖皮质激素的上升趋势，从而缓解围绝经期大鼠症状。

关键词：柴胡疏肝散；围绝经期；类固醇激素；TMAO

基于肠道代谢组学探讨朱砂删减对龟龄集抗衰老药效的影响

李洋，马瑜爽，武兴康*

（山西大学中医药现代研究中心，山西省太原市 030060）

目的：龟龄集是益寿强身内服药之首，具有强身补脑、固肾补气、增进食欲之功效。龟龄集中有一味药为朱砂，该药有一定的毒性，不宜大量服用，也不宜少量久服。然而，龟龄集的临床应用，通常需要较长服用周期才能达到预期治疗效果。因而，本研究基于肠道代谢组学探讨朱砂删减对龟龄集抗衰老药效的影响，以期为龟龄集中朱砂用药的安全性和合理性提供依据。**方法：**雄性 SD 大鼠皮下注射 D-半乳糖诱导衰老模型，采用动物行为学实验如旷场实验、新物体识别实验、转棒实验、Elisa 法、HE 染色、MASSON 染色等方法研究龟龄集全方组和龟龄集去朱砂减方组对缓解大鼠脑衰老、骨衰老、皮肤衰老、肌肉衰老的药效作用。使用 UPLC-Triple TOF 5600 仪器对大鼠的肠道内容物进行代谢组学分析，通过 HMDB 数据库、MSDAIL 软件、SCIXEOS 和 SIMCA 软件分析代谢组学数据，寻找肠道差异代谢物，进一步探索龟龄集缓解大鼠衰老的作用机制。**结果：**龟龄集能够改善 D-半乳糖致大鼠的脑衰老和骨衰老，删减朱砂对其无影响。龟龄集缓解 D-半乳糖致大鼠的皮肤衰老和肌肉衰老，其中朱砂对该作用有一定的贡献，其机制为朱砂促进胶原纤维和肌纤维的生成。进一步，肠道代谢组学发现，龟龄集和龟龄集去朱砂的差异药效相关的 9 个差异代谢物，包含醛固酮、肾上腺甾酮、顺式乌头酸等，涉及类固醇激素生物合成代谢、初级胆汁酸生物合成、牛磺酸和次黄嘌呤代谢等 6 条代谢通路。**结论：**朱砂删减对龟龄集的抗脑衰老和骨衰老的药效无贡献，在抗皮肤衰老和肌肉衰老中有一定的贡献。

关键词：龟龄集、朱砂、肌肉衰老、皮肤衰老、代谢组学

***通讯作者：**武兴康，Tel: 0351-7019178，E-mail: wuxingkang@sxu.edu.cn

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Unlocking the hidden potential: enhancing the utilization of stems and leaves through metabolite analysis and toxicity assessment of various parts of *Aconitum carmichaelii*

Yinlin Zhou[1], Can Qu[1], Tianzhe Chu[1],Cheng Peng, Yuzhu Tan[1,*]

[1] State Key Laboratory of Southwestern Chinese Medicine Resources, Pharmacy College, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

*Corresponding authors. E-mail address: tanyuzhu@cdutcm.edu.cn (Y. Z. Tan)

ABSTRACT: In this study, UHPLC-Q-Orbitrap MS/MS-based plant metabolomics was employed to analyze metabolites of the different parts of *Aconitum carmichaelii*. The cardiotoxicity and hepatotoxicity of the extracts from different parts of *Aconitum carmichaelii* were also investigated using zebrafish as animal model. Toxicity markers were subsequently identified by correlating toxicity with metabolites. The results revealed that a total of 113 alkaloids were identified from the extracts of various parts of *Aconitum carmichaelii*, with 64 different metabolites in stems and leaves compared to daughter root (Fuzi), and 21 different metabolites in stems and leaves compared to mother root (Wutou). The content of aporphine alkaloids in the stems and leaves of *Aconitum carmichaelii* is higher than that in the medicinal parts, while the content of the diester-diterpenoid alkaloids is lower. Additionally, the medicinal parts of *Aconitum carmichaelii* exhibited cardiotoxicity and hepatotoxicity, while the stems and leaves have no obvious toxicity. Finally, through correlation analysis and animal experimental verification, mesaconitine, deoxyaconitine, and hypaconitine were used as toxicity markers. In conclusion, given the low toxicity of the stems and leaves and the potential efficacy of aporphine alkaloids, the stems and leaves of *Aconitum carmichaelii* hold promise as a valuable medicinal resource warranting further development.

