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深圳医学科学院
Shenzhen Medical Academy of Research and Translation

深圳清華大學研究院
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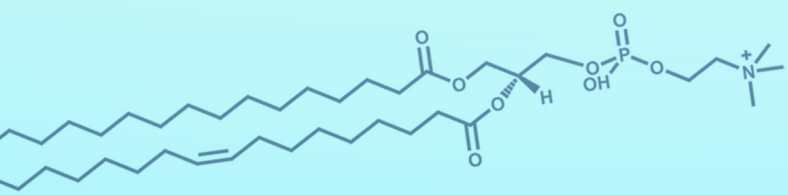
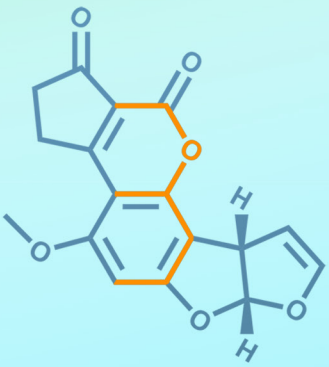
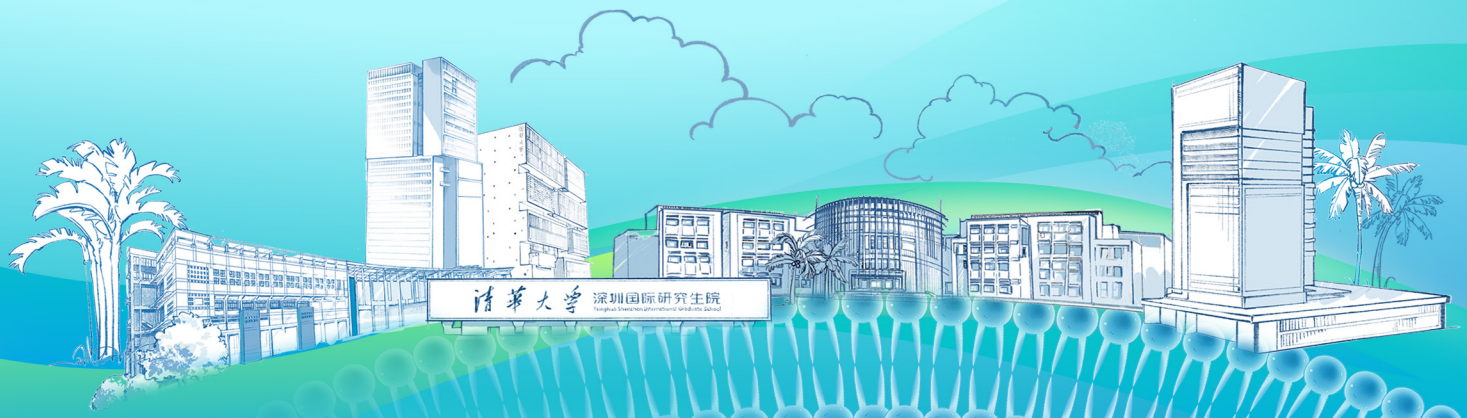
INTERNATIONAL LIPIDOMICS SOCIETY

2024 International Lipidomics Society Conference & SMART Symposium on Structural Lipidomics

October 24th-27th, 2024







AbstractBook

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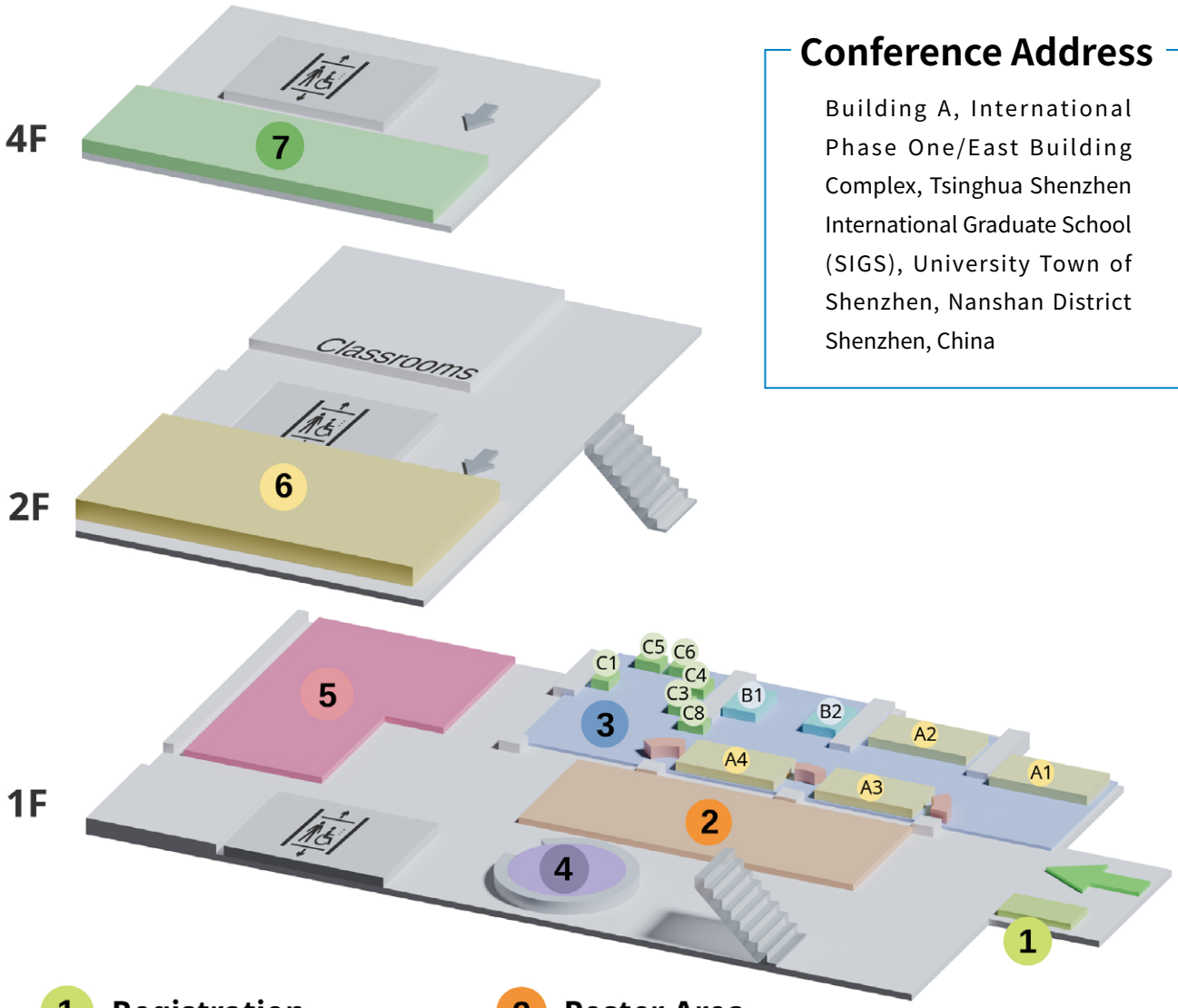
Conference Address

Building A, International Phase One/East Building Complex, Tsinghua Shenzhen International Graduate School (SIGS), University Town of Shenzhen, Nanshan District Shenzhen, China



1 Registration

2 Poster Area

3 Coffee Break & Exhibition Area

A1	BRUKER	A2	Waters™	A3	ThermoFisher SCIENTIFIC	A4	PURSPEC
B1	Create 科瑞恩特	B2	SCIEX The power of precision	C1	METWARE® 迈维代谢	C3	Agilent Trusted Answers
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4 Demo Zone

5 Lunch Seminar Zone

6 Lecture Hall

7 Dinner Area

Note

Plenary Talk: 45 min with Q&A
Talk: 20 min with Q&A
Poster session: 1:00 pm – 2:00 pm on Oct 25 and 26
Keynote Talk: 30 min with Q&A
Short Talk: 15 min with Q&A

Thursday | October 24

8:00 am – 8:30 pm — **On-site Registration**

12:00 pm — **Lunch Box Provided**

1:30 pm – 1:40 pm — **Conference Welcome** | Chair: Yu Xia

Zheng Ouyang, Dean of Tsinghua Shenzhen International Graduate School

1:45 pm – 2:00 pm — **Introduction of International Lipidomics Society** | Chair: Yu Xia

Kim Ekroos, Lipidomics Consulting Ltd.

2:00 pm – 2:30 pm — **ILS Early Career Travel Awards** | Chair: Kim Ekroos. Present the certificate on behalf of the ILS

O01
2:00 pm – 2:15 pm

Live single-cell mass spectrometry to study the metabolic mechanisms behind triple negative breast cancer cell migration

Xiaoyue Huang, Leiden University

O02
2:15 pm – 2:30 pm

Membrane lipids turnover by full organism 15N metabolic labeling and shotgun ultra-high resolution mass spectrometry

Vannuruswamy Garikapati, Max Planck Institute of Molecular Cell Biology and Genetics

2:30 pm – 4:00 pm — **Short Talks** | Chair: Jing Zhao, Thomas Ka-Yam LAM

O03
2:30 pm – 2:45 pm

Precision fatty acidomics unveils altered sebum fatty acid profiles in acne vulgaris patients

Zhen Wang, School of Public Health (Shenzhen), Sun Yat-Sen University

O04
2:45 pm – 3:00 pm

Analytical Strategy for Oxylipins in food obtained from poultry by combing chemical derivatization and liquid chromatography mass spectrometry

Xiaohui Feng, Chinese Academy of Agricultural Sciences

O05
3:00 pm – 3:15 pm

Development of oxygen attachment dissociation (OAD)-TOF system for identifying double-bond positions in lipid

Hidenori Takahashi, Shimadzu Corp.

O06
3:15 pm – 3:30 pm

A machine learning and drug repurposing approach to target ferroptosis in colorectal cancer stratified by sex and KRAS

Hong Yan, Hong Kong Baptist University, Yale University

O07 | 3:30 pm – 3:45 pm
High-field ion manipulation enables structural analysis of lipids and metabolites
Xiaoyu Zhou, Tsinghua University

O08 | 3:45 pm – 4:00 pm
Quantitative and qualitative analysis of lipids using the ZenoTOF 7600 system
Paul Baker, Sciex

4:00 pm – 4:30 pm
Coffee Break

4:30 pm – 5:30 pm
Workshop 1
Preanalytics for Lipidomics
Chaired by Xue Li Guan and Rainer Lehmann

5:30 pm – 6:30 pm
Purspec Workshop: PURSPEC Solution for Structural Lipidomics

6:30 pm –
Get Together

Friday | October 25

8:30 am – 5:00 pm
On-site Registration

8:30 am – 10:00 am
Oral Session 1: Functional Lipidomics | Chair: J. Thomas Brenna

O09 | 8:30 am – 9:00 am
***Keynote*: Concordant inter-laboratory derived concentrations of ceramides in human plasma reference materials via authentic standards**
Markus Wenk, Hamad Bin Khalifa University

O10 | 9:00 am – 9:20 am
Enhancing spatial lipidomics with large-scale structural annotation and high-throughput lipid mapping
Xiaoxiao Ma, Tsinghua University

O11 | 9:20 am – 9:40 am
The most decisive nutrient in the beginning of an independent life
Huanhu Zhu, ShanghaiTech University

O12 | 9:40 am – 10:00 am
Alzheimer's disease is likely a lipid disorder complication: An example of functional lipidomics research
Xianlin Han, University of Texas at San Antonio

10:00 am – 10:30 am
Coffee Break + Group Photo

10:30 am - 12:00 pm
Oral Session 2: Lipidomics in Health and Diseases | Chair: Xiaowei Chen

O13 | 10:30 am – 11:00 am
***Keynote*: Non-invasive lipid panel of MASLD fibrosis transition underscores the role of circulating lipids in hepatic immunomodulation**
Guanghou Shui, Institute of Genetics & Developmental Biology, Chinese Academy of Sciences

O14 | 11:00 am – 11:20 am
Preanalytical considerations on short-lived lipids in whole blood samples
Rainer Lehmann, Institut für Klinische Chemie und Pathobiochemie

O15
11:20 am – 11:40 am
High-throughput lipidomic quantitation of blood in large cohorts and application to early screening for pancreatic cancer
Michal Holcapek, University of Pardubice

O16
11:40 am – 12:00 pm
Targeting lipid unsaturation and ferroptosis in cancer metastasis
Yilong Zou, Westlake University

12:00 pm – 1:00 pm
Bruker Lunch Seminar

1:00 pm – 2:00 pm
Poster Session 1

2:00 pm – 3:00 pm
Oral Session 3: Lipid Metabolism | Chair: Tae-Young Kim

O17
2:00 pm – 2:20 pm
Bis-allylic deuterated DHA mitigates oxidation in neural tissue in vivo
J. Thomas Brenna, University of Texas at Austin

O18
2:20 pm – 2:40 pm
Bilayer equilibration at the endoplasmic reticulum governs cellular and systemic lipid homeostasis
Xiaowei Chen, Peking University

O19
2:40 pm – 3:00 pm
Technology development of lipidomics and its application in tumor immunometabolism
Shuhai Lin, Xiamen University

3:00 pm – 4:00 pm
Oral Session 4: Food Lipidomics | Chair: Baoru Yang

O20
3:00 pm – 3:20 pm
Mass spectrometry-based lipidomics as a powerful platform in foodomics research
Fang Wei, Oil Crops Research Institute of Chinese Academy of Agricultural Sciences

O21
3:20 pm – 3:40 pm
Unusual polyunsaturated fatty acids in edible marine worms identified by covalent adduct chemical ionization mass spectrometry
Donghao Wang, Sun Yat-Sen University

O22
3:40 pm – 4:00 pm
Pseudotargeted lipidomics approach for Lipid in milk
Yong-Jiang Xu, Jiangnan University

4:00 pm – 4:30 pm
Coffee Break

4:30 pm – 5:15 pm
Plenary Talk 1 | Chair: Xue Li Guan

O23
Regio- and stereospecific investigation of natural lipidomes and bioavailability of docosahexaenoic acid
Baoru Yang, University of Turku

5:30 pm – 6:30 pm
Workshop 2

Standardizing Lipidomics Reporting: A Hands-on Workshop with the Lipidomics Checklist
Chaired by Nils Hoffmann and Harald Köfeler

6:30 pm –
Dinner

Saturday | October 26

8:30 am – 10:00 am — **Oral Session 5: Isomer-Resolved MS for Lipid Analysis** | Chair: Lingjun Li

O24 | 8:30 am – 9:00 am
***Keynote*: Turning a negative into a positive: Applications of charge inversion mass spectrometry for lipid isomer resolution**
Stephen Blanksby, Queensland University of Technology

O25 | 9:00 am – 9:20 am
Full-dimensional resolution of C=C bond isomers of lipids
Suming Chen, Wuhan University

O26 | 9:20 am – 9:40 am
Lipid isomer analysis: From high-throughput characterization to point-of-care testing
Wenpeng Zhang, Tsinghua University

O27 | 9:40 am – 10:00 am
Aza-prilezhaev aziridination-enabled multidimensional analysis of isomeric lipids via high-resolution u-shaped mobility analyzer-mass spectrometry
Wenjian Sun, Shimadzu Research Lab (Shanghai)

10:00 am – 10:30 am — **Coffee Break**

10:30 am – 12:00 pm — **Oral Session 6: New Lipidomic Techniques (I)** | Chair: Xiaohui Liu

O28 | 10:30 am – 11:00 am
***Keynote*: Cutting-edge lipidomics to illuminate the host-microbiome interactions**
Makoto Arita, RIKEN Center for Integrative Medical Sciences

O29 | 11:00 am – 11:20 am
The 'ABC's of microbial lipidomics for discovery of novel lipids and functions in enterobacteriaceae
Xue Li Guan, Nanyang Technological University

O30 | 11:20 am – 11:40 am
Quantitative profiling of lipid trafficking between organelles enabled by subcellular photocatalytic labeling
Zhengjiang Zhu, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences

O31 | 11:40 am – 12:00 pm
Development of sensitive LC-MS methods for comprehensive lipidome analysis
Liang Li, University of Alberta

12:00 pm – 1:00 pm — **Waters Lunch Seminar**

1:00 pm – 2:00 pm — **Poster Session 2**

2:00 pm – 3:00 pm — **Oral Session 7: New Lipidomic Techniques (II)** | Chair: Ting Zhou

O32 | 2:00 pm – 2:20 pm
Lipidomics analysis for large-amount samples and single cells
Guowang Xu, Dalian Institute of Chemical Physics, Chinese Academy of Sciences

O33 | 2:20 pm – 2:40 pm
Optimization of skin sampling based on tape-stripping for skin lipidomics by nanoflow nUHPLC-ESI-MS/MS
Myeong Hee Moon, Yonsei University

O34 | 2:40 pm – 3:00 pm
Development of a comprehensive and quantitative lipidome analysis platform based on supercritical fluid chromatography/mass spectrometry
Takeshi Bamba, Kyushu University

3:00 pm – 4:00 pm	**Oral Session 8: Lipid Imaging** | Chair: Zhaoying Wang
O35 3:00 pm – 3:20 pm	**Unraveling the spatial lipidome using gas-phase ion/ion reactions** *Boone Prentice, University of Florida*
O36 3:20 pm – 3:40 pm	**Subcellular lipid imaging with nanoscale secondary ion mass spectrometry** *Haibo Jiang, University of Hong Kong*
O37 3:40 pm – 4:00 pm	**Subcellular resolution MALDI mass spectrometry imaging of lipids** *Jianing Wang, Hong Kong Baptist University*
4:00 pm – 4:10 pm	**ILS 2025 Introduction** *Óscar Pastor, Hospital Universitario Ramón y Cajal (IRYCIS) , Universidad de Alcalá*
4:10 pm – 4:30 pm	**Coffee Break**
4:30 pm – 5:15 pm	**Plenary Talk 2** | Chair: Xianlin Han
O38	**MALDI-2 and t-MALDI-2 mass spectrometry imaging of lipids: new applications and perspectives** *Klaus Dreisewerd, University of Münster*
5:30 pm – 6:30 pm	**Workshop 3** **Future frontiers in mass spectrometry imaging: towards high-coverage structure elucidation and quantitative analysis** *Chaired by Xiaoxiao Ma and Boone Prentice*
6:30 pm –	**Conference Dinner**

Sunday | October 27

8:30 am – 10:00 am	**Oral Session 9: Lipid Quantitation** | Chair: Guowang Xu
O39 8:30 am – 9:00 am	***Keynote*: Enabling high-throughput quantitative lipidomics via innovation in chemical tagging approaches** *Lingjun Li, University of Wisconsin – Madison*
O40 9:00 am – 9:20 am	**Quantitative lipidomics using metabolic deuterium oxide labeling** *Tae-Young Kim, Gwangju Institute of Science and Technology*
O41 9:20 am – 9:40 am	**Ultrafast quantitative measurements of circulating ceramides in human cohorts** *Federico Torta, National University of Singapore*
O42 9:40 am – 10:00 am	**Comprehensive profiling of FAHFAs by mass probe assisted liquid chromatography-mass spectrometry** *Quanfei Zhu, Wuhan Textile University*
10:00 am – 10:30 am	**Coffee Break**

10:30 am – 11:50 am — **Oral Session 10: Data Analysis in Lipidomics** | Chair: Hong Yan

O43 | 10:30 am – 10:50 am
Methods and applications of biomarker identification based on omics data and network analysis
Xiaohui Lin, Dalian University of Technology

O44 | 10:50 am – 11:10 am
FAIR computational lipidomics facilitate comprehensive data integration and analysis
Nils Hoffmann, IBG-5, Forschungszentrum Jülich

O45 | 11:10 am – 11:30 am
Oxidative lipidomic insights in cardiovascular disease: Role of lipid peroxidation and ferroptosis
Huiyong Yin, City University of Hong Kong

O46 | 11:30 am – 11:50 am
Development of PartialDB database to explore unassigned tandem mass spectra of novel lipids
Zhixu Ni, Technische Universität Dresden

12:00 pm – 1:00 pm — **Thermo Fisher Lunch Seminar**

1:00 pm – 1:30 pm — **Coffee Break**

1:30 pm – 3:30 pm — **Oral Session 11: Lipid Membranes and Membrane Proteins** | Chair: Xiaojing Pan, Long Gui

O47 | 1:30 pm – 1:50 pm
Structural basis of lipid recognition and receptor activation of lipid receptors
Yuanzheng He, Harbin Institute of Technology

O48 | 1:50 pm – 2:10 pm
The molecular basis of sphingosine-1-phosphate metabolism and structural based modulator rational design
Ruobing Ren, Fudan University

O49 | 2:10 pm – 2:30 pm
Discovery of structurally and functionally important lipids for 7TM proteins
Jiankun Xu, Shenzhen Bay Laboratory

O50 | 2:30 pm – 2:50 pm
Computational design and genetic incorporation of lipidation mimics in living cells
Shixian Lin, Zhejiang University

O51 | 2:50 pm – 3:10 pm
Regulation and modulation of RAS palmitoylation
Qi Hu, Westlake University

O52 | 3:10 pm – 3:30 pm
Maintaining outer membrane lipid asymmetry in Gram-negative bacteria
Shu-Sin Chng, National University of Singapore

3:30 pm – 4:00 pm — **Coffee Break**

4:00 pm – 4:45 pm — **Plenary Talk 3** | Chair: Yu Xia

O53
Structural basis for cholesterol delivery from NPC2 to the transmembrane domain of NPC1
Nieng Yan, Shenzhen Medical Academy of Research and Translation, Shenzhen Bay Laboratory, Tsinghua University

4:45 pm – 5:15 pm — **Award Ceremony + Closing Event + Boxed Dinner Provided**

Abstracts of Talks

Live Single-Cell Mass Spectrometry to Study the Metabolic Mechanisms Behind Triple Negative Breast Cancer Cell Migration

Xiaoyue Huang[1], Sylvia Le Devedec[2], Thomas Hankemeier[1], Ahmed Ali[1]

[1]Metabolomics and Analytics Centre LACDR, Leiden University, Leiden, Netherlands
[2]Division of Drug Discovery and Safety LACDR, Leiden University, Leiden, Netherlands

O01

ABSTRACT

Aims: Triple-Negative Breast Cancer (TNBC) is highly heterogeneous, and metastasis remains the leading cause of mortality in TNBC patients, posing significant challenges for clinical treatment and experimental investigation. Metastasis is driven by rare cells with unique cellular and molecular properties and is traditionally classified into single and collective cell migration. While single cell migration has been extensively studied, collective cell migration remains underexplored. During collective migration, leader cells detach, communicate with follower cells, determine migration direction, and pave the way for trailing cells. As cancer cells metastasize, their lipid metabolism changes, with increased lipid synthesis, storage, and uptake. However, lipid profiling in TNBC migration has been largely overlooked. The emerging field of single-cell metabolomics offers a powerful tool for understanding cell function and inter-cellular heterogeneity in rare cells, such as leader cells. Therefore, single-cell metabolomic analysis can explore the connection between migratory behavior and the lipidome in TNBC.

Methods: In our single-cell metabolomic experiment, we integrated full scan, selected ion monitoring (SIM) scan, and tandem mass spectrometry (MS2) analysis for its high sensitivity and selectivity in detecting a wide range of ions, using electrospray ionization (ESI) for molecular ion acquisition. Sixty single cells were sampled using a 5 µm nanospray tip in a natural environment for method validation. For the cell migration study, single cells were sampled in a 37 ℃ , 5% CO2 condition. Post-detection, single-cell data were compared with a feature list to illustrate differences in lipid metabolites among leader cells, follower cells, and corresponding non-migrated cells at various time points within 24 hours post-scratch wound.

Results: Using the single-cell metabolomic method we developed, a feature list was derived from the mass spectrometry spectra of serially diluted cell lysates. An average of 33 scans of approximately 755 targeted m/z values were conducted within 5 ppm in 6 minutes. We identified 34 lipids in a 1 cell/µL lysate, illustrating the 184.1 fragment in MS2. In single-cell spectra, clear signals for PC 34:1 and PC 36:2 with an average deviation within 5 ppm were observed, while blank samples showed no signals.

Conclusions: Our study describes a single-cell metabolomic method with a focus on lipids, aimed at studying TNBC cell migration to unveil lipidomic alterations in leader cells. This study underscores the significance of investigating migrated cells in single-cell lipid profiling and highlights the metabolic heterogeneity and migration dynamics in TNBC.

PERSONAL BIO

Xiaoyue Huang is a PhD candidate from Metabolomics and Analytics Center, Leiden Academic Center for Drug Research, Leiden University, the Netherlands. She won the award for the conference contribution "Live single-cell mass spectrometry to study the metabolic mechanisms behind triple-negative breast cancer cell migration".

Membrane Lipids Turnover by Full Organism 15N Metabolic Labeling and Shotgun Ultra-High Resolution Mass Spectrometry

Vannuruswamy Garikapati[1], Ronja Rehm[2], Archishman Ghosh[1], Kai Schuhmann[1], Eugenio F. Fornasiero[2], Andrej Shevchenko[1]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

[2] Department of Neuro- and Sensory Physiology University Medical Center, University Medicine Göttingen, Göttingen, Germany

O02

ABSTRACT

Assessment of lipid flux at the full-lipidome scale is pivotal for understanding the dynamics of lipid metabolism in living organisms. 15N-containing isotopic tracers offer simple isotopic profile for species of major membrane lipids and could differentiate their lipid turnover from fatty acid synthesis. However, the molecular peaks of 15N labeled lipids are not distinguishable from the first isotope (13C) peaks of endogenous lipids by conventional means of mass spectrometry.

We report a novel approach that combines in vivo organism-wide 15N metabolic labeling and shotgun ultra-high resolution mass spectrometry (sUHR MS) to determine both absolute abundance and turnover rates of membrane lipids, as well as substrates and intermediates of lipid biosynthesis in tissues and body fluids of mice. Metabolic labeling was carried out by feeding young- and old-aged mice with a 15N-enriched SILAM diet over five time points. Ultra-high mass resolution (~1.5M @ m/z 200) was attained by recording the longer transients (time-domain signals) with the help of an external data acquisition system (Booster X2) coupled to a Q Exactive Orbitrap MS. Transients were further processed using Peak-by-Peak software and lipids were identified and quantified by LipidXplorer software. Subsequently, kinetic parameters (e.g. flux rates, rate constants, and half-life times) were estimated using in-house developed MATLAB scripts.

With sUHR MS framework, we resolved 13C isotopes of unlabeled and monoisotopic peaks of 15N labeled lipid species (Δm = 0.00633 Da). We determined the molar abundance and turnover rates of over 120 nitrogen-containing species covering major classes of membrane lipids in mouse plasma, whole blood, four distinct regions of brain and liver. Further, a wide range of specimen-specific, lipid class and molecular species-characteristic turnover kinetics were observed across ages. Particularly, ethanolamine- and serine-containing lipids showed faster turnover and higher (ca. 2-fold) rate in comparison to the choline-containing lipids. Likewise, polyunsaturated molecular species unveiled distinctive kinetics across different lipid classes. Furthermore, brain regions (cortex, cerebellum, striatum, and hippocampus) exhibited much delayed and lower (ca. 4-5 fold) lipid turnover rates and discrete kinetic profiles than body fluids and liver. Eventually, we computed the kinetic parameters of intracellular precursors (e.g. choline, serine) and intermediates (e.g. P-Chol, P-Etn, P-Ser, GPC, GPE, GPS, CDP-Chol, CDP-Etn) to monitor the rate of de novo synthesis of phospho- and sphingolipids.

In summary, for the first time, we measured lipid flux rates and monitored the metabolic flow of lipid biosynthesis at the full organism level across ages, hence providing a unique resource to understand age-dependent lipid metabolism in mammals across organs and body fluids at the molecular species level.

PERSONAL BIO

I, Vannuruswamy Garikapati, a postdoctoral associate in the group of Dr. Andrej Shevchenko at Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany. My current research focuses on investigating various aspects of lipid metabolism by developing novel techniques and methodologies for the quantitative measurement of the lipid flux.

Precision Fatty Acidomics Unveils Altered Sebum Fatty Acid Profiles in Acne Vulgaris Patients

Zhen Wang[1], Lerong Qi[1], Xiangzi Li[2], Yang Guan[2]

[1] School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen, China

[2] Department of Dermatology Shenzhen Center for Chronic Disease Control Shenzhen Institute of Dermatology, Shenzhen, China

O03

ABSTRACT

The fatty acid composition and metabolism of human sebum exhibit unique characteristics: sebum is rich in branched-chain and odd-numbered carbon fatty acids, primarily in their saturated and monounsaturated forms; additionally, the sebaceous gland highly expresses fatty acid desaturase transcription factor FADS2, with limited SCD expression. Acne vulgaris, a prevalent chronic inflammatory skin disease, is closely associated with alterations in the sebum lipid composition, although its pathogenesis remains elusive. Previous studies have indicated that the upregulation of n16:1n-10 (sapienic acid) and the desaturation index n16:1n-10/n16:0, both regulated by FADS2, positively correlate with acne development.

Our previous research has revealed that the Δ6-monounsaturated fatty acid (MUFA) products regulated by FADS2 include not only n16:1n-10 but also four branched-chain fatty acids (i16:1n-10, i17:1n-11, ai17:1n-11, i18:1n-12) and one straight-chain fatty acid (n17:1n-11). To our knowledge, these Δ6-MUFA products constitute essential components of sebum lipids.

Using various precision fatty acidomics techniques, including covalent adduct chemical ionization (CACI), electron ionization (EI), and Paternò-Büchi (PB) tandem mass spectrometry (MS/MS), we systematically analyzed the double bond positions and branched-chain structures of various fatty acids in human sebum. Across both healthy and acne groups, we identified 64 fatty acids in sebum, revealing the presence of five 18:2 isomers (n18:2n-10, n18:2n-9, n18:2n-7, n18:2n-6, n18:2n-3), three 20:2 isomers (n20:2n-12, n20:2n-10, n20:2n-7), and an abundance of very-long-chain (C20-26) straight-chain and branched-chain fatty acids.

Further comparison of sebum compositions between healthy and acne groups revealed that several FADS2-regulated Δ6-MUFA (i.e. n14:1n-8, i&n15:1n-9, i&n17:1n-11) and the downstream product n18:2n-10, as well as multiple FADS2-regulated desaturation indexes (i.e. n14:1/n14:0, i&n15:1/15:0, n16:1/n16:0, i&ai&n17:1/17:0, and n18:2n-10/n18:0), were significantly upregulated in the acne group. However, no significant differences were observed in the desaturation indexes of even-numbered carbon branched-chain fatty acids (i16:1/i16:0, i18:1/i18:0). Moreover, the acne group exhibited a significantly lower level of total saturated fatty acids (SFA) and total polyunsaturated fatty acids (PUFA), accompanied by significantly elevated levels of total ai-branched-chain fatty acids (aiBCFA), total MUFA, and the total desaturation index (total MUFA/SFA). Stratified analysis revealed that patients with severe acne had significantly higher levels of n18:2n-10 and the n18:2n-10/n18:0 ratio compared to those with mild to moderate acne.

Taken together, these findings provide a methodological foundation for screening sebum fatty acid biomarkers in acne and elucidating their underlying mechanisms.

PERSONAL BIO

Zhen Wang is an Assistant Professor and Master's Supervisor at School of Public Health (Shenzhen), Sun Yat-sen University. She graduated from Cornell University with a Ph.D. degree in Food Science, specializing in Food Chemistry and Molecular Nutrition. Her research focuses on the integrated study of fatty acid nutrition, metabolism, and analysis, with a particular emphasis on the role of functional fatty acids in disease prevention and treatment. She has published 20 SCI papers in the past five years, including 9 as the first or corresponding author, with more than 500 citations. She was invited to serve as Co-Chair of the Lipid Metabolism Session at the International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2021 Annual Meeting. She is also an independent reviewer for international journals such as the *Journal of Agriculture and Food Chemistry, Food & Function,* and *BBA-Molecular and Cell Biology of Lipids*. Additionally, she holds the certification as a Registered Dietitian in China.

Analytical Strategy for Oxylipins in food obtained from poultry by combing chemical derivatization and liquid chromatography mass spectrometry

Xiaohui Feng[1], Youyou Yang[1]

[1] State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

O04

ABSTRACT

As one of the important dietary ingredients, meat from livestock and poultry is the key source for lipids and fatty acids (FAs). Composition of FAs and their positional isomers in lipid play vital roles in food nutrition. Herein, we established a method for the identification and quantitation of Sn-positional isomers of fatty acids in the lipids of meat following the procedures regarding lipid fraction, hydrolysis of lipids and fatty acids derivatization. Firstly, the lipid was extracted according to Folch's method, followed by fraction using High performance liquid chromatography (HPLC) coupled with an evaporative light scattering detector (ELSD) to obtain Triacylglycerol (TG), phosphocholine (PC) and phosphoethanolamine (PE). The obtained lipids were then hydrolyzed using various lipase, followed by derivatization using 5-(dimethylamino)-1-carbohydrazide-isoquinoline (DMAQ). We optimized the lipase used, antioxidant reagent used and concentration, reaction time and temperature, as well as derivatization conditions (coupling reagents as well as catalysts). The established method was successfully used to analyze the positional isomer of various lipid type in four different skeletal muscle samples including pork tenderloin, black pig tenderloin, chicken breast, beef sirloin and lamb tenderloin. Such applications could significantly advance research in improving meat quality.

PERSONAL BIO

Xiaohui Feng received her PhD degree in Chemical Engineering from Curtin University, Australia. She is now currently a research assistant in central laboratory in the Institute of Animal Sciences of Chinese Academy of Agricultural Sciences. Her research focuses on developing novel LC-mass spectrometry methodology in lipidomic and identifying oxylipins at low abundance based chemical derivatization method in food. Dr. Feng has published over 20 peer-reviewed research papers on *Food Chemistry, Food Research International, Chemical Engineering Journal* and *Coordination Chemistry Review* etc.

Development of Oxygen Attachment Dissociation (OAD)-TOF System for Identifying Double-Bond Positions in Lipid

Hidenori Takahashi[1], Ryosuke Nara[1], Yuta Miyazaki[1], Kaoru Nakagawa[1]

[1] Shimadzu Corp

O05

ABSTRACT

OAD is an innovative radical-induced dissociation technology utilizing charge-neutral hydroxyl radicals (OH) and oxygen atoms (O) [1-3]. Introducing OH/ O into a collision cell (q2) initiates a unique radical-induced dissociation distinct from CID. OAD provides specific fragmentations at double bonds within carbon chains (C=C). While a time-of-flight MS (TOF) is ideal for precise OAD product ion annotation, TOF systems require a high vacuum level, presenting challenges for introducing a high radical flux into q2. The chemically reactive OH/O radicals readily recombine into stable molecular forms on the metal surface of q2. To address this, we have developed an OAD-TOF system incorporating a modified q2 to increase radical density while maintaining the required vacuum level, thus enhancing OAD efficiency.

To reduce the recombination rate of OH/O on the metal surface of q2, we coated the inner walls of q2 with an insulating material, polyimide film. The recombination coefficients on metal and polyimide surfaces differ significantly, spanning several orders of magnitude. Additionally, we designed electrodes within q2 to be cut within a range that would not be affected by the electric field. Compared to the original electrodes of q2, the metal surface area has been reduced by approximately 40%. These modifications were implemented to enhance the sensitivity of OAD by preventing recombination losses of radicals within q2.

By improving OAD efficiency, it has become possible to adapt not only LC-MS analysis, which has been previously reported [3], but also atmospheric pressure MALDI mass spectrometry imaging (MSI). To evaluate the performance of MSI with OAD, several unsaturated lipids in mouse brain sections were subjected to positive and negative OAD-MS/MS. In positive ion mode, the C=C positions of several phosphatidylcholines (PC) were successfully annotated. The OAD-MS/MS spectrum of PC 16:0_18:1 ([M+H]+ m/z 760.585 and [M+K]+ m/z 798.540) revealed product ions indicative of two C=C positional isomers, PC 16:0_18:1(n-7) and PC 16:0_18:1(n-9). In the brain sections, both PC 16:0_18:1(n-7) and PC 16:0_18:1(n-9) were distributed with observed localization.

PERSONAL BIO

I am an expert in mass spectrometry with a focus on radical-induced dissociation (RID) techniques. I completed my Ph.D. in 2006 at Doshisha University, focusing on "Time Response of Negative Ions in Plasma under Laser Irradiation."

At the Koichi Tanaka Research Institute at Shimadzu Corporation, I have dedicated my career to developing and applying advanced mass spectrometry techniques. My research on atomic and molecular radicals and their reactions with ions has led to significant advancements in RID methods.

I developed Hydrogen Attachment/Abstraction Dissociation (HAD), a method inducing hydrogen addition and abstraction reactions, providing unique dissociations for structural analysis. HAD is effective for analyzing even-electron ions generated by MALDI and ESI.

Additionally, I developed a high-flux hydrogen radical source for close-proximity irradiation of target ions, integrated into a hybrid ion trap-time-of-flight mass spectrometer. This method preserves modifications like phosphorylation during peptide backbone cleavage.

I also created a modified radical source for generating O/OH radicals using water vapor. This led to the development of Oxygen Attachment Dissociation (OAD), enabling precise localization of double-bond positions in lipids.

HAD and OAD are effective for monovalent ion analysis and natural product structural elucidation, as reflected in numerous publications and patents. My pioneering work earned me the RESEARCH AWARD in 2022 from the mass spectrometry society of Japan. I remain committed to advancing mass spectrometry techniques and contributing to the scientific community.

A Machine Learning and Drug Repurposing Approach to Target Ferroptosis in Colorectal Cancer Stratified by Sex and KRAS

Hong Yan[1,2], Xinyi Shen[2], Yisha Yao[3], Sajid Khan[2], Shuangge Ma[2], Caroline Johnson[2]

[1] Hong Kong Baptist University, Hong Kong, China
[2] Yale University, New Haven, USA
[3] Columbia University, New York, USA

O06

ABSTRACT

The landscape of sex differences in Colorectal Cancer (CRC) has not been well characterized with respect to the mechanisms of action for oncogenes such as KRAS. However, our recent study showed that tumors from male patients with KRAS mutations have decreased iron-dependent cell death called ferroptosis. Building on these findings, we further examined ferroptosis in CRC, considering both sex of the patient and KRAS mutations, using public databases and our in-house CRC tumor cohort.

Through subsampling inference and variable importance analysis (VIMP), we identified significant differences in gene expression between KRAS mutant and wild type tumors from male patients. These genes suppress (e.g., SLC7A11) or drive (e.g., SLC1A5) ferroptosis, and these findings were further validated with Gaussian mixed models. Furthermore, we explored the prognostic value of ferroptosis regulating genes and discovered sex- and KRAS-specific differences at both the transcriptional and metabolic levels by random survival forest with backward elimination algorithm (RSF-BE). Of note, genes and metabolites involved in arginine synthesis and glutathione metabolism were uniquely associated with prognosis in tumors from males with KRAS mutations.

Additionally, drug repurposing is becoming popular due to the high costs, attrition rates, and slow pace of new drug development, offering a way to treat common and rare diseases more efficiently. Furthermore, increasing evidence has shown that ferroptosis inhibition or induction can improve drug sensitivity or overcome chemotherapy drug resistance. Therefore, we investigated the correlation between gene expression, metabolite levels, and drug sensitivity across all CRC primary tumor cell lines using data from the Genomics of Drug Sensitivity in Cancer (GDSC) resource. We observed that ferroptosis suppressor genes such as DHODH, GCH1, and AIFM2 in KRAS mutant CRC cell lines were resistant to cisplatin and paclitaxel, underscoring why these drugs are not effective for these patients. The comprehensive map generated here provides valuable biological insights for future investigations, and the findings are supported by rigorous analysis of large-scale publicly available data and our in-house cohort. The study also emphasizes the potential application of VIMP, Gaussian mixed models, and RSF-BE models in the multi-omics research community. In conclusion, this comprehensive approach opens doors for leveraging precision molecular feature analysis and drug repurposing possibilities in KRAS mutant CRC.

PERSONAL BIO

Dr. Hong YAN is a Research Assistant Professor at the State Key Laboratory of Environmental and Biological Analysis (SKLEBA) at Hong Kong Baptist University (HKBU). She completed her postdoctoral training at Yale University, where she gained extensive experience in advanced research methodologies. Her research focuses on tumor metabolomics and multi-omics network analysis. She is particularly interested in colorectal cancer metabolomic reprogramming, ferroptosis and KRAS mutations, precision medicine and drug repurposing, as well as bioinformatics and data mining.

High-Field Ion Manipulation Enables Structural Analysis of Lipids and Metabolites

Xiaoyu Zhou[1], Zhuofan Wang[1], Shuai Li[1], Zheng Ouyang[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, People's Republic of China.

O07

ABSTRACT

Biomolecules have complex chemical structures. Analyzing the structure of biomolecules to elucidate the relationship between structure and biological function is an important issue in chemistry and life sciences. Here, a challenging problem to address the issue is the molecular isomerism, where different isomers have identical chemical formula and molecular weights but different chemical structures. The abnormalities of isomerism in biological systems lead to diseases, such as cardiovascular and cerebrovascular diseases, neurodegenerative diseases, and tumors. In this study, we report high-field ion manipulation technologies developed in our group to enable structural analysis of lipids and metabolites.

Keywords: Ion manipulation, Isomer, Lipid, Metabolites.

PERSONAL BIO

Xiaoyu Zhou, Associate Professor of Tsinghua University. His research focuses on mass spectrometry instrumentation and structural analysis of biomolecules. He conducts research on new instruments and methodologies with original physical ideas, smart engineering design, and applications in chemistry and life science. He has published more than 50 papers in journals such as Science, Nature Methods, Nature Communications, and Analytical Chemistry.

Quantitative and Qualitative Analysis of Lipids Using the ZenoTOF 7600 System

Paul RS Baker[1]

[1] SCIEX, USA

O08

ABSTRACT

Quantitative metabolomics and lipidomics analyses are traditionally performed on triple quadrupole instruments using a multiple reaction monitoring (MRM) scan mode. This type of analysis is very sensitive, highly specific, and quantitative when paired with the appropriate chromatographic separation and internal standard strategy. One disadvantage of this type of analysis is triple quadrupole instruments have a relatively slow mass range scanning speed, so product ion analysis concomitant to quantitative analysis is impractical. Hybrid quadrupole time of flight instruments are high-resolution instruments that can acquire full product ion spectra in < 10 ms; however, they are not routinely used for quantitation due to the low sensitivity of traditional QTOF instruments. The ZenoTOF 7600 system with Zeno trapping has significantly higher sensitivity than conventional QTOF instruments, which rivals that of high-end triple quadrupole instruments and generates high-resolution, accurate mass product ion spectra during quantitative analysis. Furthermore, the ZenoTOF 7600 system has a complementary fragmentation mode, termed electron-activated dissociation (EAD), that produces as much as 20-fold more fragment ions than collision-induced dissociation (CID), which can provide greater structural specificity and qualitative for structural elucidation of isomers, isobars and unknowns.

Here, 3 examples of compound classes traditionally measured on triple quadrupole instruments were quantitated and structurally characterized using the ZenoTOF 7600 system. Using both CID and EAD-based fragmentation, the instrument was used to measure hormonal steroids, bile acids and lipid mediators in human plasma. The results demonstrate the ZenoTOF 7600 system has sufficient sensitivity to measure these compounds in vivo and can provide structurally diagnostic fragment ions via EAD to discriminate among compound isomers. This latter observation enables the development of quantitative assays that do not solely depend on chromatography to obtain compound specificity during analysis.

PERSONAL BIO

Paul Baker received his PhD in Biochemistry from Wake Forest University School of Medicine. He did his post-doctoral work at the University of Alabama at Birmingham where he helped lead the discovery of a novel class of anti-inflammatory lipid mediators—nitrated lipids. He continued to work on nitrated lipids as an assistant professor at the University of Pittsburgh School of Medicine until June of 2011 when he joined SCIEX. As a senior applications manager and the global lead scientist for Lipidomics, he pioneered the use of differential Ion mobility spectrometry and electron impact dissociation to analyze lipids. In May of 2018, Paul joined Avanti Polar Lipids as the Director of the Analytical and Lipidomics divisions, where he focused on developing new analytical and internal standard strategies for lipid analysis. Paul rejoined SCIEX in 2020 where he is now the Sr Staff Scientist liaison for lipidomics and metabolomics.

Concordant Inter-Laboratory Derived Concentrations of Ceramides in Human Plasma Reference Materials via Authentic Standards

Markus R Wenk[1]

[1] College of Health & Life Sciences, Hamad Bin Khalifa University, Education City, Doha, Qatar

O09

ABSTRACT

The inventory of lipid molecules found in blood plasma (plasma lipidome) offers insights into individual metabolism and physiology in health and disease. Plasma lipidomics based on mass spectrometry has become a powerful tool to support clinical research towards innovative approaches in precision health and medicine. However, the translation of laboratory-developed tests towards robust, rapid and quantitative tests that deliver concordant results across different analytical platforms and sites does require considerable efforts. Specifically, I will present results on behalf of an extensive community effort hosted by the International Lipidomics Society (ILS). We compared measurements between 34 laboratories from 19 countries, utilizing mixtures of stable isotope labelled authentic synthetic standards, to quantify by mass spectrometry four clinically used ceramide species in the NIST (National Institute of Standards and Technology) human blood plasma Standard Reference Material (SRM) 1950, as well as new suite of candidate plasma reference materials (RM 8231).

PERSONAL BIO

Markus Wenk has been fascinated by the structure and function of membranes since his time as a student at the Biozentrum of the University of Basel in 1994. During his time at Yale, he introduced new techniques to study how cells metabolise phospholipids. He's been a leader in using advanced methods to study lipids (lipidomics), earning recognition such as the European Lipid Science Award in 2018. Markus founded the Singapore Lipidomics Incubator (SLING) and the international Singapore Lipid Symposium (iSLS) at the National University of Singapore (NUS), focusing on innovation, education, and collaboration in lipid research. Lately, he's been working on incorporating precise molecular analysis into medical research for personalized health. Markus led the Department of Biochemistry at NUS from 2015 to 2022 and also directed the NUS Precision Medicine Translational Research Programme from 2020 to 2023. In August 2023, he assumed the role of the Dean of the College of Health and Life Sciences at Hamad Bin Khalifa University (HBKU) in Doha, Qatar.

Enhancing Spatial Lipidomics with Large-Scale Structural Annotation and High-Throughput Lipid Mapping

Xiaoxiao Ma[1], Yao Qian[1], Aolei Tan[1]

[1] Tsinghua University, Beijing, China

O10

ABSTRACT

Enhancing spatial lipidomics with large-scale structural annotation and high-throughput lipid mapping. Spatial lipidomics have increasingly played important roles in tissue segmentation, localized lipid metabolism mapping and disease diagnostics. However, one aspect of spatial lipidomics that needs technical improvement is the coverage of lipid mapping with comprehensive structural characterization. Existing works have proposed the combination of LC-MS/MS-based lipidome analysis with lipid imaging for improved lipid characterization in spatial lipidomics, but at the cost of increased time and efforts for sample analysis. The high coverage lipid characterization integrated with lipid imaging is also achieved by distributing MS/MS acquisition of lipids from many different tissue pixels. While this represents significant advancement of spatial lipidomics compared with MS1-based lipid imaging, the differential imaging of lipid isomers and isobars, which are ubiquitous in the biological lipidome, remain a technical challenge.

In this talk, I will introduce the recent progress of our group in enhancing spatial lipidomics with large-scale structural annotation and high-throughput lipid mapping. Besides lipid characterization, the distinct distributions of many lipid isomers and isobars are revealed, offering increased insights into lipid structures and distributions in spatial lipidomics that are previously challenging. By combining ion mobility separation, broad-window data-independent fragmentation, and in-house developed MS/MS deconvolution algorithm, we acquired MS/MS spectra of over 150 lipid species from each tissue pixel, and in particular archived the spatial mapping of 24 lipid isomers or isobars from the mouse brain. We also developed an MS1/MS2 fusion algorithm that can be used to efficiently convert low-resolution MS2 images (sparse sampling) to high resolution ones, thereby improving MS/MS multiplicity and quality without increasing the imaging time.