KEY WORDS: *Aconitum carmichaelii*; stems and leaves; toxicity markers; aporphine alkaloids

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Comprehensive Metabolomic Characterization of Atrial fibrillation

Chengcan Lu

Department of Pharmacy, The Affiliated Jiangning Hospital with Nanjing Medical University, Nanjing，Jiangsu 211100，China

ABSTRACT:

BACKGROUND The pathogenesis and diagnostic biomarkers for diseases can be discovered by metabolomic profiling of human fluids. Specific characterization of atrial fibrillation (AF) subtypes with metabolomics, especially in the early stages, may facilitate effective and targeted treatment.

OBJECTIVES To assess the diagnostic value of metabolomics-based biomarkers in different types of AF by investigating disturbed metabolic pathways.

METHODS A cohort of 363 patients was enrolled, including a discovery set and a validation set. Patients underwent electrocardiogram for suspected AF. Groups were divided as follows: healthy individuals (Control), suspected AF (Sus-AF), first diagnosed AF (Fir-AF), paroxysmal AF (Par-AF), persistent AF (Per-AF), and AF causing cardiogenic ischemic stroke (Car-AF). Serum metabolomic profiles were determined by liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and gas chromatography-mass spectrometry (GC-MS). Metabolomic variables were analyzed in combination with clinical information to identify relevant diagnostic biomarkers.

RESULTS 16 cross-comparisons were performed to characterize metabolic disturbances. We focused on comparisons of all types of AF (All-AFs) and Car-AF versus Control, All-AFs versus Car-AF, Par-AF versus Control, and Par-AF versus Per-AF. 117 and 96 metabolites were identified by GC/MS and LC/MS, respectively. The essential altered metabolic pathways during AF progression included D-glutamine and D-glutamate metabolism, glycerophospholipid metabolism, etc. For differential diagnosis, specific metabolomics-based biomarkers provided areas under the curve of 0.8237 to 0.9890 in the discovery phase and predictive values of 78.8% to 90.2% in the validation phase.

CONCLUSIONS Serum metabolomics is powerful for characterizing metabolic disturbances. Differences in small-molecule metabolites may reflect underlying AF and serve as biomarkers for AF early-onset, differential diagnosis, and progression.

KEY WORDS: atrial fibrillation, metabolomics, diagnostic model, risk factors, biomarker.

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水稻全生育期代谢调控网络

杨陈坤[1] 罗杰[1,2]*
1 海南大学三亚南繁研究院，三亚，572025
2 崖州湾国家实验室，三亚，572025
*通信作者： E-mail: luojie@yzwlab.cn

植物作为固着生物，在生长发育过程中产生大量的代谢产物，以适应不断变化的环境和各种胁迫。水稻（*Oryza sativa* L.）是重要的粮食作物和单子叶模式植物，目前水稻代谢组的研究大多集中在对特定组织的分析上，对于水稻整个生命周期的代谢物丰度的动态变化研究及其应用尚未报道。本研究使用籼稻品种珍汕 97 和明恢 63 覆盖整个生命周期不同组织的样品，进行广泛靶向代谢组学检测，共有 825 种代谢物被注释并定量分析。结合代谢组和同一时期的转录组数据构建了水稻代谢调控网络（Rice Metabolic Regulation Network, RMRN）。利用这个数据集，该研究成功使用两种独立的策略鉴定了新的代谢途径，分别是利用已知的转录因子作为诱饵来筛选木质素代谢调控的新网络，以及根据组织特异性无偏见地识别新的甘油磷脂代谢调控因子。这项研究表明，通过对物种整个生命周期的代谢组和转录组数据联合分析可以为揭示重要表型或代谢性状的调控提供新的思路和工具。这不仅对水稻基础研究和育种实践具有重要意义，其大数据资源也将为水稻营养品质改良和抗性品种培育提供新的方案，同时该研究策略也可作为其他作物研究的蓝图。