PERSONAL BIO

Dr. Xiaoxiao Ma is an associate professor and doctoral supervisor of the Department of Precision Instruments, Tsinghua University. In 2017, he joined the Department of Precision Instruments of Tsinghua University, mainly engaged in mass spectrometry instruments and biomedical application research, including single-cell structural lipidomics, spatial lipidomics and mass spectrometry instruments for molecular structure characterization and applied research. He has published more than 60 papers in *Nature Communications, PNAS, Angewandte Chemie, Accounts of Chemical Research*, and has been invited to write chapters for three monographs. He presided over the key R&D program of the Ministry of Science and Technology "Frontier Biotechnology" Young Scientist Project. He has won the China Instrument and Control Society-Jin Guofan Young Scholar Award and the Mass Spectrometry Youth Award Nomination Award. He was selected into the National High-level Young Talents Program and the list of the world's top 2% scientists.

The Most Decisive Nutrient in the Beginning of an Independent Life

Meiyu Ruan[1], Fan Xu[1], Na li[1], Jing Yu[2], Fukang Teng[1], Jiawei Tang[2], Cheng Huang[2], **Huanhu Zhu**[1]

[1] Shanghai Tech University, Shanghai, China

[2] Shanghai University of Traditional Chinese Medicine, Shanghai, China

O11

ABSTRACT

Postembryonic development of animals has long been considered an internally predetermined program, while macronutrients were believed to be essential solely for providing biomatters and energy to support this process. However, in this study, by using a nematode Caenorhabditis elegans model, we surprisingly discovered that dietary supplementation of palmitic acid alone, rather than other abundant essential nutrients such as glucose or amino acid mixture, was sufficient to initiate early postembryonic development even under complete macronutrient deprivation. Such a development was evidenced by changes in morphology, cellular markers in multiple tissues, behaviors, and the global transcription pattern and it occurred earlier than the well-known early L1 nutrient checkpoint. Mechanistically, palmitic acid did not function as a biomatter/energy provider, but rather as a ligand to activate the nuclear hormone receptor NHR-49/80, leading to the production of an unknown peroxisome-derived secretive hormone in the intestine. This hormonal signal was received by chemosensory neurons in the head, regulating the insulin-like neuropeptide secretion and its downstream nuclear receptor to orchestrate global development. Additionally, the nutrient-sensing hub mTORC1 played a negative role in this process. In conclusion, our data indicate that free fatty acids act as a primary nutrient signal to launch the early development in C. elegans, which suggests that specific nutrients, rather than the internal genetic program, serve as the first impetus for postembryonic development.

PERSONAL BIO

Zhu, Huanhu earned his B.S. and Ph.D. degrees from Fudan University and undertook postdoctoral research in Professor Min Han's Lab at the Howard Hughes Institute, University of Colorado Boulder. Since 2015, he has been a faculty member at ShanghaiTech University. His lab primarily uses the nematode Caenorhabditis elegans as a model organism, focusing on how essential nutritional and commensal bacterial factors regulate host development and physiology. As the corresponding author, he has published research papers in esteemed journals such as *Developmental Cell, Cell Reports, Nature Communication, Genes & Development,* and *Annual Review Genetics*.

Alzheimer's Disease Is Likely a Lipid-Disorder Complication: An Example of Functional Lipidomics Research

Xianlin Han[1]

[1] University of Texas Health San Antonio, San Antonio, USA

O12

ABSTRACT

Functional lipidomics is a frontier in lipidomics research, which identifies changes of cellular lipidomes in disease by lipidomics, uncovers the molecular mechanism(s) leading to the changed lipidomes under the condition, and investigates the sequela of these changes in the context of the disease. Sulfatide, a class of sphingolipids, is a major lipid component in the nervous system, predominantly present in the extracellular leaflet of myelin sheath and specifically synthesized by cerebroside sulfotransferase (CST) in oligodendrocytes in the CNS. We and many other laboratories have previously revealed that brain sulfatide content is specifically and dramatically reduced at the earliest clinically recognizable stages of Alzheimer's disease (AD) and in all AD mouse models examined (e.g., PMID: 12358786; PMID: 12501252; PMID: 18762354). Our recent studies using an adult-onset sulfatide-deficit mouse model have uncovered that CNS sulfatide reduction is sufficient to activate disease-associated microglia and astrocytes, leading to chronic AD-like neuroinflammation and cognitive impairment (PMID: 34526055). We also demonstrated that adult-onset sulfatide deficiency led to many other AD-like pathologies, including brain ventricular enlargement, disrupted urinary bladder control, tau hyperphosphorylation and aggregation, A metabolism disruption, etc. (e.g., PMID: 36613677; PMID: 37445661; PMID: 37239102; PMID: 37478300; doi.org/10.1002/glia.24423). In the current presentation, the sulfatide deficiency-induced AD-like phenotypes will be outlined and, selectively, sulfatide deficiency-induced bladder enlargement will be discussed in some details. Finally, the causal factors leading to sulfatide reduction in AD pathogenesis will be summarized. We believe CNS sulfatide deficiency plays a crucial role in AD pathogenesis.

PERSONAL BIO

Prof. Xianlin Han graduated from Zhejiang University, China in 1982, received his Ph.D. in Chemistry at Washington University in St. Louis in 1990, and trained as a postdoctoral fellow in Dr. Richard Gross' lab in Washington University School of Medicine (WUMS) between 1990 and 1992. Dr. Han joined WUMS as a research faculty from 1992 to 2000. He became an Assistant Professor of Medicine in 2000 and was promoted to Associate Professor of Medicine with tenure in WUMS in 2008. He was a Professor at Sanford Burnham Prebys Medical Discovery Institute between 2010 and 2017. He now is an endowed chair Professor of Medicine and the Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio. Prof. Han has authored and co-authored about 400 peer-reviewed papers with an H-index of 101 and around 40,000 citations, and is the author of the book "Lipidomics: Comprehensive Mass Spectrometry of Lipids". Prof. Han is one of the pioneers in lipidomics and the inventor of shotgun lipidomics. He is the associate editor of "J. Lipid Res.", and serves as a member of the Editorial Board of numerous international journals including *BBA - Mol. Cell Biol. Lipids, Chem. Phys. Lipids, Anal. Chim. Acta, Anal. Biochem., Life Metab.,* and *Metabolites*.

Non-Invasive Lipid Panel of MASLD Fibrosis Transition Underscores the Role of Circulating Lipids in Hepatic Immunomodulation

Guanghou Shui[1]

[1] State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China.

O13

ABSTRACT

A pressing need exists to develop non-invasive panel for differentiating mild fibrosis from non-fibrosis in MASLD to facilitate effective monitoring of pathological transition. In this work, we applied quantitative lipidomics and sterolomics (735 lipids identified from a defined library of 1207 species) on sera samples from the PERSONS cohort with biopsy-based histological assessment of liver pathology. We trained lasso regression model using quantitative omics data and clinical variables, and derived a combinatorial panel of lipids and clinical indices that differentiates mild fibrosis (Kleiner's score F>1, n=324) from non-fibrosis (F=0, n=195) with an AUROC at 0.775 (95% CI: 0.735-0.816, balanced accuracy=0.66-0.73, F1 score = 0.71-0.79). Circulating sulfatides emerged as central lipids distinctly associated with fibrosis pathogenesis in MASLD. Lipidomics analysis of distinct lipoprotein fractions revealed a redistribution of circulating sulfatides from HDLs onto LDLs in fibrosis patients, possibly ascribed to altered composition of VLDLs emanating from the liver at early fibrosis transition. We further verified that patient LDLs with reduced SL content triggered a smaller activation of Type II natural killer cells compared to control LDLs. Our results cumulatively suggest that hepatic crosstalk with systemic immunity mediated by lipoprotein metabolism underlies fibrosis progression at early-stage MASLD.

PERSONAL BIO

Guanghou Shui received his Ph.D. degree in 2004 from the National University of Singapore (NUS). Following this, he conducted his postdoctoral research in the Department of Biochemistry in 2004—2008 and worked at a senior research fellow at NUS in 2008—2012. He joined the Institute of Genetics and Developmental Biology as a principal investigator in 2013. He has co-authored over 230 SCI papers in well-known journals. The major research interests of his laboratory are: (1) developing cutting-edge lipidomic and metabolomics approaches; (2) studying lipid metabolism at systems level; and (3) dissecting the roles of lipid and metabolic dysregulation related to human diseases.

Preanalytical Considerations of Short-Lived Lipid Species in Whole Blood Samples

S. Rubenzucker[1], M. Hoene[2], L. Fritsche[2], R. Ahrends[1], **R. Lehmann**[2]

[1] University of Vienna Department of Analytical Chemistry, Vienna, Austria

[2] Institute for Clinical Chemistry and Pathobiochemistry Department for Diagnostic Laboratory Medicine University Hospital Tübingen, Tübingen, Germany

O14

ABSTRACT

During sample handling and transport, lipid species in blood samples show quite different stabilities. Consequently, depending on how whole blood samples are handled, there is a very high risk of non-reproducible results, discrepancies, and possibly misinterpretation of data in clinical lipidomics studies. Recently, as part of the Preanalytics interest group of the International Lipidomics Society (ILS), we reported the ex vivo stability of lipid species in a common lipid profile (1). In the current follow-up study, we focused on short-lived lipid species using bioinert reversed-phase liquid chromatography coupled to targeted mass spectrometry (2). We analyzed lipids from 17 different signaling lipid classes in EDTA whole blood. The samples were exposed to either 4° C or 21° C for 30 min, 60 min, 90 min or 4 h. Whole blood samples from more than 20 individuals were analyzed. Our data underline that correct and tightly controlled sample handling and transport immediately after blood collection is a must to obtain valid results in clinical lipidomics studies, especially when short-lived lipid species are of interest. Our data may support the efforts of the ILS to standardize and harmonize clinical lipidomics approaches.

PERSONAL BIO

Rainer Lehmann serves as the Vice Director of the Institute for Clinical Chemistry and Pathobiochemistry at University Hospital Tuebingen, where he also leads the technical coordination of medical technologist training. Additionally, he is a Senior Scientist at the Institute for Diabetes Research and Metabolic Diseases (IDM) of the Helmholtz Center Munich, located at the University of Tuebingen. His research interests are diverse and impactful. He focuses on clinical metabolomics and lipidomics, with an emphasis on method development, preanalytical aspects, biomarker discovery, and functional studies. His translational medical research includes work in pre-diabetes, exercise metabolism, and subcellular structures. In clinical chemistry, he explores novel diagnostic biomarkers and diagnostic panels.

High-throughput Lipidomic Quantitation of Blood in Large Cohorts and Application to Early Screening for Pancreatic Cancer

Ondej Peterka[1,2], Robert Jirásko[1], Zuzana Vaková[1], Petra Peroutková[1], Zuzana Doleková[2], Maria Kanásová[2], Beatrice Mohelníková Duchoová[3], Martin Loveek[3], Ondej Urban[3], Bohuslav Melichar[3], Jan Trna[4], Karolína Kaparová[2], Zdeněk Jirsa[2], **Michal Holcapek**[1]

[1] University of Pardubice, Czech Republic

[2] Lipidica a.s. Pardubice, Czech Republic

[3] Palack University and University Hospital Olomouc, Czech Republic

[4] Masaryk Memorial Cancer Institute Brno, Czech Republic

O15

ABSTRACT

There are two major strategies in mass spectrometry (MS) coupling with separation techniques in liquid or supercritical states, which are referred to as lipid class and lipid species separation approaches. The lipid class separation is represented by HILIC and ultrahigh-performance supercritical fluid chromatography (UHPSFC), which is an ideal strategy for high-throughput quantitation because the exogenous internal standard coelutes with lipid species from the same class, resulting in identical matrix and suppression effects [1], as illustrated by the example of UHPSFC/MS in the screening of pancreatic cancer [2]. The high level of automation can be achieved due to semiautomated data processing using the LipidQuant 2.1 software [3]. The second strategy is the lipid species separation, which is based on the use of reversed-phase UHPLC/MS, which offers higher separation selectivity resulting in the detection of more than 500 lipid species at the fatty acyl level [4], but the method has lower throughput and requires more manual supervision during the data processing.

Pancreatic cancer has the worst prognosis among all cancers, which is mainly caused by too late diagnosis in an incurable stage due to the absence of symptoms at an early stage and no existing screening test. We have demonstrated in a cohort of 830 human blood samples that lipidomic analysis allows differentiation of patients with pancreatic cancer from healthy controls with an accuracy of more than 90%, including the early stage [2], and is applicable for high-throughput analysis. The University of Pardubice and FONS company established in 2022 spin-off company Lipidica with the goal of translating the screening methodology into real clinical practice. The OPLS-DA statistical models include concentrations of ca. 150 lipid species measured by UHPSFC/MS, which provides high robustness and short analysis time (<5 min). The major dysregulations are observed for very long chain sphingomyelins, ceramides, and some lysophosphatidylcholines. Now the clinical validation of the screening test has started for high-risk groups defined by the family history of pancreatic cancer, selected genetic mutations, or hereditary pancreatitis in agreement. Our new data indicate that the LDPC test does not decrease accuracy even in the very early stages of pancreatic cancer. Measurements from patients with other types of cancer show that similar lipidomic dysregulations are observed for multiple types of cancer [5].
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PERSONAL BIO

Michal Holčapek obtained his Ph.D. in analytical chemistry from the University of Pardubice, where he currently works as a professor of analytical chemistry. His research focus is mass spectrometry and its coupling with liquid chromatography or supercritical fluid chromatography, applied mainly in lipidomic analysis and cancer biomarkers. He received several scientific awards, e.g., the Herbert J. Dutton Award from American Oil Chemists' Society (2022), the Power List of 100 most influential people in the analytical sciences from The Analytical Scientist (2020, 2015, and 2013), and ERC Advanced grant (2023). He is a coauthor of >150 research articles, coeditor of 3 books, and his h-index is 48. He is one of the founding members of the Lipidomics Standards Initiative and a vice-president for conferences in the International Lipidomics Society.

Targeting Lipid Unsaturation and Ferroptosis in Cancer Metastasis

Yilong Zou[1]

[1] Westlake University, Hangzhou, China

O16

ABSTRACT

Metastatic dissemination to distant organs demands cancer cells to possess high morphological and metabolic adaptability. However, contributions of the cellular lipidome to metastasis remain elusive. In the study to be presented in this talk, we uncover a correlation between metastasis potential and ferroptosis susceptibility in multiple cancers. Metastases-derived cancer cells exhibited higher ferroptosis sensitivity and polyunsaturated fatty acyl(PUFA)-lipid contents than primary tumor-derived cells from ovarian cancer patients. Metabolism-focused CRISPR screens in a mouse model for ovarian cancer distant metastasis established via two rounds of in vivo-selection revealed the PUFA-lipid biosynthesis pathway as a pivotal contributor to metastatic extravasation. We will discuss how the polyunsaturated-lipidome modulates tumor progression and metastasis, and how we exploit this liability to target cancer for therapeutic development.

Yilong Zou is currently a principal investigator at the School of Life Sciences, Westlake University in Hangzhou, China. Dr. Zou's research focuses on identifying metabolic vulnerabilities in cancer to develop novel anti-tumor therapeutics. Dr. Zou obtained his PhD from Memorial Sloan Kettering Cancer Center, and worked as a postdoc in Dr. Stuart Schreiber's lab at the Broad Institute before taking the independent PI position at Westlake.

PERSONAL BIO

Yilong Zou is currently a principal investigator at the School of Life Sciences, Westlake University in Hangzhou, China. Dr. Zou's research focuses on identifying metabolic vulnerabilities in cancer to develop novel anti-tumor therapeutics. Dr. Zou obtained his PhD from Memorial Sloan Kettering Cancer Center, and worked as a postdoc in Dr. Stuart Schreiber's lab at the Broad Institute before taking the independent PI position at Westlake.

Bis-Allylic Deuterated DHA Mitigates Oxidation in Neural Tissue in Vivo

Secilia Garza[1], **J. Thomas Brenna**[1]

[1] University of Texas at Austin, Austin, Texas, USA

O17

ABSTRACT

The human central nervous system simultaneously has the most highly unsaturated fatty acids (HUFA) and the highest metabolic rate among body tissue. Up to 2% of consumed O2 is converted to reactive oxygen species (ROS) that cause unregulated damage to HUFA-rich membrane phospholipids (PLs). Docosahexaenoic acid (DHA) is the brain's most unsaturated and abundant HUFA. Reinforcing the ROS-labile bis-allylic positions with deuterium (D-DHA) protects against oxidative damage in vitro and in vivo. We developed an LC-MS/MS method to detect ambient levels of nascent oxidation products of DHA and D-DHA containing PL in vivo in rat brain lipid extracts. Multiple reaction monitoring (MRM)-triggered mass spectra confirm D-DHA incorporation in D-DHA-fed rat brain PLs. D-DHA-PLs demonstrate 20%-30% lower overall oxidation in D-DHA-PL compared to DHA-PL. DHA-PL nascent oxidation products add 2 O consistent with known peroxidation reactions, while D-DHA primarily adds a single O consistent with epoxidation. jlr Our data is consistent with a mechanism of action whereby D-DHA blocks excess lipid peroxidation, leading to lower overall membrane damage. D-DHA is a unique therapeutic against neurodegenerative diseases where ROS-driven oxidation is implicated.

PERSONAL BIO

Tom Brenna, PhD, is Professor of Pediatrics, of Chemistry, and of Human Nutrition at the Dell Medical School and College of Natural Sciences at the University of Texas at Austin, and Professor Emeritus of Human Nutrition, of Food Science, and of Chemistry after 28 years as an active faculty member at Cornell University in Ithaca, NY.

Bilayer Equilibration at the Endoplasmic Reticulum Governs Cellular and Systemic Lipid Homeostasis

Xiaowei Chen[1]

[1] Institute of Molecular Medicine, College of Future Technology, Peking University, Beijing, China

O18

ABSTRACT

The intricate orchestration of lipid production, storage, and mobilization is vital for cellular and systemic homeostasis. Dysfunctional plasma lipid control represents the major risk factor for cardio-metabolic diseases, the leading cause of human mortality. Within the cellular landscape, the endoplasmic reticulum (ER) is the central hub of lipid synthesis and secretion, particularly in metabolically active hepatocytes in the liver or enterocytes in the gut. Initially assembled in the ER lumen, lipid-ferrying lipoproteins necessitate the cross-membrane transfer of both neutral and phospho-lipids onto the lumenal apolipoprotein B (APOB), in a poorly-defined process. Here we show that trans-bilayer equilibration of phospholipids, regulated by the ER protein CLCC1, determines lipid partition across the ER membrane and consequently systemic lipid homeostasis. CLCC1 partners with the phospholipid scramblase TMEM41B to recognize imbalanced bilayers and promote lipid scrambling, thereby licensing lipoprotein biogenesis and the subsequent bulk lipid transport. Strikingly, loss of CLCC1 or TMEM41B leads to the emergence of giant lumenal lipid droplets enclosed by extensively imbalanced ER bilayers, and consequently drastically accelerated pathogenesis of metabolic-dysfunction-associated liver steatohepatitis (MASH). The above results establish phospholipid scrambling at the ER as the lynchpin to maintain a dynamic equilibrium. Considering the requirement of trans-bilayer phospholipid equilibration in numerous biological processes, ranging from catabolic autophagy to viral infection, our study may enable further elucidation of a previously under-appreciated homeostatic control mechanism intrinsic to the ER function in lipid biogenesis and distribution.

PERSONAL BIO

Dr. Xiao-Wei Chen is the Boya Distinguished Professor of Molecular Medicine at the Peking University. He originally obtained his BS and BA from the Peking University, and completed his Ph.D. training with Dr. Alan Saltiel on metabolic biology at the University of Michigan. He then pursued postdoctoral study on genetics and cardiovascular biology in the laboratory of Dr. David Ginsburg, before being recruited back to the Peking University in 2014. Dr. Chen's work focuses on the genetics and cell biology of lipoprotein biology and lipid homeostasis, particularly by elucidating a receptor-mediated export program for the lipoproteins and identifying the long-sought biogenic lipid scramblase. He has also discovered a messenger role of manganese in lipid control, and conceptualized manganese therapy for intensive plasma lipid lowering to reverse exiting atherosclerotic plaques in disease models. He has published ~70 scientific papers and authored two book chapters. He is the recipient of the Young Investigator Award from the Chinese American Diabetes Association and Special Recognition Award from the Society of Heart and Vascular Metabolism, as well as the Earl Stadtman Scholar finalist from the National Institute of Health, USA and the Distinguished Young Scholar Award from the National Natural Science Foundation, China. He serves as an associate editor at the Biochemical Journal and on the editorial boards of *Life Metabolism, Journal of Lipid Research,* and *Cell Metabolism*.

Technology Development of Lipidomics and Its Application in Tumor Immunometabolism

Hao Xu[1], Pingyi Chen[1], **Shuhai Lin**[1]

[1] Xiamen University, Xiamen, China

O19

ABSTRACT

Improving annotation accuracy, coverage, speed and depth of lipid profiles remains a significant challenge in traditional spectral matching-based lipidomics. We introduce LipidIN, an advanced framework designed for comprehensive lipid annotation and reverse lipidomics. LipidIN features a 166.3-million lipid fragmentation hierarchical library that encompasses all potential chain compositions and carbon-carbon double bond locations. Developed expeditious querying module speeds up to around 70 billion times' spectral querying in less than 1 second. Furthermore, we leverage three relative retention time rules to develop lipid categories intelligence model for reducing false positive annotations and predicting unannotated lipids. More importantly, LipidIN integrates a Wide-spectrum Modeling Yield network for regenerating lipid fingerprints to further improve coverage and accuracy with a 20% estimated recall boosting. The application of LipidIN in multiple tasks demonstrated reliability and potential for lipid annotation and biomarker discovery. We also carried out the metabolic regulation of a mitochondrial protein in tumor immunity by performing LipidIN and the biological experiments.

PERSONAL BIO

Dr. Lin received his Ph.D. degree in Analytical Chemistry from Hong Kong Baptist University in 2011. From 2018, he was appointed as Professor in School of Life Sciences, Xiamen University. His current research interest mainly focuses on the development of mass spectrometry-based metabolomics technology, and its applications in cancer, SARS-CoV-2 and immunology research. Dr. Lin has published >50 peer-reviewed papers on the prestigious journals including *Nature Communications, PNAS, Autophagy, Cancer Research* with more than 3000 times in citation.

Mass Spectrometry-Based Lipidomics as a Powerful Platform in Foodomics Research

Fang Wei[1], Xin Lv[1], Dan Wang[1], Hong Chen[1]

[1] Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan, China

O20

ABSTRACT

Lipidomics, a burgeoning and critical offshoot of metabolomics, is dedicated to the in-depth analysis of lipids within biological systems. The integration of mass spectrometry (MS) within lipidomics has, in recent years, emerged as a powerful tool in foodomics, addressing complex global food chain challenges and offering profound insights into lipid-related nutrition and health. We offers a comprehensive overview of the MS-based lipidomics process, from meticulous sample preparation to advanced data processing, encapsulating the analytical strategies and chemometric tools pivotal to the field. It underscores the proven efficacy of these approaches in enhancing food traceability, ensuring quality and safety, and illuminating the health implications of dietary lipids. We further highlight the platform's robust sensitivity and capacity, when merged with chemometric tools, to detect food fraud and assess lipid-related food safety issues, while also delving into the functional roles of dietary lipids. The ongoing advancements in MS and chromatographic separation techniques are set to significantly broaden the scope of MS-based lipidomics in foodomics, promising an influx of new knowledge that will deepen our understanding of the intricate dynamics between food lipids and human health.

PERSONAL BIO

Prof. Fang Wei, doctoral Supervisor, Oil crop Research Institute, Chinese Academy of Agricultural Sciences, Executive Chief of Oil quality Chemistry and processing and Utilization team. Her current research is mainly focused on the development of lipidomics profiling methods and their applications. She has made significant progress in developing high-throughput analytical approaches of complex biological lipids based on mass spectrometry and achieved highly sensitive qualitative and quantitative analysis of trace lipids in biological samples, thus providing efficient analytical tools and comprehensive data for metabolism and nutrition research of oilseed-derived lipids. As a principal investigator, Prof. Wei has conducted more than 10 national and ministerial competitive scientific research projects (including 3 NSFC) and she has also participated in and accomplished projects funded by the Major Research Plan of the National Natura l Science Foundation of China, National Science and Technology Support Program, National High-tech R&D Program, etc. Prof. Wei has published 100 peer-reviewed papers on the prestigious journals, including 80 papers cited by SCI (with a highest IF 16) and has gotten 12 authorized national invention patents and 3 software copyrights. Participate in 5 monographs (2 English monographs). Serve as the deputy editor of *Human Nutrition & Metabolism*. She has won the Youth Science and Technology Innovation Award of Chinese Academy of Agricultural Sciences and the First Prize of Hubei Technical Innovation Award in 2018. She has been selected as Second Level Candidates for High Level Talents in the New Century of Hubei Province in 2012 and Academic Candidates for Cultivation Project of "The Young Talents Program" under Chinese Academy of Agricultural Sciences and the "3551 Optical Valley Talent Program" candidates. She has won 100 outstanding female scientific and technological innovation talents in Hubei Province in 2020.

Unusual Polyunsaturated Fatty Acids in Edible Marine Worms Identified by Covalent Adduct Chemical Ionization Mass Spectrometry

Donghao Wang[1], Hao Yang[1], Tingxiang Yang[1]

[1] Sun Yat-sen University, Shenzhen, China

O21

ABSTRACT

Unusual seafood, such as spoon worms (Urechis unicinctus) and peanut worms (Sipunculus nudus), serve as both delicacies and potential solutions to the global food insecurity crisis. Despite being consumed primarily in parts of China, Korea, and Japan, these marine worms face challenges in wider acceptance, in part, due to the poor characterization of their fatty acids. To address this obstacle, we employed covalent adduct chemical ionization (CACI) tandem mass spectrometry (MS/MS) for the de novo identification of their unusual polyunsaturated fatty acids (PUFA). CACI is one of the advanced MS methods that derivatizes a double bond in an unsaturated lipid via an inline mode, with the Paternò-Büchi (PB) reaction and ozone-induced dissociation (OzID) being the most well-known techniques. Leveraging CACI-MS/MS, we identified several PUFA with polymethylene-interrupted (PMI) double bond configurations, including 22:3(7Z,13Z,16Z), a novel PUFA derived from sciadonic acid (20:3(5Z,11Z,14Z)). 22:3(7Z,13Z,16Z) exhibits a fragmentation pattern similar to that of 20:3(5Z,11Z,14Z), featuring an ω diagnostic ion at m/z 246, which pinpoints the Δ7 double bond position. U. unicinctus has an exceptionally low n-6/n-3 PUFA ratio of 0.15, suggesting its potential as a medicinal food to counterbalance the n-6/n-3 imbalance in modern diets. Additionally, S. nudus boasts notably high concentrations (~3%, wt/wt) of branched chain fatty acids (BCFA), exceeding typical levels found in dairy products (~2%, wt/wt).

PERSONAL BIO

Dr. Wang graduated from the Department of Food Science at Cornell University, completed a four-year postdoctoral fellowship at the University of Texas at Austin, and is now an Associate Professor at Sun Yat-sen University. For the past ten years, he has been engaged in research related to food analytical chemistry and lipids. His work primarily focuses on using mass spectrometry to reveal subtle structural differences in lipid molecules, such as double bond positions and the presence of methyl-branched chains, as well as addressing the quantitative challenges of a wide variety of lipid molecules. During his postdoctoral studies in the United States, Dr. Wang's work on lipidomics was sponsored by a pharmaceutical company due to his successful establishment of a mass spectrometry method capable of revealing the deuterium distribution of a newly developed deuterated DHA drug. Dr. Wang has further developed covalent adduct chemical ionization mass spectrometric methods (CACI-MS), initially invented by his advisor, Dr. Tom Brenna. He established a systematic method for identifying polymethylene-interrupted double bonds in fatty acids and applied the method for food analyses. He has also established an approach to quantify trace-level fatty acids using CACI-MS. Dr. Wang has published a series of articles in Analytical Chemistry and the Journal of Agricultural and Food Chemistry. Recently, he contributed to a highly anticipated book in the field of lipidomics: Mass Spectrometry for Lipidomics: Methods and Applications (Wiley, 2023), edited by Michal Holcapek, where he wrote chapters related to fatty acid mass spectrometry analysis. Dr. Wang's current research interests include elucidating the structures of complex fatty acids in certain marine organisms and the underlying mechanisms of their biosynthesis.

Pseudotargeted Lipidomics Approach for Lipid in Milk

Yong-Jiang Xu[1], Hailong Wang[1], Jinnan Lei[1], Shuang Zhu[1], Yuan He[1], Xiaoyin Chen[1]

[1] School of Food Science and Technology, Jiangnan University, Wuxi, China.

O22

ABSTRACT

Lipidomics is a powerful method for the analysis of lipids in milk, but reducing false positives and obtaining structurally accurate annotations of lipids in conventional method remain a significant challenge. In this study, we developed a novel pseudotargeted lipidomics approach for determining lipids in milk. The developed approach exhibited a competitive alternative to the acknowledged pseudotargeted strategy, including wider coverage, higher repeatability, better linearity, and similar sensitivity. The validated method was further employed to identify lipid biomarkers to distinguish milk from different varieties and origins. The experimental and computational approach is an efficient method for rapidly and specifically screening lipids.

Keywords: Pseudotargeted, Lipidomics, Lipid, milk

PERSONAL BIO

Yong-Jiang Xu, Professor, Director of the Oil and Protein Research Center in School of Food science and technology, Jiangnan University, Member of council in Nutrition Sub-Association of the Chinese Cereals and Oils Association, Deputy minister of the Youth Committee of the Jiangsu Society of Agricultural Engineering, Standing committee member of the Dietary and Nutritional Health Management Committee of Jiangsu Health Management Association, Distinguished professor in Jiangsu Province, Innovative leading talent in the "Taihu Talent Plan". He has worked in University of California, National University of Singapore and University of Antwerp for six years. His research interest is foodomics and its application in the field of oil safety, nutrition and processing. So far, he has received several research funding supporting, such as National Key R&D Program of China, Key R&D Projects of Shandong Province, and National Natural Science Foundation of China. He published more than 100 papers and more than 20 patents. He has obtained first prize in Science and Technology of China Chamber of Commerce and the first prize in Grain and Oil Technology of Jiangsu Province. He participated in a series of book writing, such as *Future Food Science and Technology, Food Chemistry, Food Lipids* and *Food Nutrition and Health*.

Regio- and Stereospecific Investigation of Natural Lipidomes and Bioavailability of Docosahexaenoic Acid

Mikael Fabritius[1], Pontus Bostrm[1], Md Al Sazzad[1], Yuqing Zhang[1], Marika Kalpio[1], Qizhu Zhao[1], Gudmundur Haraldson[2], Harald Gudmunsson[2], Yumei Zhang[3], **Baoru Yang**[1]

[1] Food Sciences Faculty of Technology, University of Turku, Turku, Finland

[2] Science Institute, University of Iceland, Reykjavík, Iceland

[3] Department of Nutrition and Food Hygiene School of Public Health, Peking University, Beijing, China

O23

ABSTRACT

Lipidomics is a fast growing field of research in food, nutriton and health. Triacylglycerols (TAGs) and glycerophospholipids (GPLs) are major components of natural lipidomes. The regioisomeric and sterespecific structures of TAGs and GPLs in food influences food quality as well as the digestion, absorption and metabolism of dietary lipids. Due to the complexity of TAGs and GPLs in natural lipidome, comprehensive analysis of regiosiomeric profiles of food lipids has been a major challenge of lipid analysis. Combining modern UHPLC-MS/MS method and machine learning technology, we recently developed novel methods enabling non-targeted analysis of TAG and GPL regioisomers of natural lipidomes of various sources. Human milk is the optimal food for infants, and the unique composition and structural profiles of human milk fat plays an important role in infant nutrition and wellbeing. Analysing regioisomers of lipidomes of human milk and alternative sources are important for improving the current knowledge in lipid biochemistry and for developing next generation infant formula. Driven by sustainable and healthy diet, consumption of proteins from alternative sources is increasing. Shifting from animal-based diet to plant-based diet inevitably alters the profiles of dietary lipids. Phospholipids as important structural and functional components of cell membranes are of special interest. In this study, we compared the regioisomeric profiles phosphatidylcholine species of food resources of animal and plant origins, creating new insights on the nutritional quality of different dietary patterns. Despite the wide interest in lipidomics in health and diseases, regio-specific profiles of lipids in organs and tissues have remained largely unresolved. Even less is known about the influence of dietary supply of n-3 PUFAs on regiospecific profiles of lipids in tissue and organs. Using the newly developed methods, the regioisomeric profiles of phosphatidylcholines in various organs and tissues in rats were revealed for the first time. Feeding with n-3 PUFA deficient diet resulted in major changes in molecular species and regiosiomer profiles of glycerophospholipids, which were effectively reversed by adding docosahexaenoic acid to the diet.

PERSONAL BIO

Professor Baoru Yang received her doctoral degree in Food Chemistry, at the University of Turku, Finland. She is the Director of Food Sciences, and the Vice Dean of the Faculty of Technology at the University of Turku. Professor Yang is a member of the Finnish Academy of Science and Letters and the Academic Chair of the Finland-China Food and Health Network. Professor Yang's research is in the field of Food Chemistry and Food Development, with special focus on the composition and health effects of food from sustainable resources. Professor Yang's research on lipids focuses on developing high throughput methods for comprehensive analysis regio- and stereoisomeric profiles of natural lipids and application of these methods to study structure-function relationships of lipids.Professor Yang has published > 270 peer-reviewed publications with a total number of citation > 13000, h-index 67.

Turning a Negative into a Positive: Applications of Charge Inversion Mass Spectrometry for Lipid Isomer Resolution

Stephen J. Blanksby[1], Berwyck L. J. Poad[1], Rhiannon J. McVeigh[1], David L. Marshall[1], Samuel C. Brydon[1]

[1] Queensland University of Technology, Brisbane, Australia

O24

ABSTRACT

Introduction: Despite recent advances in mass spectrometry-based lipid structure elucidation, some classes of isomerism still present a challenge for structural lipidomics. Branched chain fatty acids, and lipids carrying them, present one such setback, with contemporary mass spectrometry methods unable to unequivocally identify site(s) of chain branching using collisional activation. Contemporary research has indicated such lipids may have a positive impact in digestive health, yet the precise lipid structures remains an active debate. Recent developments exploiting gas-phase ion-ion reactions have demonstrated the efficacy of this approach for lipid structural elucidation, however signal-to-noise on diagnostic fragments can sometimes be low. Here we introduce a novel reagent for gas phase ion-ion reactions that provides a 10-fold enhancement compared to other reagents.

Methods: Experiments were undertaken on an ion mobility enabled quadrupole time-of-flight mass spectrometer (Waters Synapt G2-Si). Fatty acid samples were introduced by electrospray ionisation through a negatively biased ESI probe, while doubly charged 4′-nitro-2,2′:6′,2′′-terpyridine magnesium (II) reagent ions [(4-NO2Terpy)2Mg]2+ were introduced through a positively biased ESI probe. The native electron transfer dissociation capability was modified to mass select and store negatively charged fatty acids in the trap region of the instrument, and subsequently combine these with mass selected doubly charged reagent cations. The resulting singly charged ion-ion complexes [(4-NO2Terpy)(FA-H)Mg]+ were then separated by ion mobility and collisionally activated in the transfer region prior to mass analysis by time-of-flight.

Preliminary Data: Complexation of the dication reagent ions with the deprotonated fatty acid (FA) anions resulted in a singly charged positive adduct of the form [(4-NO2Terpy)MgFA]+. Collision-induced dissociation (CID) of the resulting ion-ion complexes in the transfer region of the instrument (post ion mobility) yielded rich radical directed fragmentation of the fatty acyl chain with product ion peaks spaced 14 Da apart. For fatty acids containing methyl branching, this regular pattern was interrupted at site(s) of branching with the absence of a product ion peak, enabling explicit identification of the branching sites. For unsaturated fatty acids, an enhancement of the product ions formed by fragmentation either side of the carbon-carbon double bond permitted identification of the site(s) of unsaturation. Moreover, loss of H2 was observed for cis double bonds from the precursor ion in significantly higher abundance than trans double bonds, enabling assignment of double bond stereochemistry.

Coupling the ion-ion strategy with high performance reversed-phase liquid chromatography (Waters Acquity i-Class) permitted analysis of complex mixtures containing multiple fatty acid isomers. The

LCMS separation was optimised using a mixture of three isomeric fatty acid standards containing methyl branches at either the iso (15-methyl-hexadecanoic acid) or anteiso (14-methyl-hexadecanoic acid) positions, along with the straight chain heptadecanoic acid. Extracted ion chromatograms for the diagnostic ion-ion fragmentation products enabled identification of the separated fatty acids. Application of this method to hydrolysed lipid extracts of fermented dietary supplements from Bacillus subtilis showed that this was significantly enriched in branched chain fatty acids, with the majority being branched at the anteiso position. Future coupling of this approach with in-source fragmentation of intact negatively charged glycerophospholipids with subsequent mass selection of the liberated fatty acyl chains will enable explicit linking and identification of the branched fatty acids to their respective glycerophospholipid carriers.

PERSONAL BIO

Stephen Blanksby completed his PhD (1999, U. Adelaide) in the field of gas phase ion chemistry before undertaking postdoctoral research in Europe (1999, TU Berlin) and the USA (2000-2002, U. Colorado). He held a faculty position in the School of Chemistry at U. Wollongong (Wollongong, Australia, 2002-2013) before relocating to the Queensland University of Technology (Brisbane, Australia 2014-present) where he is currently Pro-Vice Chancellor (Research Infrastructure). Stephen's research is focused on putting the fun back into fundamental gas phase ion chemistry and developing new applications in analytical mass spectrometry with a focus on structural lipidomics. He is an enthusiastic advocate for mass spectrometry within the international community serving as associate editor of the *International Journal of Mass Spectrometry* and vice-President of the International Mass Spectrometry Foundation.

Full-Dimensional Resolution of C=C Bond Isomers of Lipids

Suming Chen[1], Guifang Feng[1], Qiongqiong Wan[1], Rongrong Fu[1]

[1] The Institute for Advanced Studies, Wuhan University, Wuhan, China

O25

ABSTRACT

The structural diversity of lipids confers important biological functions, and unsaturated lipids are a subclass of lipids that contain C=C bonds in the fatty acyl chain. The greatest challenge facing structural lipidomics research at present is the accurate characterization of information such as double bond position, cis-trans isomerism, and stereospecific numbering (sn) of fatty acids in lipid isomers. We pioneered the use of visible light reaction for lipid isomer analysis and developed visible light-induced [2+2] cycloaddition reactions of C=O and C=C bonds, which realized mild activation and enhanced fragmentation of double bonds in lipids, and improved the comprehensive analysis and accurate identification of lipid double bond isomers in complex biological systems. In order to further solve the problem of ionization efficiency of double-bond derivatives, we innovatively used the aziridination reaction for the selective derivatization of unsaturated lipid C=C bonds. We also proposed the new concept of increasing the chromatographic feature dimension of double-bond cis-trans isomers by photoisomerization, which realized the precise characterization of the stereo-configuration of the double bonds of unsaturated lipids. Through the development of a novel visible-light-catalyzed sensitized isomerization reaction, a single cis-trans isomer was converted into a mixture of cis-trans isomers, which led to the generation of multidimensional characteristic chromatographic peaks and then to the precise characterization. The reaction system can simultaneously realize the photocycloaddition of double bonds, thus realizing the full dimensional identification and analysis of the stereo configuration and position of unsaturated lipid double bonds.

PERSONAL BIO

Dr. Suming Chen is a professor at Wuhan University and a young expert of National Talent Program. He received his Ph.D. degree from the Institute of Chemistry, Chinese Academy of Sciences in 2010. After working there as an associate professor for another four years, he moved to The Ohio State University and Johns Hopkins University to conduct the postdoctoral research. At the end of 2018, he joined The Institute for Advanced Studies, Wuhan University. His current research focuses on the deep structure-resolved mass spectrometry analysis. He has published more than 70 papers, including correspondent/first author research papers in prestigious journals such as *Nature Nanotechnology, Nature Communications, Science Advances, Journal of American Chemical Society, Angewandte Chemie*, etc. He is also a member of the editorial board of Chinese Chemical Letters and the editorial board of *Journal of Chinese Mass Spectrometry Society*.

Lipid isomer analysis: From high-throughput characterization to point-of-care testing

Wenpeng Zhang[1]

[1] Department of Precision Instruments Tsinghua University, Beijing, China

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ABSTRACT

Mapping the complete molecular composition of a lipidome is a crucial objective in lipidomics for understanding the pathways and mechanisms behind lipid homeostasis. Previously, we implemented the PaternòBüchi (PB) reaction online with LC-MS/MS for the large-scale identification of lipid C=C location isomers. This approach successfully identified over 200 molecular species of phospholipids at the C=C locations from biological samples. It has been utilized to screen lipid biomarkers for diseases such as breast cancer, type 2 diabetes, glioma, and viral infections. Recently, we integrated the PB reaction into capillary electrophoresis (CE)-MS, which enabled the highly efficient separation of various lipid classes and enhanced the characterization of phospholipid C=C isomers in raw biological samples and small cell populations. To detect lipid isomers rapidly, we developed fast lipid enrichment methods such as polymer coating transfer enrichment and polyporous membrane microsampling. Coupled with photochemical derivatization and direct MS/MS, these methods allowed for the identification and relative quantification of lipid C=C isomers in biofluid and tissue samples. This approach has been applied to the rapid analysis of potential lipid biomarkers of diseases such as type 2 diabetes and gliomas.

PERSONAL BIO

Wenpeng Zhang is currently an associate professor of the Department of Precision Instrument, Tsinghua University. He received Ph.D. in Pharmaceutical Science from Wuhan University. He was a postdoctoral scholar at Purdue University from 2015 to 2020 and joined Tsinghua University in 2021. His research focuses on biological mass spectrometry, lipidomics and clinical applications of mass spectrometry. Dr. Zhang has lead projects such as the Excellent Youth Fund of NSFC, and the National Key R&D Program of China. He has published over 80 papers in journals such as Nature Communications, PNAS, and Angewandte Chemie. He also serves as associate editor of Frontiers in Chemistry and editorial board member of Green Analytical Chemistry. He is the recipient of awards such as Jin Guofan Prize for Excellent Youth of China Instrument Society and Zhu Liangyi Analytical Instrument Innovation Award.

Aza-Prilezhaev Aziridination-Enabled Multidimensional Analysis of Lipids and Related Applications via High-Resolution U-Shaped Mobility Analyzer-Mass Spectrometry

Yuling Li[1], Yiming Wang[1], Kang Guo[1], Kuo-feng Tseng[1], Xiaoqiang Zhang[1], Wenjian Sun[1]

[1] Shimadzu Research Laboratory (Shanghai) Co. Ltd.

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ABSTRACT

A metal- and additive-free aza-Prilezhaev aziridination (APA)-enabled ion mobility mass spectrometric method is developed for probing multiple levels of unsaturated lipid isomerization with high-sensitivity. Both unsaturated polar and nonpolar lipids can be efficiently labeled in the form of N-H aziridine without significant side reactions. The signal intensity can be increased by up to three orders of magnitude, especially for non-polar lipids, achieving nM detection limit. Abundant site-specific fragmentation ions can be obtained to indicate C=C location and sn-position in MS/MS spectra. Better yet, stable mono-aziridination product is dominant, simplifying the spectrum for lipids with multiple double bonds. To further boost the LC on-line coupling capability of this method, a second generation reagent was developed to speed up the reaction rate and minimize the peak broadening effect.

U-shaped mobility analyzer is one kind of counter-flow ion mobility spectrometers in which ions can be separated by their mobilities along a U-shaped trajectory. One unique feature is that it can be operated as a high-resolution mobility filter, which enables high dynamic range and good quantitation capability.

Coupled with a U-shaped mobility analyzer, identification of geometric isomers and separation of different lipid classes can be achieved. Additionally, a unique pseudo MS3 mode with UMA-QTOF MS boosts the sensitivity for generating diagnostic fragments. Applications in analysis of isomeric lipids with this method will be demonstrated. Overall, the current method provides a comprehensive solution for deep-profiling of lipidome, which is valuable for lipid marker discovery in disease monitoring and diagnosis.

PERSONAL BIO

Dr. Wenjian Sun received his Ph.D. in 2007 from Texas A&M University under the supervision of Prof. David Russell for analytical chemistry research and instrumentation. Since then he joined Shimadzu Research Laboratory (Shanghai) for the development of ion mobility and mass spectrometry related technologies. Dr. Sun became the managing director of the company in 2012 and he continuously supervised a variety of projects in development of hardware, software and applications for multi-omics and small drug molecules. Dr. Sun constantly publishes in research journals for the new discoveries and developments, and he is also inventor or co-inventor of 70+ original patents in the fields of mass spectrometry and ion mobility spectrometry.

Cutting-Edge Lipidomics to Illuminate the Host-Microbiome Interactions

Makoto Arita[1,2,3,4]

[1] Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[2] Division of Physiological Chemistry and Metabolism, Keio University Faculty of Pharmacy, Tokyo, Japan
[3] Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q), Keio University, Tokyo, Japan
[4] Graduate School of Medical Life Science, Yokohama City University, Yokohama, Japan

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ABSTRACT

Abnormal lipid metabolism is often a background factor of diseases, which may lead to the discovery of new seeds for drug discovery and medical applications such as early diagnosis and treatment. Recent advances in mass spectrometry have provided a major impact on lipid biology, suggesting that the lipid molecules analyzed in the past are only the tip of the iceberg. Host-microbiome interactions create a unique metabolic milieu that modulates intestinal environments. By combining non-targeted mass spectrometry with feature-based molecular spectrum networking technology, we revealed that lipids with complex structures are produced by gut microbiota. Correctly capturing such molecular groups that have been overlooked by conventional targeted analysis will lead to understanding the significance of bacterial species that correlate with various diseases. The method developed in this study is a technology that enables us to view such changes in the bacterial flora as changes in the lipidome environment from a bird's eye view, and is expected to contribute to the identification of functional metabolites that mediate the host-microbiome interactions.

PERSONAL BIO

Dr. Makoto Arita received his Ph.D. from the Graduate School of Pharmaceutical Sciences, University of Tokyo in 1997. Currently, he is a Dean of the Faculty of Pharmacy and a Professor of Physiological Chemistry and Metabolism at Keio University, and a Team Leader of RIKEN Center for Integrative Medical Sciences. Dr. Arita has experience leading multidisciplinary research teams as a principal investigator for "Biology of LipoQuality" a program project supported by JSPS Grant-in-aid for Scientific Research on Innovative Areas (FY2015-2020). He serves as an Executive Editor for the Progress in Lipid Research. Now he is leading JST-ERATO Lipidome Atlas Project (FY2021-2026) to pioneer the spatiotemporal biology of lipid diversity through a creation of the Lipidome Atlas, and to discover unknown molecules associated with important biological processes. Also, he is a Core Director of the Keio University World Premier International Research Center Initiative (WPI-Bio2Q) (FY2022-2032) to integrate human biology, microbiome research, and quantum computing to explore the foundations of healthy longevity.

The 'ABC's of Microbial Lipidomics for Discovery of Novel Lipids and Functions in Enterobacteriaceae

Xueli Guan[1]

[1] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

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ABSTRACT

Lipids play instrumental role for the survival of a bacterium by serving as both an energy source as well as a protective barrier against its external environment. In recent years, it is becoming increasing evident that lipids produced by bacterium also serve as signalling molecules involved in host-microbe interactions. With the great lipid diversity exhibited by different microbes, novel approaches are instrumental to probe this biochemical space for discovery of lipids and their functions in human health. Here, I will present the development and application of a combinatorial 'ABC' approach involving analytical chemistry, biology and computation to probe the complex lipid repertoire of Enterobacteriaceae, and discovery of novel lipid biochemistry and functions in infectious disease. I will further share the ongoing international inter-laboratory efforts to define a reference bacterial lipidome for future standardization in the field.

PERSONAL BIO

Dr Xue Li Guan is a Nanyang Assistant Professor in Lee Kong Chian School of Medicine, Nanyang Technological University (Singapore). Dr Guan obtained her PhD from the National University of Singapore in 2009 and was a recipient of an EMBO short term fellowship during her PhD to work in University of Geneva. She further pursued her postdoctoral training in systems biology within a SystemsX.ch consortium, LipidX, in Switzerland. In 2011, she secured the competitive Ambizione career grant from the Swiss National Science Foundation and became a Group Leader of Lipidomics and Systems Biology in Swiss Tropical and Public Health Institute (Swiss TPH). In 2016, she was awarded the Elite Nanyang Assistant Professorship to pursue her research on systems biology of lipid metabolism and functions in human health and diseases. Dr. Guan is currently the Vice President of Early Career Representative, and a member of the steering committee for the microbial lipids interest group within the International Lipidomics Society. Her laboratory drives development and application of lipidomics tools to elucidate the impact of alterations of host and microbial metabolism on outcome of diseases and treatment response. The new insights in biology and the biochemical resources generated will contribute to modern medicine, particularly in drug target and biomarker discovery.