关键词：水稻，代谢组，转录组，转录因子，共表达，甘油磷脂

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基于 NMR 谱峰拟合的白茶代谢组学数据分析

黄羽菲[1]，王梓鉴[1]，郭阳[1]，鲁菲菲[2]，董继扬[1]，黄彪[2,*]，崔晓红[1,*]

1. 厦门大学电子科学系，中国厦门
2. 福建省农业科学院农产品质量标准与检测技术研究所，中国福州

基于 NMR 的代谢组学可以提供稳健的可重复指纹图谱，从而对生物样品进行相对甚至绝对定量[错误!未找到引用源。]。然而，由于生物样品多包含大量的代谢物，其一维 NMR 谱图存在显著的谱峰重叠，从而导致无法对其进行简单、直观的代谢物信息提取[错误!未找到引用源。]。因此，对于生物 NMR 谱处理，主要有两种方法，一种是靶向分析，该方法对每种代谢物通过特征峰进行鉴别与定量。另一种是分段积分方法，通过采用固定的化学位移间隔，对一维 NMR 谱图进行分段积分从而进行谱图信息的分割和提取，之后再结合多元统计分析方法识别感兴趣的光谱区域。然而，由于样品间的 pH 值和离子浓度的差异会导致相同化合物在不同样品谱中的谱峰漂移，再加上 NMR 谱本身存在的谱峰重叠问题，当利用上述两种谱图处理方法时会出现随之而来的谱峰归属和代谢物定量的不可靠和不准确，为之后的分析带来严重影响。

本文提出一种基于谱峰拟合的 NMR 数据处理方法。首先，对于可归属的代谢物，通过其标准谱得到谱峰的化学位移、线宽以及谱峰面积等关键信息。随后，基于同一代谢物中峰之间面积比不变的原则和谱峰的裂分峰形，将标准谱与样品谱进行匹配，个别谱峰无法对齐的可对标准谱中该峰的化学位移进行微调，匹配好后即可根据标准谱对样品谱图进行拟合。对于高于噪声水平的无法归属的谱峰，则逐个利用洛伦兹线型进行单峰手动拟合，并归为未知峰集。该方法由于使用已知单一化合物的标准谱和未知单峰对样品谱峰进行拟合，通过保持代谢物谱峰面积比不变微调单个谱峰化学位移可有效解决谱峰随机漂移问题。同时，由于采用代谢物谱峰整体拟合和未知峰单峰拟合的方式从而很大程度上减小了谱峰重叠的影响。我们以不同储藏年份（1 年、3 年和 7 年）的三种白茶（银针、寿眉、牡丹）为例对该方法的可行性进行了检验，共归属出了胆碱、儿茶素、茶氨酸等 22 种代谢物，拟合得到了 219 个未知峰。通过 ANOVA 方差分析、单变量分析、多变量分析等统计分析方法将所提峰拟合策略与传统 NMR 数据分析方法进行对比，其较高的相对标准偏差 RSD 值和 Q2 值（表征模型预测能力）表明峰拟合策略可以有效避免谱峰漂移以及重叠对分析结果的影响，并且可以捕获更多的差异特征信息。基于该方法得到的代谢物信息对三种不同年份的白茶分别建立储存年份回归预测模型，预测结果如图 1 所示。所建立的各模型预测误差范围为 0.17±0.17 至 0.32±0.26，表明可有效预测白茶存储年份。

关键词：NMR；代谢组学；峰拟合；白茶；储存年份预测

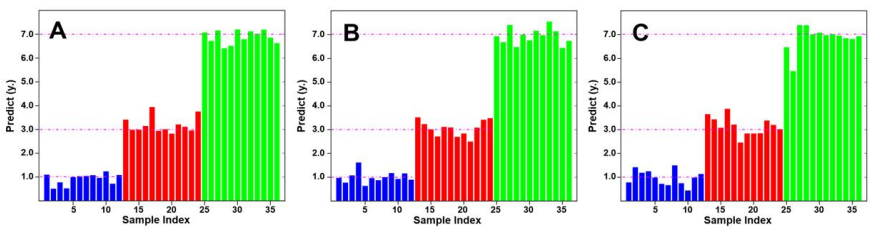

图 1. 不同品种白茶的存储年份预测。(A) 牡丹; (B) 寿眉; (C) 银针.