Quantitative Profiling of Lipid Trafficking Between Organelles Enabled by Subcellular Photocatalytic Labeling

Zheng-Jiang Zhu[1]

[1] Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China

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ABSTRACT

Subcellular lipid composition and trafficking significantly influence the physiological and pathological functions of both cells and organelles. However, lipid trafficking between organelles remain poorly understood due to a lack of methods for selectively labeling lipids in organelles. Here, we develop a subcellular photocatalytic labeling strategy that enables the organelle-selective lipid analysis by mass spectrometry and the quantitative profiling of lipid trafficking between organelles. Fatty acyl-dependent trafficking of phosphatidylethanolamine (PE) and phosphatidylserine (PS) lipids is quantitatively characterized between the endoplasmic reticulum (ER) and mitochondria or nucleus. Relative contributions of various biosynthesis pathways and trafficking routes to the PE and PS lipid compositions in mitochondria and nucleus are unraveled. Notably, a previously uncharacterized PE trafficking route from ER to mitochondria is uncovered, contributing ~20% of PE lipids in mitochondria. Together, subcellular-localized photocatalytic labeling of lipids quantitatively deciphers the subcellular lipid composition and trafficking, enhancing our understanding of lipid metabolism in living organisms.

PERSONAL BIO

Dr. Zheng-Jiang Zhu received his B.S. degree in Chemistry from Nanjing University in 2006, and his Ph.D. degree in Chemistry from University of Massachusetts at Amherst, United States in 2011. Then, he moved to The Scripps Research Institute as a Postdoctoral Research Associate with Prof. Gary Siuzdak from 2011 to 2013. From 2013, he was appointed as Principal Investigator and group leader for Laboratory for Mass Spectrometry and Metabolomics, affiliated with Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (CAS). Please visit Dr. Zhu website (www.zhulab.cn) for more information.

Development of Sensitive LC-MS Methods for Comprehensive Lipidome Analysis

Liang Li[1]

[1] The Metabolomics Innovation Centre and Department of Chemistry, University of Alberta, Edmonton, Canada

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ABSTRACT

The lipidome comprises a diverse array of lipid species, making profiling the full spectrum of lipids an analytical challenge. To address this, we are developing an advanced analytical platform for comprehensive lipidome analysis. Our approach employs ultra-high-performance liquid chromatography (UHPLC) and high-resolution mass spectrometry (HRMS), incorporating new and improved sample preparation processes, optimized chromatographic separation, and enhanced ionization protocols. We are also developing data processing strategies for precise quantitation and structural elucidation of lipid species. Combined with advanced bioinformatics, our platform significantly expands the detectable lipidome, including the profiling of low-abundance lipids. This presentation will highlight recent advances in our lipidomics platform and workflow.

PERSONAL BIO

Dr. Li obtained his BSc in Chemistry from Zhejiang University in 1983 and his PhD from the University of Michigan, Ann Arbor, Michigan. He joined the University of Alberta in July 1989, where he is Professor of Chemistry and Adjunct Professor of Biochemistry. He is the Co-Director of the Metabolomics Innovation Centre (TMIC) of Canada. He is an elected fellow of the Royal Society of Canada (Academy of Science). Dr. Li was Tier 1 Canada Research Chair in Analytical Chemistry from 2005 to 2019. He served as Director, Alberta Cancer Board Proteomics Resource Laboratory, from 2000 to 2005. He was Chair of Analytical Chemistry Division at the University of Alberta from 2007 to 2019. He was a Co-PI of the Human Metabolome Database (HMDB) Project; his laboratory generated the HMDB MS/MS spectral library of the endogenous human metabolites which has been widely used by the metabolomics community. His laboratory is a pioneer in developing the high-performance chemical isotope labeling liquid chromatography mass spectrometry (HP-CIL LC-MS) platform for quantitative and comprehensive metabolome profiling of bio-systems. Dr. Li has received a number of national and international awards and honors. He is an editor of *Analytica Chimica Acta*, an international journal on analytical chemistry, since 2005.

Lipidomics analysis for large-amount samples and single cells

Guowang Xu[1]

[1] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

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ABSTRACT

Recently lipidomics and metabolomics have been applied in many different fields by using untargeted and targeted strategies. It is expected to obtain more metabolites or lipid species information including isomer resolution by using high resolution mass spectrometry (MS) and the accurate quantitation results by using TQ MS. Unfortunately in untargeted analysis the data repeatability and batch effect exist, therefore, in most of papers less than 100 samples were analyzed, leading to poor biomarker discovery and validation. It should be emphasized that a scientific clinic-related results should be based on large-scale samples, therefore, the analytical throughput also becomes an important issue.

On the other hand, to understand mechanism cell lipidomics and metabolomics are necessary. In some situations, the study should at the single cells level, but big challenges exist, especially in sensitivity and throughput.

Facing above these issues, our lab has established a comprehensive metabolomics and lipidomics platform. It can be used to analyze the large-scale clinic samples for the metabolic reprogramming study, disease subtyping and biomarker discovery, in the meantime single cells lipid/metabolite analysis can also be carried out based on our nanomate-ESI method or a serpentine channel microfluidic device coupled with a pulsed electric field-induced electrospray ionization-high resolution MS. This lecture will report our new results.

PERSONAL BIO

Prof. Dr. Guowang Xu received his Ph.D in Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS) in Jun., 1991. He was the administrative vice-director of Biotechnology Division (2016.4-2023.11), and is director of CAS Key Laboratory of Separation Science for Analytical Chemistry in DICP. He is also president of Chromatography Committee of Chinese Society of Chemistry, deputy-president of the Tumor Metabolism Committee of Chinese Society of Anticancer and a member of permanent scientific committee of HPLC. Prof. Xu has co-written 5 books and published 540+ peer-reviewed papers in the 'Web of Science Core Collection' (WoS) indexed journals including *PNAS, Nature Protocols, Nature Commun., Nature Methods, Cell Metab., Advance Science, Hepatology, Cancer Res., Anal. Chem.,* etc., and holds more than 100 China patents. His main research fields are in the chromatography-mass spectrometry (MS) technique development and the MS-based metabolomics/exposomics applications in disease biomarker discovery, traditional Chinese medicines and food safety.

Optimization of Skin Sampling Based on Tape-Stripping for Skin Lipidomics by Nanoflow nUHPLC-ESI-MS/MS

Seung Hee Shin[1], **Myeong Hee Moon**[1]

[1] Yonsei University, Seoul, Republic of Korea

O33

ABSTRACT

In human skin, lipids play a primary physical barrier against the external environment. Since alterations in cutaneous lipid profiles can be related with the numerous inflammatory condition of skin, skin lipidomics is of an increasing interest. In order to sample skin lipids, a tape stripping method using an adhesive tape can be utilized to collect the stratum corneum layers which is the outermost layer of epidermis. Since a number of variables in the sampling of skin may affect the efficiency in the analysis of skin lipidome, a proper guideline of skin sampling is needed but there is no standardized procedure of skin sampling for lipidomic analysis.

This presentation will show an optimization of skin sampling based on the tape stripping for lipidomic analysis with nanoflow ultrahigh performance liquid chromatography-electrospray ionization-tandem mass spectrometry (nUHPLC-ESI-MS/MS). Lipid analysis was accomplished by the structural determination of individual lipid molecules, mostly ceramides and glycerolipids which are the major constituents of stratum corneum, based on collision-induced dissociation experiments using MS/MS followed by targeted quantification. Optimization of the tape stripping method for high-throughput lipidomic analysis was made by comparing the lipid composition and amounts of lipids at different sampling locations, among the adjacent spots in the same location, upon consecutive sampling at the same location, and a minimum number of pooling tapes for quantitation purpose. Additionally, investigations into the matrix effect and extraction efficiency upon pooling layers indicated that pooling up to five layers did not significantly impact ionization suppression or reduce extraction recovery. The limit of detection and the limit of quantitation in the skin lipid analysis were examined.

PERSONAL BIO

Myeong Hee Moon completed his undergraduate studies in chemistry at Yonsei University in 1987 and earned his Ph.D. from the University of Utah in 1991 under Prof. Calvin J. Giddings. He began his academic career at Kangnung National University in 1994 and joined Yonsei University's chemistry department in 2003. His research focuses on developing methodologies in flow field-flow fractionation (FFF) for separating and characterizing biological macromolecules, lipid analysis with LC-ESI-MS/MS, and hyphenating FFF with MS for direct analysis of lipids.

Development of a Comprehensive and Quantitative Lipidome Analysis Platform Based on Supercritical Fluid Chromatography/ Mass Spectrometry

Takeshi Bamba[1]

[1] Kyushu University, Fukuoka, Japan

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ABSTRACT

With advancements in chromatographic separation techniques using HPLC and mass spectrometry, comprehensive measurements of lipid molecular species have become feasible. Particularly, RP-LC/MS employing reversed-phase supports is widely used due to the hydrophobic interactions between octadecylsilyl (ODS) supports and lipid molecules, facilitating the separation of individual lipids, including isomers. However, this separation complicates quantitative analysis due to the matrix effects in mass spectrometry, which are challenging to correct accurately. Conversely, normal phase chromatography (NPLC) and hydrophilic interaction chromatography (HILIC) can separate lipid classes by recognizing the head structures of lipids, such as glycerophospholipids and glycolipids. By adding internal standards for each lipid class to the sample extract, quantitative measurement becomes possible. Nonetheless, NPLC and HILIC face challenges with comprehensive separation and retention time reproducibility. Moreover, NPLC's high hydrophobic mobile phase makes it unsuitable for mass spectrometry.

Supercritical fluid chromatography (SFC) emerges as a suitable method for lipidome analysis, offering high-resolution separation of lipid classes using a normal-phase column and a mobile phase compatible with sensitive mass spectrometry analysis. Our group developed a new lipidome analysis method leveraging SFC coupled with triple quadrupole mass spectrometry (QqQ-MS) for comprehensive and quantitative lipid analysis. Using a DEA (diethylamine) column in a normal-phase SFC system, we achieved the separation of 22 lipid classes within 20 minutes with high resolution. SFC's advantage over conventional NPLC and HILIC lies in its ability to separate a broad range of lipid classes more rapidly. Notably, SFC allowed for the chromatographic separation of lysophospholipids and regioselective isomers of neutral lipids (MG, DG), which mass spectrometry alone cannot discriminate. Additionally, detailed fragmentation analysis of various lipid classes revealed that structural isomers of diacylphospholipids could be separated by mass spectrometry with appropriate MRM settings. Tests on the recovery of individual lipid molecules showed rates exceeding 70%, confirming the capability for comprehensive and quantitative lipid analysis. Analyses of animal cells, plasma, and various tissues demonstrated the quantitative analysis of over 400 lipid molecular species.

We are currently developing MRM transition list creation software and quantitative data analysis software based on in-silico databases, while also conducting non-targeted analysis using SFC coupled with high-resolution MS. I would like to sharing the latest findings from our research and development with you.

PERSONAL BIO

Takeshi Bamba is a professor at the Division of Metabolomics, Medical Research Center for High Depth Omics, Medical Institute of Bioregulation, Kyushu University, Japan. He obtained his Ph. D. degree in engineering from Osaka University. He spent five years doing a NEDO project postdoc at Hitachi Zosen Co. He held an assistant professor in the Graduate School of Pharmaceutical Science and an associate professor in the Graduate School of Engineering at Osaka University, and became the professor at Kyushu University in 2015. His research revolves around an analytical science approach to metabolomics. His laboratory focuses on the development of various metabolomics technologies such as sample preparation, instrument analysis and data mining, and their application researches in various fields. He has published more than 200 articles in scientific journals/books.

Unraveling the Spatial Lipidome Using Gas-Phase Ion/Ion Reactions

Boone M. Prentice[1]

[1] University of Florida, Gainesville, USA

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ABSTRACT

Imaging mass spectrometry enables the visualization of molecular pathology directly in tissue. However, the complexity of the spatial lipidome results in the presence of many isomeric compounds that complicate spectral analysis. Each individual component in this mixture may possess a unique cellular function and spatial distribution, necessitating means to effect lipid isomer separation. Conventional chromatographic methods are often incompatible with pixel-by-pixel imaging mass spectrometry timescales. Instead, investigators have turned to tandem mass spectrometry (MS/MS) to enable isomer separation in the gas-phase. Conventional MS/MS approaches performed using low-energy collision induced dissociation (CID) of protonated or deprotonated lipid ion types result in limited structural information, necessitating alternative MS/MS approaches.

MS/MS structural information is dependent on the dissociation method and ion type. Previous reports leveraged metal-cationized lipid ion types to produce complementary fragmentation information upon CID. Herein, we build on that work by forming divalent metal-ligand-lipid ion types. We have achieved metal cationization in the condensed-phase by spraying salt mixtures onto the tissue surface using a robotic sprayer prior to matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry. We have also achieved metal cationization in the gas-phase by performing charge inversion ion/ion reactions between singly charge lipid analyte ions and multiply charged metal-ligand reagent ions. These gas-phase transformations are fast, efficient, and specific, making them ideally suited for implementation into imaging mass spectrometry workflows. Our prior work has shown ion/ion reactions to enable the identification of multiple sn-positional phosphatidylcholine isomers as well as the separation of isobaric phosphatidylserines and sulfatides.

We have used divalent metal cationization to reveal sn and double bond (C=C) isomers in biological tissues. For example, CID of [phosphatidylcholine+metal(II)+anion]+ ion types eliminates the headgroup loss fragmentation channel and favors fragmentation at the fatty acyl chain positions. Further, we have used CID of [phosphatidylcholine+metal(II)+anion]+ ion types to improve carbon-carbon bond fragmentation efficiency roughly 10-fold along the fatty acyl tail to identify C=C position. We have also used magnesium tris-phenanthroline reagent dications to affect a gas-phase ion/ion reaction with fatty acid analyte monoanions. The resulting [FA-H+MgPhen2]+ metal-ligand complex produces charge remote fragmentation upon subsequent CID, enabling C=C localization. Finally, we demonstrate imaging of [sulfatide-H]- beta and alpha isomers using a [Sr(Phen)3]2+ ion/ion reaction reagent.

PERSONAL BIO

Boone Prentice is Assistant Professor in the Department of Chemistry at the University of Florida. He received his B.S. in Chemistry from Longwood University (Farmville, VA), and completed his Ph.D. in Chemistry at Purdue University (West Lafayette, IN) under the mentorship of Prof. Scott McLuckey studying gas-phase ion/ion reactions and ion trap instrumentation. He then completed his postdoctoral work in the Department of Biochemistry at Vanderbilt University (Nashville, TN) as an NIH NRSA fellow under the guidance of Prof. Richard Caprioli before joining the faculty at UF in 2018. He was awarded an NIH Focused Technology Research and Development R01 grant in 2020 and a JDRF Innovation Award in 2023 to support his research developing gas-phase reactions and imaging mass spectrometry technologies to study the molecular pathology of diabetes, infectious disease, neurodegeneration, and neuropharmacology. He was also awarded the 2022 Young Investigator Award from Eli Lilly and Company, which is an unsolicited award given annually by Eli Lilly's Analytical Chemistry Academic Contacts Committee to recognize a "rising star" in analytical chemistry. He has been highlighted as a 2023 Emerging Investigator by the Journal of the American Society for Mass Spectrometry, a 2023 Young Investigator in (Bio-)Analytical Chemistry by Analytical and Bioanalytical Chemistry, a 2023-2024 Early Career highlight by the Journal of Mass Spectrometry, and a 2024 Young Scientists Feature by the International Journal of Mass Spectrometry.

Subcellular Lipid Imaging with Nanoscale Secondary Ion Mass Spectrometry

Haibo Jiang[1]

[1] The University of Hong Kong, Hong Kong, China

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ABSTRACT

Tracing the trafficking of lipids in cells and tissues has been challenging. The nanoscale secondary ion mass spectrometry (NanoSIMS) technique has provided a feasible method to trace lipids due to its capability of mapping elements and stable isotopes with high spatial resolution and high sensitivity. NanoSIMS utilizes a focused primary ion beam to bombard the sample surface, generating high-resolution chemical information and enabling the tracing of stable-isotope labelled lipids in cells and tissues with the organelle level resolution. This presentation will discuss our work developing NanoSIMS-based analytical workflows, combining multimodal imaging techniques to visualize a range of lipid nutrients in biological systems and the applications in investigating the functions of key proteins in lipid trafficking.

PERSONAL BIO

Dr. Haibo Jiang is an Associate Professor in the Department of Chemistry at The University of Hong Kong and the Director of the JC STEM Lab of Molecular Imaging. He obtained a bachelor's degree from Shanghai Jiao Tong University and a Ph.D. from the University of Oxford. His subsequent academic positions included roles as a Lecturer and Group Leader at The University of Western Australia and Visiting Assistant Professor at the David Geffen School of Medicine at UCLA. His work focuses on the development and application of bioimaging methods to study molecular trafficking and metabolism.

Subcellular Resolution MALDI Mass Spectrometry Imaging of Lipids

Jianing Wang[1] , Chengyi Xie[1], Lei Guo[1], Zongwei Cai[1]

[1] State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

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ABSTRACT

In biomedical research, molecular mass spectrometry imaging techniques, especially those based on Matrix-Assisted Laser Desorption/Ionization (MALDI), are becoming increasingly important. The advanced design of the optical system has successfully enhanced the resolution of MALDI imaging to 1.2 m, with oversampling tested at 0.6 m intervals. This advancement represents significant progress in technology. However, challenges persist in achieving high-quality matrix deposition at high resolution and maintaining system stability. Currently, the highest resolution available for commercial MALDI MS imaging systems is 5 m. If unmodified commercial systems could achieve subcellular resolution, it would not only significantly enhance their application but also maintain high stability and usability.

Improvements in sample preparation methods and strategies can achieve higher signal-to-noise ratios and image contrast in the sample at a certain resolution, thereby maximizing the effective resolution of the instrument. We have developed optimization methods and data analysis strategies, significantly enhancing the sensitivity and coverage of single-cell imaging at a 5 m resolution. Through multidimensional statistical analysis, we have demonstrated subcellular resolution lipid localization changes associated with the cell cycle and environmental stress. Furthermore, we have developed a tenfold (10x) expansion MS imaging technique that has enhanced the imaging resolution of unmodified commercial mass spectrometers to the sub-micrometer level, achieving an imaging resolution of 500 nm. This enhanced resolution allows for the visualization of tissue structures very similar to those observed with optical microscopes, enabling more precise analysis of the correlation between structure and function. Notably, this technology not only identifies individual neurons in mouse brain tissue but also successfully displays the dendritic structure of Purkinje cells with widths less than 1 m. Additionally, the technology reveals subcellular structural details within single cells, opening new research opportunities to explore the biological functions of lipids at the subcellular level.

In summary, our research has achieved an unprecedented level of detail in subcellular lipid mass spectrometry imaging, significantly enhancing our ability to study complex biological systems and utilize mass spectrometry imaging for cellular biology research.

PERSONAL BIO

Dr. Jianing Wang is an experienced and accomplished researcher in the field of mass spectrometry imaging and biological mass spectrometry. He is currently a Research Assistant Professor at the State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University. Dr. Wang received his B.Sc. in Chemistry from Xiamen University, M.Sc. in Cell Biology from Beijing Normal University, and Ph.D. from the Institute of Chemistry, Chinese Academy of Sciences. He continued his research as a postdoctoral fellow at the Sanford Burnham Prebys Medical Discovery Institute and the University of Texas Health Science Center.

Dr. Wang has made significant contributions to the development and application of mass spectrometry imaging, particularly in high spatial resolution and isomer-resolved mass spectrometry imaging. He has authored 47 peer-reviewed publications and holds 8 patents. His innovations in MALDI mass spectrometry imaging have substantially improved the coverage and sensitivity of lipid and metabolite imaging, advancing MALDI-TOF MS imaging and small molecule detection.

Among his notable achievements is the development of the high-resolution NEDC-assisted MALDI MSI method, which has gained widespread adoption globally. The NEDC matrix is highly regarded for its robust performance and has become a preferred choice in metabolite mass spectrometry imaging, facilitating significant advancements in biomedical research.

In recent years, Dr. Wang's research has focused on high spatial resolution and isomer-resolved mass spectrometry imaging. He established a method for ion mobility mass spectrometry imaging that simultaneously distinguishes 19 chiral amino acids and developed a technique that triples the coverage of lipid C=C double bond isomer mass spectrometry imaging.

Furthermore, Dr. Wang has advanced high-sensitivity and high-coverage single-cell imaging methods and developed a tenfold expansion mass spectrometry imaging technique, enhancing the imaging resolution of commercial mass spectrometers to 500 nanometers. These technological breakthroughs have enabled the detailed observation of subcellular structures, significantly enhancing the research capabilities for studying complex biological systems.

MALDI-2 and t-MALDI-2 Mass Spectrometry Imaging of Lipids: New Applications and Perspectives

Klaus Dreisewerd[1]

[1] University of Münster, Institute of Hygiene, Robert-Koch-Str. 41, 48149, Münster, Germany

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ABSTRACT

Arguably, matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI in short) is the most widely used chemical imaging modality for the spatially-resolved analysis of various classes of lipids in tissue sections and in cell culture. Recent technology advancements, such as MALDI with laser-based postionization (MALDI-2) and transmission mode t-MALDI-2 MSI,1,2 now enable sensitive lipid profiling at a cellular to partially sub-cellular resolution and pixel sizes in the low to one micrometer range. Moreover, the direct in-source coupling of t-MALDI-2-MSI with bright-field and fluorescence microscopy, as recently achieved in our laboratory (unpublished data), now enables the co-registration of cellular lipids and stained proteins at high spatial confidence.

Following a brief excursion into the historical roots of the MALDI technique at Münster University, I will in may talk depict some of these current developments and their adaptation to state-of-the-art mass spectrometers.3 I will discuss their analytical abilities and current limitations with examples from ongoing interdisciplinary collaborations. This will include the (correlative) MALDI- and t-MALDI-2-MS imaging of lipids and further compounds and microscopy-based cell markers from healthy and tumorous tissues and isolated single cells. To illustrate how highly-resolved MALDI-2-MSI can also help to obtain new insights in the multicellular mechanisms of bacterial biofilm formation, I will present recent data from an interdisciplinary study on the model system Bacillus subtilis.

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3: Soltwisch J et al., Anal Chem 2020, 92, 8697-8703. DOI: 10.1021/acs.analchem.0c01747

PERSONAL BIO

Klaus Dreisewerd is a Professor of Biophysics at the University of Münster and head of the research group Biomedical Mass Spectrometry at Münster Medical School. He graduated under the supervision of two of the early MALDI pioneers, Franz Hillenkamp and Michael Karas, before he moved to the Free University of Amsterdam to participate in one of the first MALDI-TOF research projects on single cell profiling. Since his return to Münster University in 1997, he continues to work on methodological and instrumental advancements of the MALDI technique with a particular interest in the physical and physicochemical fundamentals of the laser-based method. Since several years, a strong application-driven research focus of Klaus Dreisewerd and his group is on the advancement of highly-resolved MALDI imaging and its coupling with correlative microscopy. For this, the team collaborates closely with numerous partners from industry and academia.

Enabling High-Throughput Quantitative Lipidomics via Innovation in Chemical Tagging Approaches

Lingjun Li[1] , Shuling Xu[1], Zhijun Zhu[1], Ting-Jia Gu[1], Peng-Kai Liu[1], Zicong Wang[1]

[1] University of Wisconsin-Madison, Madison, USA

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ABSTRACT

Lipids are crucial for cellular homeostasis, with their functions closely linked to their structures and concentrations. Accurate lipid identification and quantification are key to studying their roles, but their diverse structures make comprehensive quantification challenging. Here, we present several chemical labeling approaches that enable multiplex quantitative lipidomics in complex biological samples with high throughput and improved accuracy.