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质谱代谢组学揭示 C-异戊二烯香豆素代谢与活性的规律

程燕[1]，颜冬梅[1]，朱卫丰[1]，李飞[2,*]

1. 江西中医药大学院士工作站
2. 四川大学华西医院疾病相关分子网络前沿科学中心

C-异戊二烯香豆素（C-PYCs）是一类具有相似结构和多种生物活性的化合物，广泛分布于药用植物中，例如枳壳。枳壳是一种具有已用于治疗消化不良、肥胖、高血压、结石和抑郁症的中药[1-4]。迄今为止，C-PYCs 在体内的代谢特征以及代谢和生物活性之间的关系仍不清楚。本研究采用超高效色谱电喷雾电离四极杆飞行时间基于质谱的代谢组学（UPLC-ESI- QTOF-MS），首先确定在枳壳中的三种 C-PYCs meranzin 水合物 (MH)、isomeranzin (ISM)和 meranzin (MER)的代谢特征，总共鉴定出 52 种新的代谢物。这些代谢物中，10 个来自 MH，22 个来自 ISM，20 个来自来自 MER。这些 C-PYCs 的主要代谢途径是羟基化、脱氢、去甲基化，以及与半胱氨酸、N-乙酰半胱氨酸和葡萄糖醛酸结合。MH 代谢的发生率远低于 ISM 和 MER，在 MLM 中仅为 27.1%以及在 HLM 中为 8.7%。研究发现半胱氨酸、乙酰半胱氨酸、葡萄糖醛酸和羟基化-葡萄糖醛酸络合物是 C-PYCs 的主要 II 期代谢产物，其中葡萄糖醛酸可以与多种有害物质结合发挥解毒作用，半胱氨酸可参与肝脏磷脂代谢和细胞减少。因此，我们推测结合代谢物是 C-PYCs 形成的具有多种生物活性代谢物。此外，重组细胞色素 P450 (CYP) 筛选表明 CYP1A1、CYP 2B6、CYP 3A4 和 CYP 3A5 是参与代谢物形成的主要代谢酶。进一步的生物活性测定表明，这三种 C-PYC 均表现出抗炎活性，但是 ISM 和 MER 的效果略高于 MH，同时显着降低巨噬细胞 RAW264.7 中脂多糖（LPS）诱导细胞炎症因子的转录水平[5]。综上所述，三种 C-PYCs 的代谢特征表明，异戊二烯基链可能影响 C-PYCs 的代谢和生物活性。

关键词: 代谢组学，C-异戊二烯香豆素，质谱分析，抗炎

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A comprehensive strategy for prediction and quality evaluation of standardized planting herbs based on plant metabolomics coupled with extreme learning machine: Astragali Radix as an example

Chenyue Li[1], Yangbin Lv[1], Wei Gao[1], Keji Mao[2], Weiguang Shan[1], Shengqiang Tong[1], Xiaolin Xing[3], Feijun Qiu[3], Zhengyan Xia[4,*], Chu Chu[1,*]

1. College of Pharmaceutical Science, Zhejiang University of Technology, 310000, China; Chenyueli0119@163.com; lyb0127@126.com; 2722650919@qq.com; swg@zjut.edu.cn; sqtong@zjut.edu.cn; chuchu@zjut.edu.cn