First, we propose a diazobutanone-assisted isobaric labeling strategy as a rapid and robust platform for multiplexed quantitative lipidomics across a broad range of lipid classes, including various phospholipids and glycolipids. Our method demonstrates excellent performance in terms of labeling efficiency, detection sensitivity, quantitative accuracy, and broad applicability to various biological samples. Next, we performed a 6-plex quantification analysis of lipid extracts from lean and obese mouse livers. In total, we identified and quantified 246 phospholipids in a high-throughput manner, revealing lipidomic changes that may be associated with obesity in mice.

Furthermore, we also explore the utility of N,N-dimethyl leucine (DiLeu)-based chemical tagging strategies for relative and absolute quantification of multiple lipid subclasses. By employing a novel 12-plex isobaric dual-reactive dimethylaminopyridine-activated N,N-dimethyl leucine- (DiLeu-DMAP) tags the detection sensitivity for nonpolar subclasses can be increased by up to 200-fold, enhancing annotation accuracy. Utilizing this method, we analyzed brain tissue lipid changes in a mouse model of Alzheimer's disease, quantifying 243 lipid species across 11 subclasses. Finally, we explore functionalization of the free primary amine groups of aminophospholipids (APLs) using amine-reactive isotopic N,N-dimethyl leucine (iDiLeu) and employ high-resolution ion mobility mass spectrometry (IM-MS) to develop a novel method for sensitive discernment and accurate quantification of APL sn-isomers, which have long been considered as the "dark matter" of traditional lipidomics analyses. Specifically, 5-plex iDiLeu labeling enables construction of an internal four-point calibration curve and therefore absolute quantification of APL sn-isomers in a single run.

PERSONAL BIO

Dr. Lingjun Li is a Vilas Distinguished Achievement Professor and the Charles Melbourne Johnson Distinguished Chair Professor of Pharmaceutical Sciences and Chemistry at the University of Wisconsin-Madison (UW-Madison). Dr. Li received her Ph.D. degree in Analytical Chemistry/Biomolecular Chemistry from the University of Illinois at Urbana-Champaign in 2000. She then did joint postdoctoral research at the Pacific Northwest National Laboratory and Brandeis University before joining the faculty at UW-Madison in December 2002. Dr. Li's research interests include the development of novel mass spectrometry (MS)-based tools such as new isotopic and isobaric labeling strategies that enable multiplexing for quantitative proteomics, peptidomics, lipidomics, metabolomics, and glycomics, and their applications in neuroscience and cancer research. Professor Li has established a highly productive research program and published more than 400 peer-reviewed research journal papers (with H-index of 67, and more than 17,299 citations) and has given more than 300 invited talks. Dr. Li has been recognized with numerous awards, including American Society for Mass Spectrometry (ASMS) Research Award, NSF CAREER Award, NSF Special Creativity Award, Sloan Fellowship, PittCon Achievement Award, and ASMS Biemann Medal, and was named one of the Top 50 most influential women in the analytical sciences in 2016 and featured in the 2019, 2021, and 2024 Power List by the Analytical Scientist. Dr. Li is currently serving as an Associate Editor for the Journal of the American Society for Mass Spectrometry (JASMS) and sitting on the Advisory Board for Analytical and Bioanalytical Chemistry and Mass Spectrometry Reviews. Dr. Li served on the Board of Directors for the US Human Proteome Organization (US HUPO) 2015-2018. Dr. Li was also the President for the Chinese American Society for Mass Spectrometry (CASMS has more than 1000 members in the US) for 2015-2017, and is currently the Chair for the CASMS Board of Directors.

Quantitative Lipidomics Using Metabolic Deuterium Oxide Labeling

Tae-Young Kim[1]

[1] Gwangju Institute of Science and Technology, Gwangju, Republic of Korea

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ABSTRACT

Understanding changes in lipid levels between healthy and diseased states is crucial for uncovering disease mechanisms and identifying potential markers for lipid-related diseases. The quantitative analysis of these lipid changes offers insights into the underlying pathophysiology and can aid in the identification of biomarkers associated with various lipid metabolism-related conditions. Mass spectrometry, when combined with stable isotope labeling techniques, serves as a powerful tool for quantifying changes in lipid composition and dynamics.

Deuterium oxide (D2O), also known as heavy water, stands out as a particularly attractive labeling agent for quantitative lipidomics. Compared to traditional methods that rely on complete replacement of light isotopes with heavier counterparts, D2O offers several advantages. First, it is significantly more cost-effective due to its readily available nature. Second, its administration is simple and non-invasive, making it amenable to various experimental designs. Finally, D2O boasts a unique advantage in its ability to uniformly label all major lipid classes, providing a comprehensive picture of the lipidome.

This presentation will explain the key features and uses of D2O labeling in quantitative lipidomics, including how it allows us to measure how quickly lipids are replaced within a cell or organism and their relative abundance. Furthermore, we detail the principle of D2O labeling for global omics relative quantification (DOLGOReQ), an innovative quantitative omics strategy that utilizes partial metabolic D2O labeling. DOLGOReQ enables the relative quantification of various biomolecules beyond lipids, broadening its application in multi-omics studies.

PERSONAL BIO

Professor Tae-Young Kim received his B.S. and M.S. degrees in Chemistry from Seoul National University in 1999 and 2001, respectively, and acquired Ph.D. in Analytical Chemistry with a Biochemistry minor under Prof. James P. Reilly at Indiana University, Bloomington, USA, in 2009. The major project during his Ph.D. research was to develop a novel hybrid ion trap/time-of-flight mass spectrometer enabling energy/time-dependent vacuum UV photodissociation of biomolecules such as peptides, lipids, and carbohydrates. He worked as a postdoc at Caltech in the lab of Prof. Jesse L. Beauchamp from 2009 to 2010 and at UCLA under Prof. Peipei Ping from 2010 to 2013. He began his independent career in teaching and research at Gwangju Institute of Science and Technology in 2013. His research goal is to advance environmental science by developing novel analytical platforms for highly sensitive and selective detection of trace amounts of toxic substances in complex matrices by coupling mass spectrometry with chromatography.

Ultrafast Quantitative Measurements of Circulating Ceramides in Human Cohorts

Federico Torta[1]

[1] Cardiovascular and Metabolic Disorders Program Duke-NUS Medical School, Singapore

[2] Precision Medicine Translational Research Programme and Department of Biochemistry Yong Loo Lin School of Medicine, National University of Singapore, Singapore

[3] SLING Singapore Lipidomics Incubator Life Sciences Institute, National University of Singapore, Singapore

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ABSTRACT

Ceramides (Cer) are sphingolipids accumulating in human blood plasma during metabolic disorders, insulin resistance and diabetes. Monitoring levels of four circulating ceramides, Cer d18:1/16:0, Cer d18:1/18:0, Cer d18:1/24:0 and Cer d18:1/24:1 can predict the risk for cardiovascular disease (CVD) events and death. Using a RapidFire instrument, we present an online Solid Phase Extraction-Tandem Mass Spectrometry (SPE–MS/MS) methodology for ultrafast (~15 seconds/sample) measurements of these ceramides in human plasma. Addition of authentic deuterated standards enables absolute quantitation of each ceramide species. The applicability of the novel methodology was demonstrated by testing the association of plasma ceramides with different outcomes in patients with type 2 diabetes.

PERSONAL BIO

Federico Torta is a Research Assistant Professor at Duke-NUS Medical School and at the Singapore Lipidomics Incubator (SLING), National University of Singapore. He is also the director of the lipidomics unit of SingMass, the Singapore National Laboratory for Mass Spectrometry. He received his Ph.D. degree in Biochemistry from the University of Parma, Italy. His expertise includes sphingolipidomics, high-throughput technologies for the assessment of lipidomes in large human cohorts and the study of protein-lipid complexes.

Comprehensive Profiling of FAHFAs by Mass Probe Assisted Liquid Chromatography-Mass Spectrometry

Quanfei Zhu[1,2], Yuqi Feng[1,2]

[1] Wuhan Textile University, Wuhan, China
[2] Wuhan University, Wuhan, China

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ABSTRACT

Fatty acid esters of hydroxy fatty acids (FAHFAs) are a new class of lipid mediators with promising anti-diabetic and anti-inflammatory properties. Comprehensive screening and identification of FAHFAs in biological samples would be beneficial to the discovery of new FAHFAs and enable greater understanding of their biological functions. Here, we report the comprehensive screening of FAHFAs in rice and Arabidopsis thaliana by chemical isotope labeling assisted liquid chromatography mass spectrometry (CIL-LC-MS). Multiple reaction monitoring (MRM) was used for screening of FAHFAs. With the proposed method, we detected 49 potential FAHFA families, including 262 regioisomers, in tissues of rice and Arabidopsis thaliana, which greatly extends our knowledge of known FAHFAs. In addition, we proposed a strategy to identify FAHFA regioisomers based on their retention on a reversed-phase LC column. Using the proposed identification strategy, we identified 71 regioisomers from 11 FAHFA families based on commercial standards and characteristic chromatographic retention behaviors. The screening technique could allow for the discovery of new FAHFAs in biological samples. The new FAHFAs identified in this work will contribute to the in-depth study of the functions of FAHFAs.

PERSONAL BIO

Dr. Quan-Fei Zhu earned a Ph.D. in Analytical Chemistry from Wuhan University's College of Chemistry and Molecular Sciences in 2017. From 2018 to 2021, she conducted postdoctoral research at Wuhan University and Macau University of Science and Technology. Following this, she served as a Distinguished Associate Researcher at Wuhan University's School of Public Health from 2022 to 2023. Since 2024, Dr. Zhu has been a Professor at the School of Biological Engineering and Health, Wuhan Textile University. Her research mainly focuses on developing chemical derivatization-assisted liquid chromatography-mass spectrometry methods for metabolite analysis, constructing metabolite databases, and establishing advanced platforms for targeted and untargeted metabolomics. Dr. Zhu has published over 50 SCI papers, including 23 as the first or co-corresponding author, and has been awarded several prestigious grants and honors, such as the National Natural Science Foundation of China (Youth Project) and Wuhan City's Talent Program. She also actively involved in various editorial and committee roles.

Methods and Applications of Biomarker Identification Based on Omics Data and Network Analysis

Xiaohui Lin[1] , Yanhui Zhang[1]

[1] Dalian University of Technology, Dalian, China

O43

ABSTRACT

The advancement and application of omics technology have generated a large amount of omics data, propelling the progress of life science study. Omics data are usually high dimensional and contain rich information. Defining the key information from the complex omics data is of great significance for the deep understanding of disease pathological mechanisms, identification of potential biomarkers, and promotion of disease warning and diagnostic research.

The existing omics data processing methods mainly adopt univariate and multivariate analysis techniques, which primarily focus on defining the differential molecules and ignore the correlations and interactions between molecules. Thus, the identified potential biomarkers usually have problems such as difficult validation and false positives. It is known that the disease occurrence and development are usually caused by the dysregulation of a group of biomolecules. Therefore, systematically exploring the changes in molecular relationships under different physiological and pathological states can help to uncover the important features that reflect the disease process and identify the disease biomarkers having a strong generalization ability.

In our study, network analysis is conducted to develop omics data analysis methods. By exploring the complex and diverse synergistic effects between molecules, we build the molecular synergistic networks, disease-related differential networks, etc., and the knowledge base information is integrated to optimize the network. Based on the established molecular networks, we systematically analyze the omics data by the methods such as reinforcement learning and greedy searching to identify the key information modules, yet define the reproducible and important biological features and biomarkers that can reflect the mechanisms of disease occurrence and development.

PERSONAL BIO

Xiaohui Lin is a professor in the School of Computer Science and Technology, Dalian University of Technology. Her research focus is on mass spectrometry-based metabolomics data processing, artificial intelligence. She has published more than 60 peer-reviewed papers in the 'Web of Science Core Collection' (WoS) indexed journals and holds 10 China patents.

FAIR Computational Lipidomics Facilitate Comprehensive Data Integration and Analysis

Nils Hoffmann[1,2], Dominik Kopczynski[2], Jacobo Miranda[3], Andrej Shevchenko[3], Fadi al Machot[4], Daniel Krause[5], Dominik Schwudke[5], Robert Ahrends[2]

[1] IBG-5 Forschungszentrum Jülich GmbH, Jülich, Germany

[2] Analytical Chemistry Universitt Wien, Vienna, Austria

[3] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

[4] Norwegian University of Life Sciences, Ås, Norway

[5] Division of Bioanalytical Chemistry Research Center Borstel, Leibniz Lung Center, Borstel, Germany

O44

ABSTRACT

Lipidomics as a discipline has seen a steady increase in research output throughout the last decade. With the advent of high-throughput metabolomics-platforms based on chromatography and high-resolution mass spectrometry, the need has increased for a central, well connected and comprehensive resource for both experimental and computational scientists.

With LipidCompass, we want to offer a FAIR resource to simplify the exploration of quantitative lipidomics data from different angles, following the structural hierarchy induced by the shorthand nomenclature, as established by LIPID MAPS and SwissLipids, refined by the recent update to the shorthand nomenclature for lipids. Lipidomics and Metabolomics tools that support mzTab-M as an output format can submit their data to LipidCompass. Usually, additional reviewing and curation will be needed to map tool-specific CV parameters to canonical ones from NCIT, PSI-MS, NCBITaxon and other commonly used controlled vocabularies. Lipid names are being parsed using Goslin to simplify mapping against LIPID MAPS, SwissLipids and other external databases. Automatic conversion allows for easier import and mapping of legacy data and reduces the risk of manual translation errors. LipidCompass provides comprehensive data exploration, comparison and interactive visualization features that simplify the detection of differences between samples within the same study, but, for the first time, also allow analysis and visualization of similarities and differences on a large scale within and between studies.

LipidCompass will be the central integration hub for multiple lipid-related web services, such as LipidXplorer and LipidSpace as part of the Lipidomics informatics for life-science (LIFS) project.

Further collaboration with the International Lipidomics Society interest groups on standardization and clinical lipidomics will integrate support with the upcoming lipidomics checklist. (Anonymized) results from clinical lipidomics ring trials will also be integrated into the database in the near future for interactive comparison of results.

PERSONAL BIO

I currently work as the Compute and Interoperability-Platform Coordinator foe ELIXIR Germany and as a Post-Doctoral Researcher at the Institute of Bio- and Geosciences, Forschungszentrum Jülich, located in Bielefeld, Germany. I am responsible for networking and coordinating activities among projects within the German Network for Bioinformatics Infrastructure (de.NBI) and the European life-sciences Infrastructure for Biological Information (ELIXIR). In this role, I focus on scientific cloud-computing infrastructures and the integration of bioinformatics tools, services, and resources.

My research primarily centers on lipidomics bioinformatics and data science, where I have developed a graph-based reference database and a comparative platform for quantitative lipidomics data, based on multiple tools that we developed as part of the "Lipidomics Informatics for Life Science" (LIFS) project. I am also an active collaborator with the Human Proteome Organization's Proteomics Standards Initiative (HUPO-PSI) and the Metabolomics Society, focusing on the standardization and harmonization of data exchange formats such as mzTab-M and mzQC. Additionally, I work closely with international colleagues within the International Lipidomics Society (ILS) to enhance standardized reporting of lipidomics experiments, with an emphasis on clinical applications.

Before joining Forschungszentrum Jülich, I worked as a Research Associate/Postdoctoral Fellow with the Lipidomics research group at the Leibniz Institute for Analytical Sciences - ISAS - e.V. in Dortmund until February 2021. There, I coordinated the "Lipidomics Informatics for Life Science" (LIFS) project, funded by the German Ministry for Education and Research (BMBF), and contributed to the development and maintenance of lipidomics tools such as LipidCreator and the Goslin libraries.

Oxidative Lipidomic Insights in Cardiovascular Disease: Role of Lipid Peroxidation and Ferroptosis

Huiyong Yin[1,2,3]

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

[2] School of Life Science and Technology, Shanghai Tech University, Shanghai, China

[3] Department of Biomedical Sciences, Jockey Club College of Veterinary Medicine and Life Sciences, Tung Biomedical Science Center, State Key Laboratory of Marine Pollution (SKLMP), The Shenzhen Research Institute and Futian Research Institute, City University of Hong Kong, Hong Kong, China

O45

ABSTRACT

Background: As an iron-dependent form of regulated cell death caused by lipid peroxidation, ferroptosis has been implicated in ischemic injury but the underlying mechanisms in acute myocardial infarction (AMI) remain poorly defined. Acetaldehyde dehydrogenase 2 (ALDH2) catalyzes detoxification of lipid aldehydes derived from lipid peroxidation and acetaldehydes from alcohol consumption. The Glu504Lys polymorphism of ALDH2 (rs671, ALDH2 *2), affecting around 8% world population and 40% East Asians, is associated with increased risk of MI. This study aims to investigate the role of ALDH2 and ferroptosis in MI.

Methods: A Chinese cohort of 177 acute heart failure patients with ALDH2 and ALDH2*2 were enrolled. MI mouse model of left anterior descending coronary artery ligation (LAD) was conducted on wild type, ALDH2*2, and mice with cardiomyocyte-specific knock down of eukaryotic translation initiation factor 3 subunit E (eIF3E) by adeno-associated virus. Lipid peroxidation products were measured by mass spectrometry-based lipidomics and metabolomics in human plasma and in mouse serum and heart tissues.

Results: Human ALDH2 *2 carriers exhibit more severe heart failure post-AMI with features of ferroptosis in blood samples in lipidomic analysis, including increased levels of multiple classes of oxidized phospholipids, serum heme, and decreased levels of antioxidants, such as Coenzyme Q-10 (Co-Q10) and tetrahydrobiopterin (BH4). Similar features were observed in MI mouse models of ALDH2 *2, whereas ferroptosis inhibition by Fer-1 significantly improved heart functions and reversed ferroptosis markers. Importantly, ALDH2*2 led to significantly decreased protein levels of ALDH2, whereas ferroptosis related proteins including Transferrin receptor (TFRC), Acyl-CoA synthetase long chain family member 4 (ACSL4), and Heme oxygenase 1 (HMOX1) were upregulated specifically in the infarct heart tissues. Mechanistically, ALDH2 physically interacted with eIF3E to modulate translation of critical proteins involved in ferroptosis, and ALDH2 deficiency in ALDH2 *2 mutant predisposes cardiomyocytes to ferroptosis by promoting Tfrc/Acsl4/Hmox1 translation. Consistently, cardiomyocytes-specific eIF3E knock down restored ALDH2 *2 cardiac function by attenuating ferroptosis in MI.

Conclusions: ALDH2 *2 aggravates acute heart failure in MI through promoting cardiomyocytes ferroptosis, and targeting ferroptosis may be a potential therapeutic target for treating AMI, especially for ALDH2 *2 carriers.

PERSONAL BIO

Dr. Huiyong YIN is a tenured Professor in the Department of Biological Sciences and Associate Dean （Research） of the Jockey Club College of Veterinary Medicine and Life Sciences at City University of Hong Kong. He also serves as the Associate Dean for Research for JCC and Chair of College Research Committee. Before joining CityU, Prof. Yin was the Distinguished Principal Investigator and Group Leader of Lipid Metabolism in Human Nutrition-related Diseases at Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai, China. He was also the Distinguished Adjunct Professor in School of Life Sciences and Technology in ShanghaiTech University since 2013. Prof. YIN is one of the leading scientists in the field of redox regulation of glucose and lipid metabolism in human metabolic diseases including atherosclerosis, liver cancer, hyperuricemia and gout (http://www.cityu.edu.hk/bms/profile/huiyongyin.htm). He has published over 170 manuscripts in SCI journals, including *Science, Nature, Cell Metabolism, Nature Cancer, JACS, Hepatology, JCI, Redox Biology*, with > 14,900 citations and H-index of 66 (Google Scholar, Sept. 2024). He has been listed as the top 2% of the Most-Cited Scientists in the world by Stanford University and was awarded prestigious “Senior International Scientists” in 2021 by Chinese National Natural Science Foundation (NSFC).

Development of PartailDB Database to Explore Unassigned Tandem Mass Spectra of Novel Lipids

Zhixu Ni[1], Chetin Baloglu[2], Matthew Conroy[3], Miguel Gijón[4], Simon Andrews[2], Valerie B O'Donnell[3], Maria Fedorova[1], Laura Goracci[5]

[1] TU Dresden, Dresden, Germany
[2] Babraham Institute, Babraham, UK
[3] School of Medicine, Cardiff University, Cardiff, UK
[4] Cayman Chemical USA
[5] DCBB DAISY lab, University of Perugia, Perugia, Italy

O46

ABSTRACT

Recent developments in high resolution MS instruments and corresponding analytical methods have enhanced the quality and quantity of MS2 spectra contained in untargeted lipidomics datasets. The mainstream lipid identification tools cover lipid (sub)classes according to the classification system defined by the widely used LIPID MAPS Structure Database (LMSD). However, due to the diversity and complexity of lipid structure and corresponding fragmentation patterns, the currently available algorithms (such as spectra matching, class specific rule-based identification, and structure based in silico predictions) are not fully sufficient to cover all lipid subclasses. Thus, novel lipid structures that are not yet included in commonly-available databases or mainstream software tools cannot be identified in generic workflows. For instance, epilipids, formed by modifications of regular lipid classes, are generally not included in curated databases and thus much less covered by the identification software due to their distinct and diverse fragmentation patterns compare to the unmodified parent lipids. Currently available pipelines also face the difficulties in assigning the exact modification type and site on lipids, thus only partial structural information can be reported, based on the MS2 spectra and LC behaviors. Similar problems also prevent deeper investigation of less studied lipid classes such as LipidA and novel lipid subclasses observed in bacteria, algae, and other organisms. To address this challenge and boost the discovery of novel lipid species, we propose the PartialDB project to collect scattered information of these partially assigned lipid species including the characteristic peaks and corresponding experimental tandem mass spectra. By joining community efforts, the spectra and metadata will be collected in PartialDB, presenting a valuable resource for spectral matching, neutral loss and fragment ions search to support discovery of novel lipids.

PERSONAL BIO

Zhixu Ni is a postdoctoral researcher at Center of Membrane Biochemistry and Lipid Research at the Medical Faculty of the TU Dresden. He received his B.Sc degree in Pharmacy from Wuhan University in China. And then he completed his M.Sc. and his Ph.D. in Chemistry at Leipzig University in Germany. Since his master thesis in 2012, he started to work with identification of oxidized lipids under supervision of Dr. Maria Fedorova. After few years training in epilipid analysis using LC-MS and shotgun MS, he started to program software tools to accelerate the data analysis phase of epilipidomics workflow. In the past few years at Fedorova research group, Ni developed several tools namely LipidHunter, LPPtiger, LipidCircos, and LipidLynxX for the identification, visualization, and data integration of epilipidomics data. Currently, at the research group LMAI (Lipid metabolism: analysis and integration), his research interests include LC-MS based epilipidomics methods development, data integration of epilipidomics data, and epilipidomics software development.

Structural Basis of Lipid Recognition and Receptor Activation of Lipid Receptors

Yuanzheng He[1]

[1] Harbin Institute of Technology, Harbin, China

O47

ABSTRACT

Lipids are not only fundamental components of cell membranes and energy storage for the entire body, but they also function as key signaling molecules that regulate a wide range of physiological processes, including immune response, neuronal development, and metabolic activities. In recent years, our lab has resolved multiple structures of lipid receptors, including Sphingosine-1-phosphate (S1P) receptors, Lysophosphatidic acid (LPA) receptors, Lysophosphatidylserine (LPS) receptors, Leukotriene receptors, and Free fatty acid receptors. The structural information uncovered by our research provides a framework for understanding lipid receptor signaling and serves as a rational basis for drug design and development.

PERSONAL BIO

Dr. Yuanzheng He is an advanced PI of HIT Center for Life Sciences at Harbin Institute of Technology. Dr. He's research interests are G-protein coupled receptors (GPCRs) signaling and the related drug discovery, focusing on lipid receptors and orphan receptors. In addition, Dr. He is also interested in other membrane proteins of critical physiologies. Dr. He's overall research goal is to uncover structural insights into pivotal signaling and utilize this knowledge to design therapeutic solutions for associated human diseases. Dr. He has published more than 40 peer review papers, including *Cell, Nature, Science, Molecular Cell, Nature Chemical Biology, Cell Research, Nature Communications, PNAS*.