2. College of Computer Science and Technology, Zhejiang University of Technology, 310000, China; maokeji@zjut.edu.cn

3. Zhejiang Xinguang Pharmaceutical Co., Ltd., 312000, China; 739578021@qq.com; 906469949@qq.com

4. School of Medicine, Zhejiang University City College, 310000, China; xiazy@zucc.edu.cn

ABSTRACT: Standardizing the planting process is an effective way to control the quality stability of plant resource with food/pharmaceutical uses, which are susceptible to external environmental factors (*e.g.* moisture, soil, etc.)[1]. However, how to scientifically and comprehensively assess the effects of standardized planting on plant quality and quickly determine unknown samples has not been addressed. In this study, an efficient strategy using UHPLC-ESI-Q-TOF-MS based on plant metabolomics combined with extreme learning machine has been developed for the efficient distinguishing and predicting Astragali Radix (AR) after standardized planting. Moreover, a comprehensive multi-index scoring method has been developed for the comprehensive evaluation of the quality of AR. The results confirmed that AR after standardized planting was significantly differentiated, in which the content of 43 differential metabolites was more stable. An extreme learning machine model (ELM) was established based on LC-MS data, and the accuracy could reach more than 90% in predicting unknown planting samples. As expected, higher total scores which indicating a much better quality were obtained after standardized planting of AR. This is the first report describing the change in chemical profile and quality evaluation of standardized planting AR. A dual system for evaluating the impact of standardized planting on the quality of plant resources was established, which will significantly contribute to innovation in the quality evaluation of medicinal herbs with supporting the selection of optimal planting conditions.

KEY WORDS: Astragali Radix (AR), extreme learning machine (ELM), plant metabolomics, quality evaluation, standardized planting.

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Study on the mechanism of Chinese classical formula Qingxin Lianzi Yin Decoction on Diabetic nephropathy based on metabolomics and network pharmacology

Wenya Gao,Yanyan Zhou, Xiaolu Wei, Hongjie Wang, Baolin Bian, Nan Si*, Haiyu Zhao*
Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

ABSTRACT: Diabetic nephropathy (DN) was one of the most significant microvascular complications associated with diabetes mellitus. Qingxin Lianzi Yin Decoction (QXLZY) was a traditional Chinese classical formula, suitable for chronic urinary system diseases, and had good clinical efficacy in neurasthenia and early DN. This study aimed to establish the index component content determination method of QXLZY and explore the mechanism of QXLZY on DN by metabolomics and network pharmacology studies. *Methods:* The content determination methods of QXLZY were established with calycosin-7-*O*-β-D-glucoside, acteoside, baicalin and glycyrrhizic acid as index components. *Db/db* mice were used to observe the biochemical indices and histopathological changes in the kidney to evaluate the pharmacological effects of QXLZY on DN. Untargeted metabolomics studies were performed to investigate by UHPLC-LTQ-Orbitrap MS on urine, serum, and kidney samples. The key targets and pathways were analyzed by network pharmacology. For the pathways enriched by untargeted metabolomics, targeted metabolomics by UHPLC-QQQ-MS/MS was performed in urine, serum, and kidney samples for validation. A "compound-reaction-enzyme-gene" interaction network was constructed. The effect of QXLZY on the improvement in DN from the perspective of *in vivo* metabolism of small molecules was described. *Results:* A method of the simultaneous determination of multiple index components in QXLZY was established, which passed the comprehensive methodological verification. It was simple, feasible, and scientific. QXLZY could improve the degree of histopathological damage to the kidney and remarkably ameliorate the level of urinary microalbumin/creatinine ratio. The pathways related to amino acid metabolism were focused by untargeted metabolomics in *db/db* mice, suggesting metabolic dysfunction in this pathway. Treatment with QXLZY could reverse metabolite abnormalities, and influence the energy metabolism and amino acid metabolism pathways. Through network pharmacology analysis, the effect pathways were related to lipid and amino acid metabolism and signal transduction. It affected the endocrine system and immune system. *Db/db* mice were screened for a series of endogenous metabolites. Amino acid metabolism was disturbed in *db/db* mice, which could be callback by QXLZY, such as quinolinic acid, arginine, and asparagine. This is the first attempt to elucidate the mechanism of the effect of QXLZY on DN using metabolomics and network pharmacology. *Conclusion:* In conclusion, this research provided useful information and evidence in support of the clinical use of QXLZY for the treatment of DN.

KEY WORDS: Qingxin Lianzi Yin Decoction, Diabetic nephropathy, Metabolomics, Network pharmacology.