The Molecular Basis of Sphingosine-1-Phosphate Metabolism and Structural Based Modulator Rational Design

Ruobing Ren[1], Bin Pang[1], Rujuan Ti[2], Leiye Yu[1], Lizhe Zhu[2]

[1] Fudan University, Shanghai, China

[2] The Chinese University of Hong Kong Shenzhen, Shenzhen, China

O48

ABSTRACT

S1P (Sphingosine-1-phosphate) is an essential bio-active lipid in promoting T/B cells egress, maintaining cardiovascular integrity, and facilitating neuronal development. S1P is synthesized in cells from sphingosine by S1P kinases, secreted to circulating system by S1P transporters such as Spns2 and MFSD2b, and sensed by cell surface receptors S1PR1-5. The detailed S1P transport mechanism crossing the cell membrane is unknown, and the selectivity of downstream signaling transduction is lacking of structural and biochemical evidences. These limit the rational design of therapeutic molecules targeting S1P metabolism. Among these five receptors, S1PR1 is widely expressed in a variety of tissues and validated as a therapeutic target. In the past decade, several S1PR1 agonists have been approved by FDA to treat auto-immune diseases such as multiple sclerosis (MS) and inflammatory bowel diseases (IBD). However, these lunched drugs will non-specifically activate other S1PR sub-types and cause side-effects like bradycardia and vascular leakage. Besides, the balanced downstream signaling activation, both for G protein and β-arrestin, will limit the indication extension of S1PR agonists. Here, we will report the recent progress on the molecular basis of S1P transport and sensing, and use a computational-aided rational design strategy to obtain a potent, S1PR1 selective, and G protein signaling biased agonist. It provides new insights in GPCR biased agonist discovery and is of great potential to broader therapeutic purposes by regulating S1P signaling.

PERSONAL BIO

Dr. Ren, Ruobing received his PhD degree at Tsinghua University in 2015, focusing on the molecular mechanism of cholesterol metabolism regulation. During 2015-2017, he worked as an associate scientist at Amgen Asia R&D Center. After that, he joined in the Chinese University of Hong Kong, Shenzhen School of Life and Health Sciences and Kobilka Institute of Innovative Drug Discovery as tenure-track assistant professor until end of 2021. Now he is the investigator of Institute of Metabolism and Integrative Biology (IMIB) at Fudan University. He focuses on the structures and molecular mechanisms of membrane proteins with biological significance using a combination of state-of-the-art structural (X-ray crystallography, cryo-EM) and biochemical/biophysical techniques for over 10 years. The overriding interests of his lab are the synthesis, transport, and signaling transduction of steroid molecules, fatty acids, and sphingolipids.

Discovery of Structurally and Functionally Important Lipids for 7TM Proteins

Jiankun Xu[1]

[1] Shenzhen Bay Laboratory, Shenzhen, China

O49

ABSTRACT

The membrane environment plays a critical role in the structure and function of membrane proteins. Native mass spectrometry (nMS) is a powerful tool that enables the isolation of protein-lipid complexes from solution, facilitating the investigation of lipid functions. In this presentation, I will explore the use of nMS in conjunction with other MS techniques to study two classes of membrane proteins: G protein-coupled receptors (GPCRs) and microbial rhodopsins, both of which consist of seven transmembrane (TM) domains. Our findings reveal correlations between lipids and protein conformation, protein-protein interactions, signaling cascade regulation, and protein folding and maturation. For GPCRs, we found that lipids can stabilize the active conformation and transducer interactions of the receptors, highlighting the role of lipids in regulating GPCR signaling cascades. Additionally, our studies on microbial rhodopsins demonstrated how different membrane mimetics affect protein folding states and lipid interactions, providing insights into the maturation process of photoreceptor proteins. These results underscore the importance of the lipid environment in membrane protein functionality and offer new perspectives on drug targeting.

PERSONAL BIO

Dr. Hoi is a researcher specializing in native mass spectrometry (nMS). He holds a BSc from Queensland University of Technology and an MSc from the University of Melbourne. Dr. Hoi earned his PhD at Oxford University, where he also completed postdoctoral research in the lab of Prof. Dame Carol Robinson. Currently, he is a junior PI at the Shenzhen Bay Laboratory. His research focuses on developing nMS methods to capture and elucidate various biomolecular interactions, with a special emphasis on protein-lipid interactions.

Computational Design and Genetic Incorporation of Lipidation Mimics in Living Cells

Shixian Lin[1]

[1] Zhejiang University, Hangzhou, China

O50

ABSTRACT

Protein lipidation, which regulates numerous biological pathways and plays crucial roles in the pharmaceutical industry, is not encoded by the genetic code but synthesized post-translationally. In this talk, we report a computational approach for designing lipidation mimics that fully recapitulate the biochemical properties of natural lipidation in membrane association and albumin binding. Furthermore, we establish an engineered system for co-translational incorporation of these lipidation mimics into virtually any desired position of proteins in Escherichia coli and mammalian cells. We demonstrate the utility of these length-tunable lipidation mimics in diverse applications, including improving the half-life and activity of therapeutic proteins in living mice, anchoring functional proteins to membrane by substituting natural lipidation, functionally characterizing proteins carrying different lengths of lipidation and determining the plasma membrane-binding capacity of a given compound. Our strategy enables gain-of-function studies of lipidation in hundreds of proteins and facilitates the creation of superior therapeutic candidates.

PERSONAL BIO

Dr. Shixian Lin is a principal investigator at the Life Sciences Institute, Zhejiang University since 2017. The Lin lab focuses on developing new chemical reactions, molecular probes, and tools to precisely manipulate protein PTM in living cells at the molecular level and applying these innovative chemical biology methods to biological discoveries. In the past five years, Dr. Lin has published papers in Science, Nat. Cell Biol., Nat. Chem. Biol., Nat. Chem., Nat. Struct. Mol. Biol., Nat. Commun., and JACS as corresponding authors. He has received the Young Chemical Biologists Award from the International Chemical Biology Society, the Young Scientist Finalist Award from the Chinese Society for Cell Biology, and has been selected as a scientist of the Feng Foundation of Biomedical Research. He has applied for more than ten patents in the intersection of chemistry and biomedicine, five of which have been transferred.

Regulation and Modulation of RAS Palmitoylation

Anlan Yang[1], Shengjie Liu[1], Yuqi Zhang[1], Jiakai Zhu[1], Ruiying Guo[1], Jianping Wu[1], **Qi Hu**[1]

[1] Westlake University, Hangzhou, China

O51

ABSTRACT

The RAS family proteins HRAS and NRAS are key regulators of cell proliferation and oncogenesis, with their activities being tightly controlled by S-palmitoylation. We recently determined the cryo-EM structure of the human ZDHHC9-GCP16 complex, which is known to catalyze the S-palmitoylation of HRAS and NRAS. The structure explains why the accessory protein GCP16 is required for the function of the palmitoyltransferase ZDHHC9 and shows that phospholipid binding to an arginine-rich region of ZDHHC9 and palmitoylation on three residues (C24, C25, and C288) are essential for the catalytic activity of the ZDHHC9-GCP16 complex. Furthermore, we reveal that ZDHHC14 and ZDHHC18 also require GCP16 as an accessory protein to catalyze RAS palmitoylation. A second focus of our study is to develop chemical approaches to modulate RAS palmitoylation. By fusing NRAS with a depalmitoylase, acyl-protein thioesterase 1 (APT1), we create a system where NRAS palmitoylation and signaling can be reversibly controlled by an APT1 inhibitor, ML348. This strategy allows for observation of temporal changes in RAS protein localization. Our findings provide insights into the regulatory mechanisms of RAS palmitoyltransferases and suggest potential routes for controlling RAS and other small GTPase signaling via modulation of palmitoylation.

PERSONAL BIO

Dr. Qi Hu received his bachelor's degree in pharmaceutical sciences and master's degree in medicinal chemistry from the School of Pharmaceutical Sciences, Peking University, Beijing, China. He then joined the School of Life Sciences, Tsinghua University and received his doctoral degree in biology in 2014. In 2015, he joined Prof. Kevan Shokat's lab in the University of California, San Francisco for postdoctoral training. Dr. Hu was awarded the prestigious Damon Runyon Postdoctoral Fellowship in 2015. Since 2019, Dr. Hu has been a tenure-track assistant professor in the School of Life Sciences at Westlake University.

Dr. Hu is interested in using biological and chemical tools to study the molecular mechanisms of cell signaling, and developing chemical tools and drugs based on the understanding of the molecular mechanisms. The research in the Hu lab is currently focusing on the following directions:

1. Protein lipidation and lipid metabolism:
(1) To understand the structure, function and regulatory mechanism of enzymes involved in protein lipidation and lipid metabolism.
(2) To explore the effects of protein lipidation on protein localization and function.
(3) To design chemical tools to study protein lipidation and lipid metabolism.
(4) To develop selective regulators of disease-related protein lipidation enzymes.

2. Small molecule drug development:
(1) To develop small molecule inhibitors targeting essential enzymes of coronaviruses.
(2) To explore new strategies for anticancer drug development.

Maintaining Outer Membrane Lipid Asymmetry in Gram-Negative Bacteria

Shu-Sin Chng[1]

[1] National University of Singapore, Singapore

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ABSTRACT

Gram-negative bacteria can survive in harsh environments in part because of the presence of the outer membrane (OM), which comprises lipopolysaccharides (LPS) and phospholipids (PLs) in the outer and inner leaflets, respectively. This asymmetric distribution of lipids renders the OM a very effective permeability barrier against toxic compounds, including antibiotics and bile salts. In this talk, I will share key findings by our group elucidating how Gram-negative bacteria maintain OM lipid asymmetry via the retrograde transport of PLs from the OM to the IM. Understanding the mechanisms of OM stability and homeostasis has applications in the future development of new antibiotics to fight the bacterial antimicrobial resistance.

PERSONAL BIO

Associate Professor Shu-Sin Chng completed his Ph.D. degree in Chemistry and Chemical Biology at Harvard University. Following a short postdoctoral stint at the Harvard Medical School, Shu-Sin returned to the National University of Singapore (NUS) in 2011 as an Assistant Professor in the Department of Chemistry, and was appointed Principal Investigator at SCELSE since 2012. In 2018, Shu-Sin was promoted to Associate Professor with tenure. His research group has established a strong reputation and interest in elucidating the mechanisms of cell envelope biogenesis and inter-membrane lipid trafficking in Gram-negative bacteria and mycobacteria, and in characterizing relevant protein machines in these bacteria for the development of new antibiotics. For his exceptional work, Shu-Sin has received the 2019 Walter Shaw Young Investigator Award in Lipid Research, a recognition conferred by the American Society of Biochemistry and Molecular Biology. Beyond research, Shu-Sin is a passionate educator who adopts an active learning classroom approach in his courses, and has won multiple NUS Annual Teaching Excellence Awards, including the Honour Roll. In addition, he currently serves as Vice Dean (Student Life and Alumni Relations) at the Faculty of Science, and the NUS Associate Director for the Singapore Center for Environmental Life Sciences Engineering (SCELSE).

Structural Basis for Cholesterol Delivery from NPC2 to The Transmembrane Domain of NPC1

Nieng Yan[1,2,3]

[1] Shenzhen Medical Academy of Research and Translation (SMART), Shenzhen, China
[2] Shenzhen Bay Laboratory, Shenzhen, China
[3] Tsinghua University, Beijing, China

O53

ABSTRACT

Lysosomal cholesterol egress requires two proteins, NPC1 and NPC2, whose defects are responsible for Niemann-Pick disease type C (NPC). I will present systematic structural characterizations that reveal the molecular basis for cholesterol delivery from NPC2 to the transmembrane domain of NPC1. Similar structures of NPC1 were obtained in nanodisc and in detergent micelles at resolutions of 3.6 Å and 3.0 Å, respectively. The high-resolution structure allows for precise mapping of 218 NPC-related disease mutations and provides important insight into the pathogenic mechanism for dozens of them. A tunnel connecting the N-terminal domain (NTD) and the transmembrane sterol-sensing domain (SSD) is unveiled. The structure of the NPC1-NPC2 complex at 4.0 Å resolution was obtained, elucidating the molecular basis for cholesterol handoff from NPC2 to NPC1(NTD). Our structural studies establish a framework for structure-function relationship study of the hundreds of NPC disease mutants, and, thus, represent a major step forward in the mechanistic understanding of NPC1/2-mediated lysosomal egress of cholesterol. We hope this endeavor will contribute to the therapeutic development for NPC.

PERSONAL BIO

YAN Nieng received her Bachelor's Degree from the Department of Biological Science and Technology at Tsinghua University in 2000 and Ph.D. from the Department of Molecular Biology at Princeton University in 2004, where she continued her postdoctoral research. She joined School of Medicine, Tsinghua University as a professor in 2007. Ten years later, in 2017, she was recruited back by Princeton University and became the inaugural Shirley M. Tilghman Professor of Molecular Biology. In 2022, Yan Nieng served as the Founding President of Shenzhen Medical Academy of Research & Translation (SMART). In 2023, she served as the Director of Shenzhen Bay Laboratory (SZBL).

Dr. Yan's primary research interest has been in the structural and mechanistic investigation of membrane transport proteins that are of tremendous physiological, pathophysiological, and pharmaceutical significance. She reported the first structures of the human glucose transporters GLUT1 and GLUG3, the eukaryotic voltage-gated sodium and calcium channels, and a number of proteins involved in sterol metabolism. Her present research program focuses on structure-guided mechanistic understanding and drug discovery for pain relief. Her achievements have won her numerous accolades.

Abstracts of Posters

List of Posters

Odd number posters are scheduled to present on Friday (October 25) and even number posters are scheduled to present on Saturday (October 26).

P01	**Interactive Lipidome: A Characterization of Host and Virus Lipids During Viral Infection**	*Xin Li*
P02	**Single Cell Mass Spectrometry-Based Lipidomics on Gut Epithelial Cells**	*Qianying Xu*
P03	**A Facile Lipidomic Method for Qualitative and Quantitative Analyses of Sebum Fatty Acids and Squalene**	*Lerong Qi*
P04	**High Coverage and Accuracy Plasma Lipidomics Analytical Strategy of Phospholipids in Clinical Samples by Hydrophilic Interaction Liquid Chromatography with Data Independent Acquisition Tandem Mass Spectrometry**	*Jie Wang*
P05	**Trapped Ion Mobility Spectrometry-Mass Spectrometry Improves the Coverage and Accuracy of Four-Dimensional Untargeted Lipidomics**	*Xi Chen*
P06	**High-Resolution Hybrid Tandem Mass Spectrometer for Lipid Analysis of Trace Samples**	*Xiaomin Fan*
P07	**Lipidomics Profiling of NAFLD Cells Treated with Aurantiochytrium sp. Extract**	*Jen Kit Tan*
P08	**Effect of Long-Term Exercise Intervention on Plasma Lipids in Young Sedentary Adults in the ACTIBATE Randomized Controlled Trial**	*Yu Zhang*
P09	**Impact of Cuproptosis and m6A-Associated lncRNAs on the Lipidomic Pathway in High-Risk Hepatocellular Carcinoma Patients**	*Yuezhi Zhu*
P10	**A Deep Lipidomics Strategy for Evaluation of Cancer Therapy**	*Haoyue Zhang*
P11	**Lipid Metabolism in Gliomas by Using Arterial-Venous Analysis**	*Wen Li*
P12	**Unveiling COPD Phenotypes with Targeted Metabolomics Approaches-Taking Signaling Lipids as a Stepping Stone**	*Lu Zhang*
P13	**Kinetic Isotope Tracing Combined with MS Methods Reveals De Novo Synthesis and Metabolism of Phospholipids C=C Isomers**	*Zhuoning Xie*
P14	**Analysis of Single-Cell Lipid Metabolism in Mixed Microalgal Populations Using Combined Fluorescence-Activated Cell Sorting and Label-Free Mass Cytometry**	*Yu Qiao*

P15	Analysis of Lipid Components in Five Kinds of Cordyceps Based on Lipidomics	*Yang Yang*
P16	Sphingolipid Remodeling in the Plasma Membrane Is Essential for Osmotic and Insectic Stress Tolerance in Arabidopsis	*Yong-kang Li*
P17	Revealing the Hydrophobic Interactions Between Lipid Bilayers and Transmembrane Peptides via Photo-Tagging and Mass Spectrometry	*Jing Zhao*
P18	Deep Profiling of Gangliosides Enabled by Selective Enrichment and Liquid Chromatography- Trapped Ion Mobility-Mass Spectrometry	*Yichun Wang*
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Interactive Lipidome: A Characterization of Host and Virus Lipids During Viral Infection

Xin Li[1], Hao Yang[1,2], Meng Gong[1]

[1] Institutes for Systems Genetics, Frontiers Science Center for Disease-Related Molecular Network, West China Hospital, Sichuan University, Chengdu, China

[2] Division of Abdominal Tumor Multimodality Treatment, Cancer Center, NHC Key Lab of Transplant Engineering and Immunology, Regenerative Medicine Research Center, West China Hospital, Sichuan University, Chengdu, China

P01

ABSTRACT

Lipids play a crucial role in host antiviral immunity and efficient viral replication, as they are essential components exploited by both hosts and viruses during infection. To achieve a comprehensive characterization of virus-host interactions, we developed a metabolomics-based strategy employing both non-targeted and targeted approaches via liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/MS (GC/MS) to determine lipid alterations during infection in a time-course manner.

Utilizing this strategy, we identified over 1000 lipids involved in fatty acid, carnitine, phospholipid, sphingolipid, and glycerolipid metabolism. Temporal expression analysis and reconstruction of metabolic networks involving these lipids enabled the identification of critical perturbing molecules associated with viral infection. Furthermore, application of biofilm separation technology allowed us to analyze the lipid composition of viral particles, providing insights into indispensable molecules for intervening in viral infection and proliferation from a novel perspective.

Single Cell Mass Spectrometry-Based Lipidomics on Gut Epithelial Cells

Qianying Xu[1], Hadeer Mattar[2], Emmanuelle Claude[3], Scarlet Ferrinho[3], Lee Gathings[3], Emily Fraser[4], Melanie Bailey[4], E.N.C. Mills[1,5]

[1] Division of Infection Immunity & Respiratory Medicine, Manchester Institute of Biotechnology, University of Manchester, Manchester, UK

[2] Albaha University, Al Baha, Saudi Arabia

[3] Waters Corporation, Wilmslow, UK

[4] School of Chemistry and Chemical Engineering, University of Surrey, Guildford, UK

[5] School of Bioscience and Medicine, University of Surrey, Guildford, UK

P02

ABSTRACT

The Caco-2 cell line is commonly used as a model for the gut epithelial layer, while the HT29-MTX cell line produces the mucus. Consequently, their co-culture system is widely used as an in vitro gut uptake model. However, cell heterogeneity in culture is not fully understood. Despite numerous studies highlighted its importance, gaps still remain in understanding the lipid distribution of the co-culture system and achieving the single-cell level resolution in the analysis. This study applies desorption electrospray ionisation (DESI) mass spectrometry imaging (MSI) to address the heterogeneity in 2D culture cell mode and a further onsite validation of cell heterogeneity validated by single cell level LC-MS/MS based lipidomics.

Methodology and results: Caco2 and HT29-MTX cells were cultured either singular or co-cultured for 21 days to allow full differentiation, followed by analysis using DESI MS imaging (Waters Corp.) under both positive and negative ionisation. The co-culture lipid profile was primarily discovered through DESI MS imaging, which requires the minimal sample treatment, revealed the two-dimensional distribution of the lipid profile. To explore the single-cell workflow for LC-MS based proteomics and lipidomics, serial dilution of the differentiated cells was then applied to allow the isolation of single cell. Following this, single cell was sampled by Yokogawa SS2000 and analysed by LC-MS/MS for further single cell analysis to provide a detailed characterisation of these cells. However, due to the intercellular tight junction, the cell monolayer was treated with trypsin for 3 – 4 min under monitoring to enable sampling. Distinctive patterns and lipidomes were also identified from the two cell lines. The lipidome profile from DESI imaging indicated that the Caco2 cell line was more heterogenous and rich in cholesterol esters and triglycerides whilst HT29-MTX cells has a significant lipidome related to phosphatidylethanolamines, phosphatidylinositols and odd chain lipids. From the single cell lipidomics characterisation, around 100 to 150 lipid IDs were identified from each of the single cell sample, the presence of a membrane lipid feature PC34:1 also indicated the success of the single cell level lipidomics.

Conclusions: The DESI MS imaging proved to be an efficient and sensitive tool for investigating the lipidome for cell cultures with minimal treatment. It revealed distinctive lipid distributions in Caco2 and HT29-MTX, even when they were co-cultured. The integration of single-cell techniques allowed for the characterisation of gut model cells, revealing the heterogeneity of these cells under co-culture conditions and investigating its impact on the role of cell models.

A Facile Lipidomic Method for Qualitative and Quantitative Analyses of Sebum Fatty Acids and Squalene

Lerong Qi[1], Xiangzi Li[2], Yachen Ren[1], Tingxiang Yang[3], Yang Guan[2], Dong Hao Wang[3], Zhen Wang1

[1] School of Public Health (Shenzhen), Shenzhen Campus of Sun Yat-sen University, Shenzhen, China

[2] Department of Dermatology, Shenzhen Center for Chronic Disease Control, Shenzhen Institute of Dermatology, Shenzhen, China

[3] School of Agriculture and Biotechnology, Shenzhen Campus of Sun Yat-sen University, Shenzhen, China

P03

ABSTRACT

Background: Sebum fatty acids play a critical role in the pathogenesis of dermatological conditions through their compositional alterations. Therefore, a facile method for the analysis of sebum fatty acids is pivotal for elucidating their contribution to the pathogenesis of skin diseases. However, sebum fatty acids contain many alterations in their structures such as chain length, double bond positions, and the presence of a methyl branch. Previous researches mainly identified certain categories of them, yet lack of comprehensive qualitative and quantitative analysis based on gas chromatography (GC) of diverse sebum fatty acids. For instance, many branched-chain isomers and double bond isomers of long-chain and very long-chain fatty acids remain inaccurately identified.

Objectives: This study is aimed to establish a facile lipidomic method for qualitative and quantitative analyses of fatty acids and squalene in facial sebum, including unusual fatty acids that have not been reported before, probably due to their trace amounts and unavailability of commercialized standards. At the same time, it could provide a comprehensive report on the sebum fatty acid and squalene compositions. Furthermore, the established analytical workflow could serve as a reference method for subsequent lipid research in skin diseases.

Methods: Sebum from 20 of dermatologically healthy individuals was extracted and covalent adduct chemical ionization (CACI), electron ionization (EI) and Paternò-Büchi (PB) tandem mass spectrometry (MS/MS) techniques were employed for the identification of fatty acids with varied methyl branch and double bond positions, along with squalene.

Results:

(1) Totally 64 of sebum fatty acids were identified using combined CACI-MS/MS, EI-MS/MS, and PB-MS/MS techniques, which is much more than the reported in previous researches. Sebum fatty acid composition is generally similar between genders, with variations in specific fatty acids. For instance, the levels of n17:0 and n19:1n-11 were higher in males, while i12:0 and n20:0 were higher in females, probably attributed to sex hormones.

(2) The double bond position of branched monounsaturated fatty acids i15:1n-9 was newly identified via combination of CACI-MS/MS and EI-MS/MS techniques. Unusual diunsaturated fatty acid isomers such as n18:2n-7, n18:2n-3, n20:2n-12 and n20:2n-7 were newly characterized via PB-MS/MS.

(3) Various novel branched long-chain and very-long-chain fatty acids, mainly with19-26 carbons in chain, were identified in sebum by CI-MS/MS.

(4) The structural characterization of squalene was established by PB-MS/MS and verified by CACI-MS/MS.

Conclusions

In a word, this study established a facile lipidomic method for comprehensive qualitative and quantitative analysis analyses of sebum fatty acids and squalene, which facilitate the dermatological research by introducing advanced lipidomic methodologies.