Dietary synbiotic ameliorates constipation through the modulation of gut microbiota and its metabolic function

Hui-Hui Su[1], Ping-Jun Zhang[1], Jun-Sheng Huang[1], Zhan-Dong Yang[2,*]

1. Institute of Biological and Medical Engineering, Guangdong Academy of Sciences, Guangzhou, 510316, China

2. Guangzhou National Laboratory, Guangzhou International Bio Island, Guangzhou 510005, China

ABSTRACT: The purpose of this study is to investigate the mitigatory effect of a novel synbiotic (SBT) on constipation from the perspective of gut microbiome and metabolome. Here, intake of SBT effectively attenuated diphenoxylate induced constipation, recuperated colonic epithelial integrity and increased serum levels of gastrointestinal excitatory neurotransmitters (P substance, vasoactive intestinal peptide, motilin, gastrin and serotonin). 16S rRNA sequencing showed that SBT intake rehabilitated the composition and functionality of gut microbiota. Relative abundances of short-chain fatty acids (SCFAs)-producing bacteria including Lactobacillus, Faecalibaculum and Bifidobacterium were elevated by administration of SBT. The gas chromatography-mass spectrometry analysis confirmed that fecal concentrations of propionate and butyrate were significantly increased in the rats intervened with SBT. In addition, SBT ingestion reduced the relative levels of opportunistic pathogens, such as Oscillibacter, Parasutterella and Parabacteroides. Microbial functional prediction showed that the relative abundances of lipopolysaccharide (LPS) biosynthesis and arachidonic acid metabolism were downregulated with SBT administration, which were in accordance with the serum metabolomics results. Furthermore, serum levels of LPS, tumour necrosis factor alpha and interleukin 6 were significantly decreased, indicating that SBT supplementation suppressed inflammatory responses. Therefore, this study demonstrated that consumption of SBT ameliorated constipation possibly by regulating gut microbiota, promoting the SCFAs production and inhibiting inflammatory responses in rats. Our study also indicated that SBT may provide a novel alternative strategy for the treatment of constipation clinically in future.

KEY WORDS: Constipation, Synbiotic, Gut microbiota, Short-chain fatty acids, Inflammatory response, Metabolomics

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羊草对不同浓度盐碱胁迫的代谢响应差异

闫阁 [1], 穆春生 [1,*]
工作单位: 东北师范大学生命科学学院草地科学研究所
邮编: 130024
Email 地址: Yang674@nenu.edu.cn（闫阁）
Mucs821@nenu.edu.cn（穆春生）

摘要：为探究草地多年生植物对不同浓度盐碱胁迫的代谢响应差异，采用气相色谱质谱技术（GC-MS）对根茎型多年生耐盐碱羊草（*Leymus chinensis*）的代谢组进行测定。结果显示羊草通过积累小分子渗透溶质来降低胁迫伤害，尽管积累的溶质种类因胁迫类型和浓度梯度而异；在低浓度盐胁迫下，可溶性糖、氨基酸和甜菜碱含量显著增加，甘氨酸和脯氨酸为显著差异代谢物，甘氨酸、丝氨酸和苏氨酸代谢、乙醛酸和二羧酸代谢受到显著影响；在高浓度盐胁迫下，氨基酸、甜菜碱和有机酸含量显著增加，柠檬酸、异柠檬酸和乳酸 3 种有机酸，以及乙醇胺和甘油为显著差异代谢物，TCA 循环、乙醛酸和二羧酸代谢受到显著影响；在低浓度碱胁迫下，可溶性糖、氨基酸、甜菜碱和有机酸含量均显著增加，葡萄糖胺、异麦芽糖、乳果糖和肌醇 4 种可溶性糖，以及磷酸为显著差异代谢物，丁酸代谢、丙氨酸，天冬氨酸和谷氨酸代谢受到显著影响；在高浓度碱胁迫下，有机酸和甜菜碱含量显著增加，奎宁酸、柠康酸、柠檬酸、异柠檬酸、衣康酸、苹果酸和丙二酸 7 种有机酸，以及甘油为显著差异代谢物，乙醛酸和二羧酸代谢、TCA 循环和 C5-支链二元酸代谢受到显著影响。上述结果表明，羊草不但响应盐胁迫和碱胁迫的代谢物不同，而且在响应同种胁迫不同浓度时，也会采取不同的代谢响应对策；有机酸不但在响应碱胁迫时发挥重要作用，也是响应高浓度盐胁迫的重要物质；甜菜碱在羊草响应不同浓度的盐和碱胁迫中均发挥了重要作用；参与能量代谢的 TCA 循环和乙醛酸与二羧酸代谢在重度盐碱胁迫下均受到显著影响，说明能量供应是羊草响应重度盐碱胁迫的主要途径之一。

关键词: 可溶性糖、氨基酸、甜菜碱、有机酸、能量供应