High Coverage and Accuracy Plasma Lipidomics Analytical Strategy of Phospholipids in Clinical Samples by Hydrophilic Interaction Liquid Chromatography with Data Independent Acquisition Tandem Mass Spectrometry

Jie Wang[1]

[1] Key Laboratory of Oilseeds Processing of Ministry of Agriculture, Hubei Key Laboratory of Lipid Chemistry and Nutrition, Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan, China

P04

ABSTRACT

Phospholipids are vital biomolecules widely present in organisms, serveing as the main components of cell membranes and possess diverse biological functions. Lipidomics has attracted considerable attention in recent years with the development of chromatography and mass spectrometry techniques. Sequential windows acquisition of all theoretical spectra (SWATH) is a data-independent acquisition that employs multiple narrow Q1 isolation windows to acquire MS/MS fragments for all precursor ions. This study presents and systematically evaluates a lipidomics analytical strategy for human plasma based on HILIC SWATH-MS/MS technology. 30 variable Q1 isolation windows and 30 ms accumulation time were utilized to achieve the best qualitative coverage. The optimal quantitative results were attained through an integrated approach that combines MS1 with selected MS2 fragment ions for quantitation. Furthermore, compared to conventional information-dependent acquisition (IDA), this strategy demonstrated a 21% increase in qualitative coverage and a 10% improvement in quantitative accuracy. Notably, for lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and phosphatidylinositol (PI), the unique MS2 quantitation capability of SWATH yielded accuracy increases of 24%, 17%, and 71%, respectively. Ultimately, applying this HILIC SWATH-MS/MS lipidmoics strategy to dyslipidemia plasma analysis. A total of 266 phospholipid molecular species were identified, with low-abundance PI species showing significant up-regulated. This not only underscores the strategy novelty and precision but also paves the way for a more nuanced understanding of lipidomics in clinical diagnostics, potentially transforming our approach to the early detection and treatment of dyslipidemia and other lipid-related disorders.

Trapped Ion Mobility Spectrometry-Mass Spectrometry Improves the Coverage and Accuracy of Four-Dimensional Untargeted Lipidomics

Xi Chen[1,2], Yandong Yin[1], Yuping Cai[1], Zheng-Jiang Zhu[1]

[1] Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China

[2] University of Chinese Academy of Sciences, Beijing, China

P05

ABSTRACT

Lipids play vital roles in many physiological and pathological processes in living organisms. Due to the high structural diversity and the numerous isomers and isobars of lipids, high-coverage and high-accuracy lipidomic analysis of complex biological samples remain the bottleneck to investigate lipid metabolism. Here, we developed the trapped ion mobility spectrometry-mass spectrometry (TIMS-MS) based four-dimensional untargeted lipidomics to support accurate lipid identification and quantification in biological samples. We first demonstrated that the TIMS based multi-dimensional separation improved the differentiations of isomeric and isobaric lipids, and increased the purity of precursor ion isolation and the quality of MS/MS spectra. Hyphenation of TIMS and PASEF technologies significantly improved the coverages of MS/MS spectra. These technological advantages jointly improved the coverage and accuracy of lipid identification in untargeted lipidomics. We further demonstrated that the CCS values of lipids acquired using TIMS were highly consistent with those from drift tube ion mobility spectrometry (DTIMS). Lipid identification and quantification results of NIST human plasma samples were also verified with inter-laboratory reports. Finally, we applied the TIMS-MS based untargeted lipidomics to characterize the spatial distributions of 1,393 distinctive lipids in the mouse brain, and demonstrated that diverse lipid distributions and compositions among brain regions contributed to different functions of brain regions. Altogether, TIMS-MS based four-dimensional untargeted lipidomics significantly improved the coverage and accuracy of untargeted metabolomics, thereby facilitating a system-level understanding of lipid metabolism in biological organisms.

High-Resolution Hybrid Tandem Mass Spectrometer for Lipid Analysis of Trace Samples

Xiaomin Fan[1], Zheng Ouyang[1]

[1] Tsinghua University, Beijing, China

P06

ABSTRACT

Introduction:Lipids play a crucial role in biological research. In recent years, lipid analysis techniques for trace samples, such as single-cell analysis and mass spectrometry imaging, have garnered significant attention. However, traditional analysis platforms generally have low ion utilization efficiency, typically analyzing only one target ion per injection, resulting in the waste of other ions. To improve the utilization of trace samples and achieve a broader identification depth, we have developed a high-resolution hybrid tandem mass spectrometry platform based on linear ion trap in this study, enabling comprehensive analysis of trace samples.

Methods: We coupled a linear ion trap with a quadrupole-time-of-flight mass spectrometer to create a high-resolution tandem mass spectrometry platform. The inclusion of the linear ion trap enables a variety of ion manipulation and analysis modes. For instance, in a single injection, all precursor ions can be stored in the ion trap, and then individual precursor ions or a wide window can be sequentially selected for transfer and fragmentation. This allows obtaining MS/MS information for all precursor ions of interest, thereby improving sample utilization while maintaining high-resolution analytical performance. Consequently, this platform is particularly suitable for the analysis of trace samples.

Preliminary results: We have completed the construction of the high-resolution tandem mass spectrometry platform, including vacuum testing, ion trap assembly design, and system control. The preliminary installation and debugging phases have also been completed. The ion trap's basic functions, such as ion transmission, ion accumulation, ion storage, and mass-selective transmission, have been successfully implemented. We are currently optimizing the performance of the comprehensive MS/MS analysis mode for single injections and developing additional analysis modes. Soon, we will be ready to utilize this platform for lipid-related analyses, with the ultimate goal of applying it to lipid analysis of trace samples, such as single cells.

Lipidomics Profiling of NAFLD Cells Treated with Aurantiochytrium sp. Extract

Jen Kit Tan[1], Kartthigeen Tamel Selvan[1], Ayaka Nakashima[2], Kengo Suzuki[2], Yu Inaba[2], Jo Aan Goon[1], Hiroshi Tsugawa[3], Suzana Makpol[1]

[1] Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

[2] Research and Development Department, Euglena Co. Ltd, Tokyo, Japan

[3] Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Tokyo, Japan

P07

ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) pathogenesis is related lifestyle modifications. Natural products are an importance source for novel drug discovery and development. Aurantiochytrium sp., a marine microalga, has unexplored potential in NAFLD management. This study aimed to determine the lipidomic alterations caused by Aurantiochytrium sp. extract on a NAFLD cell model. Our findings showed that the extract reduced fat accumulation in HepG2 cells overloaded with free fatty acids, based on staining and ELISA methods. LCMS analyses using untargeted metabolomic and lipidomic approaches suggested that amino acid metabolism and various lipid classes were altered by the extract in NAFLD cells. In particular, triacylglycerol subclasses (TGs, OxTGs and EtherTGs), glycerophosphocholine subclasses (PCs and LPCs), PGs, PAs, PIs and sterols levels were reversed by the extract in NAFLD cells compared to untreated NAFLD cells. Our findings imply that Aurantiochytrium sp. extract exhibits an anti-steatosis effect by modulating lipid metabolism. Further studies are warranted to explore the roles of the lipids involved in this anti-steatosis effect.

Effect of Long-Term Exercise Intervention on Plasma Lipids in Young Sedentary Adults in the ACTIBATE Randomized Controlled Trial.

Yu Zhang[1], Lucas Jurado Fasoli[2], Zhengzheng Zhang[1], Charles Clark[1], Nicolas Drouin[1], Amy Harms[1], Thomas Hankemeier[1]

[1] Leiden University, Leiden, Netherlands

[2] University of Granada, Granada, Spain

P08

ABSTRACT

Background: Regular physical activity is important not only for physical health but also for mental health. Systemic changes in metabolic profiles can be seen resulting from physical activity, and the study of lipid metabolism is especially important in understanding if and how physical activity affects the health of sedentary subjects1. Although it has become clear that resistance and endurance exercises use lipids as an energy source2, the role of lipidome has not been fully defined.

Study design: A randomized controlled trial was conducted to investigate changes in the plasma lipidome after a 24-week supervised exercise training program in young adults. After baseline examination, participants were assigned to one of three groups:(i) a control group (no exercise), (ii) a moderate-intensity exercise group, and (iii) a vigorous-intensity exercise group. After 24-week exercise(resistance and endurance training), plasma samples were collected after an overnight (10 hours) fast, and samples were aliquoted and stored at 80° C.

Methods: Lipidomics profiling analysis was done using a previously published method.3 Plasma lipids were extracted using the Matyash method and analyzed with Hydrophilic interaction liquid chromatography (HILIC)-MS/MS. Acquired data were evaluated using SCIEX OS Software and an in-house developed software tool (mzQuality) was used for batch correction and assessing data quality. The data was statistically analyzed using partial least square discriminant analysis (PLS-DA), volcano plot, one-way ANOVA, correlations, enrichment analysis and pathway analysis in R(4.2.2).

Results: After conducting a quality control check, we could report 794 lipids across 18 subclasses.

Compared to control and vigorous-intensity exercise group, many lipids undergo significant changes after moderate exercise. Phosphatidylethanolamines (PE), Phosphatidylcholines (PC) and Triglycerides (TG), were significantly increased in the plasma lipidome after moderate exercise. This may reflect increased lipolysis and fatty acid mobilization from adipose tissue leading to elevated circulating triglyceride levels. Gender differences in the increase of TG levels after exercise were observed and this may be related to hormonal variations, insulin sensitivity and metabolic rate. In addition, several lipids exhibited a strong correlation with handgrip strength, brown adipose tissue(BAT), glucose and Apolipoprotein AI(APOA1). In conclusion, our study offers valuable insights into the complex interplay between exercise, lipid metabolism, and physiological parameters.

Impact of Cuproptosis and m6A-Associated lncRNAs on the Lipidomic Pathway in High-Risk Hepatocellular Carcinoma Patients

Yuezhi Zhu[1], Jen Kit Tan[1], Jo Aan Goon[1]

[1] Universiti Kebangsaan Malaysia, Selangor, Malaysia

P09

ABSTRACT

Hepatocellular carcinoma (HCC) is associated with a poor prognosis, and the lack of clear prognostic markers remains a challenge. While cuproptosis and N6-methyladenosine (m6A) modifications have demonstrated potential in predicting prognosis in cancer, their roles in HCC are still not well understood. This study aims to identify long non-coding RNAs (lncRNAs) related to cuproptosis and m6A, and to develop a risk assessment model that explores the impact of these lncRNAs on lipidomics in HCC across different risk groups. Our results revealed that among the RNA-Seq data and clinical information from 375 HCC samples, 916 lncRNAs were found to be associated with both m6A modification and cuproptosis. Through LASSO survival regression analysis, five lncRNAs were found to be independently associated with overall survival (OS). The constructed risk assessment model revealed heightened activity in Glycolysis/Gluconeogenesis, Carbon metabolism, and PPAR signaling pathways in the high-risk group. These findings suggest that lncRNAs related to cuproptosis and m6A play crucial roles in regulating metabolic gene expression, potentially impacting lipidomic diversity and metabolic pathways. Our study indicates that lncRNAs associated with cuproptosis and m6A may influence HCC progression by modulating lipid metabolism, and this progression can be predicted using the model. With the declining incidence of virus-related HCC and the increasing prevalence of obesity and T2DM, non-alcoholic steatohepatitis (NASH) has emerged as a significant contributor to HCC. In comparison to other causes, NASH-related HCC is characterized by more pronounced disturbances in lipid metabolism. Therefore, it is imperative to investigate the mechanisms and predictive roles of cuproptosis and m6A-related lncRNAs in NASH-related HCC using integrated genomics and lipidomics approaches. This will facilitate the identification of specific lipid alterations and offer novel targets for monitoring and treating NASH-related HCC.

A Deep Lipidomics Strategy for Evaluation of Cancer Therapy

Haoyue Zhang[1], Yikun Liu[1], Donghui Zhang[1], Jiani Wang[2], Fei Ma[2], Wenpeng Zhang[1], Zheng Ouyang[1]

[1] State Key Laboratory of Precision Measurement Technology and Instrument, Department of Precision Instruments, Tsinghua University, Beijing, China

[2] Department of Medical Oncology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

P10

ABSTRACT

In the realm of oncology, neoadjuvant therapy (NAT) is widely used to treat breast cancer, primarily enhancing the rates of breast-conserving surgeries and improving overall survival. The ideal outcome, known as pathological complete response (pCR), serves as a critical success indicator. Traditionally, determining pCR involves invasive tissue biopsies, which are risky, painful, and costly. Given these challenges, there is a pressing need for dynamic and non-invasive methods like liquid biopsy, which could revolutionize the evaluation of treatment responsiveness without the risks associated with traditional methods.

Lipids, vital components of living organisms, play diverse physiological roles including forming cell membranes, serving as signaling mediators, storing energy, and contributing to epithelial barriers. Disruptions in lipid homeostasis are often associated with pathological states, frequently observed in conditions such as cancer, insulin-resistant diabetes, and cardiovascular diseases.

Previously, we developed an LC-PB-MS/MS approach enabling comprehensive characterization of phospholipid C=C isomers and sn-isomers in biological samples. While this technique has unveiled potential lipid isomer biomarkers in small-scale disease studies like those involving type 2 diabetes, its application in large-scale biomarker screening and disease diagnosis requires the development of massive data identification and robust digital models.

In this study, we introduce a sophisticated deep lipidomics strategy to evaluate the efficacy of NAT for breast cancer. Our approach merges high-throughput lipidomic profiling with advanced machine learning techniques to non-invasively predict treatment outcomes. Utilizing a novel LC-PB-MS/MS platform, we conducted comprehensive lipid profiling, identifying 268 unique lipids, including detailed C=C isomer annotations across various lipid subclasses such as Glycerophosphocholines (PC) and Glycerophosphoethanolamines (PE). The Lipid Omega Analyzer (LipidOA) was pivotal in this process, offering a robust method for de novo lipid C=C location sequencing. This tool enhances our workflow by enabling precise data cleaning and identification at multiple levels: lipid class, molecular species, C=C bond positions, and sn-positions. Additionally, we established a digital modeling approach based on deep lipidomics for disease diagnosis. By integrating a machine learning strategy with a Cox proportional hazards model, we incorporated structural lipid histological features into clinicopathological characterizations. This multi-omics fusion model was constructed and successfully applied to predict NAT treatment response with an accuracy of 96%, and to forecast disease-free survival (DFS) in breast cancer patients.

Lipid Metabolism in Gliomas by Using Arterial-Venous Analysis

Wen Li[1], Wenpeng Zhang[1], Zheng Ouyang[1]

[1] Tsinghua University, Beijing, China

P11

ABSTRACT

Glioma is the most common and lethal primary brain tumor. Most malignant brain gliomas (MBGs) are associated with dismal outcomes, mainly due to their late diagnosis. Current diagnostic methods rely on imaging and histological examination, which limits their early detection. Hence, the development of high-precision and sensitive biomarkers for glioma is crucial.

Here, we aimed to identify reliable plasma lipid biomarkers for glioma diagnosis by comparing arterial supply and venous drainage in combination with deep structural lipidomics. After performing targeted and untargeted lipidomics analysis through paired comparison of arterial-venous plasma (n=30) based on five levels (phospholipid (PL) class, PL subclass, fatty acid (FA) sn-position, double bond (DB) location of PL and free fatty acid (FFA)), we can exclude the interpatient variation that is present in plasma samples.

A Total of 208 lipid subclasses were tentatively identified, data showed that nearly 50% of the lipids exhibited significant differences between arterial and venous plasma. In addition, we quantified the FFA and FFA isomers, surprisingly, Significant differences in FFA levels were observed, with all significantly different FFAs reduced in venous plasma through glioma, the decreased FA levels may be correlated with GBM dependency on FA as build material and energy source in addition to glucose derived from anaerobic glycolysis. Futher we found that FA isomers showed inconsistent variation, Certain FFAs (e.g., FFA 18:1 (n-7), FFA 20:1 (n-6), and FFA 16:1 (n-9)) were produced in larger amounts by gliomas. Other FFAs (e.g., FFA 18:1 (n-9), FFA 20:1 (n-5), and FFA 16:1 (n-6)) were consumed in larger amounts by gliomas.

This study underscores the potential of arterial-venous lipid analysis combined with deep lipidomics as a strategy for identifying sensitive and specific biomarkers for glioma. Currently we are validating these findings by establishing external cohort and conducting metabolic mechanism studies.

Unveiling COPD Phenotypes with Targeted Metabolomics Approaches-Taking Signaling Lipids as a Stepping Stone

Lu Zhang[1], Jean Marie Wernet[1], Susy Braun[2], Emmylou Beekman[2], Darcy Ummels[2], Tanja Luxzenburg[2], Wei Yang[1], Lieke Lamont[1], Alida Kindt[1], Amy Harms[1], Thomas Hankemeier[1], Herman van, Wietmarschen[3]

[1] Metabolomics and Analytics Centre, Leiden Academic Centre for Drug Research, Leiden University Leiden, Netherlands

[2] Zuyd University of Applied Sciences, Heerlen, Netherlands

[3] Louis Bolk Institute, Utrecht, Netherlands

P12

ABSTRACT

Aims: Chronic Obstructive Pulmonary Disease (COPD) is one of the most mortal and prevalent diseases, leading to the enormous health burden which is expected to increase due to the aging of population, air pollution and exposure to the biomass fuel waste. It has developed diverse treatments including non-pharmacological interventions such as smoking cessation, exercise training and pulmonary rehabilitation, and pharmacotherapies such as antibiotics, β2 agonists, corticosteroids, etc. However, the complexity and heterogeneity of COPD led to the obstacle of COPD management. To clarify different types of COPD and facilitate clinical practice as well, the concept of phenotypes has been introduced. Although the phenotypes have been investigated and developed, the underlying mechanisms still remain unclear. This study applied the signaling lipid assay to unveil the signaling pathways and mechanisms behind COPD subtypes and discover COPD metabolic patterns.

Methods: First, COPD patients were recruited by Zuyd University of Applied Sciences followed by the collection of basic demographic information and COPD-related clinical indicators. The patients were required to fill out diverse questionnaires and perform physical capacity tests including six minute walk test. Plasma samples from participants were collected and analyzed with the signaling lipids assay to obtain metabolic patterns of participants. After data acquisition, the ordinal regression model, analysis of covariance, and Spearman correlation were performed to identify the key metabolites in COPD classifications.

Results: This study revealed metabolic patterns in Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) stages, Koninklijk Nederlands Genootschap voor Fysiotherapie (KNGF) profiles and questionnaire-based subtypes. Specifically, lysophospholipids, oxylipins and bile acids were associated with different COPD classifications. Lysophosphatidylethanolamine, lysophosphatidylserine and lysophosphatidic acid are correlated with GOLD stages and KNGF profiles. The oxidation products of fatty acids via lipoxygenases (LOX) are linked with KNGF profiles and questionnaire-based subtypes. Additionally, the bile acids are specially connected with GOLD stages. More importantly, a cluster of oxylipins were found to be positively correlated with lung attacks and antibiotic treatment, among which are driven by LOX-12.

Kinetic Isotope Tracing Combined with MS Methods Reveals De Novo Synthesis and Metabolism of Phospholipids C=C Isomers

Zhuoning Xie[1], Zheng Ouyang[1], Xiaoxiao Ma[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

Recently, the analysis of unsaturated lipid C=C double bond position isomers has been increasingly emphasized, especially regarding their roles in cancer cell metabolism. However, due to the complexity of the phospholipid structure, analysis of C=C isomers is limited to static relative proportions.

In this study, we combined 13C-glucose isotope tracing with LC-PB-MS/MS system to comprehensively analyze the de novo synthesis process of phospholipids in human breast cancer cells. For the first time, we revealed the labeling rates of phospholipid C=C isomers, casting light on the dynamics of phospholipid synthesis and metabolism at C=C location level. We also accurately determined the 13C labeling sites in the molecule and explored the changes in de novo synthesized lipids at the C=C isomer level under SCD1 inhibition.

Breast cancer cells (MCF7 and MDA-MB-468) were analyzed, with over 10 sampling points from 0h to 130h at different labeling times.

At the phospholipid subclass level, we over 20 lipids, including PCs, PEs, PSs and SMs. The global labeling rate of PCs surpasses that of PEs, indicative of a higher turnover rate for PCs. In the acyl chains, the highest labeling rate is observed in C18:1, followed by C18:0, C16:0, and C16:1.

As for the 13C labeling sites, initially, the 3-carbon unit of G-3-P skeleton is rapidly labeled. With increasing labeling time, the additional 13C predominantly incorporates into acyl chains, with minimal in head groups. PB-MS/MS analysis reveals that at short labeling time, 13C predominantly localizes to the carboxyl end of the acyl chains, due to the later integration of 13C-labeled CoA during the de novo synthesis of palmitate-CoA.

As for the C=C isomers, five lipid species (PC36:1, PC36:2, PC34:1, PC32:1, PE36:1) with representative and higher abundances were chosen for analysis. It's worth noting that for these phospholipids, the LE of n7, n9 isomers shows a consistent trend over time, indicating that their turnover rates are in accordance with static ratios. Furthermore, we posit that compared to the elongation and desaturation processes, the glucose uptake and the synthesis of palmitoyl-CoA is relatively slow, resulting in uniform labeling trends of FA C=C isomers. Though free fatty acids (FFA) reach a labeling steady state in less than a day, downstream integration into phospholipids like PC and PE is more protracted.

Analysis of Single-Cell Lipid Metabolism in Mixed Microalgal Populations Using Combined Fluorescence-Activated Cell Sorting and Label-Free Mass Cytometry

Yu Qiao[1], Shiji Xue[1], Xiuchang Zhu[1], Hetian Jia[1], Xusheng Huang[1], Yue'e Peng[1]

[1] China University of Geosciences (Wuhan), Wuhan, China

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ABSTRACT

Microalgae are among the earliest life forms on Earth and play a crucial role in the ecosystem. As unicellular organisms, microalgae exhibit significant heterogeneity both inter- and intra-specifically, making single-cell studies an ideal approach to understanding their physiology. Single-cell metabolomics, especially lipidomics, can uncover the differences in metabolite composition and concentration within individual microalgae, which directly reflect their responses to the surrounding environment. Therefore, developing single-cell lipid analysis methods provides a powerful tool for investigating microbe-environment interactions. Label-free Mass Cytometry (CyESI-MS) has enabled high-throughput detection of metabolites in pure culture of microalgae, making it suitable for single-cell lipidomic analysis. However, in natural environments, microalgae often coexist in diverse communities, and interpreting signals from mixed microalgal systems without prior knowledge remains challenging. To address this, we propose a novel approach combining fluorescence-activated cell sorting (FACS) with CyESI-MS to study the phenotypic differentiation of mixed microalgal populations. Specifically, we explored the Chlorella-Microcystis allelopathy system as a case study.We optimized the CyESI-MS platform to achieve a detection throughput of 30 cells per minute and analyzed the lipidomics of single cells of both Chlorella (820 cells) and Microcystis (386 cells) in the co-cultured system, obtaining corresponding single-cell mass spectrometry data. Based on these data, we further developed a Python-based software for the preprocessing and machine learning analysis of massive single-cell mass spectrometry datasets. Our results showed that both Chlorella and Microcystis displayed changes in their lipidomes under allelopathic conditions. Additionally, on the fourth day, Chlorella developed two subtypes, which was consistent with the results of fluorescence-activated cell sorting. Based on these data, we conclude that FACS combined with CyESI-MS provides a powerful tool for analyzing single-cell lipidomics in mixed microalgal communities. This research was supported by the National Natural Science Foundation of China (NSFC) under Grant No. 21775141.

Analysis of Lipid Components in Five Kinds of Cordyceps Based on Lipidomics

Yang Yang[1]

[1] Shen zhen Bao'an Authentic TCM Therapy Hospital, Shenzhen, China

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ABSTRACT

Objective: In recent years, the of Cordyceps sinensis (CS), which has been listed as a national second level endangered species, the production of CS is low, the demand is high, and it is easy to be adulterated. Since the 2000 edition of the Chinese Pharmacopoeia, the quality standards for CS have been established based on the content of adenosine as an indicator. However, the adenosine is also commonly present in other fungi, lacking specificity. Therefore, it is incomplete to take adenosine as a quality indicator for CS. Lipids are a diverse group of compounds with many key roles enabling them to serve as forms of energy storge, cellular membrane and signaling molecules.Guo et al. have demonstrated that fatty acid composition might be sensitive makers for determinantion of CS geographical origins. We attempt to identificate and achieve a preliminary evaluation of the quality of CS through the research of lipidomics in this study.

Method: Were analyzed using an LC-ESI-MS/MS system ,the lipids of wild Cordyceps sinensis (YS), cultivated Cordyceps sinensis(RG), Cordyceps taii (DS), Liangshan Cordyceps sinensis (LS), and Cordyceps hawkesii (YXB) were analyzed from the perspective of lipidomics.Result: A total of 671 lipid components, were identified in all samples, among which the highest number of lipid components of TG was detected as 341. It was found that the detection of lipid components of LDGTS and DGGA classes is expected to be the key lipid type components for the identification of CS.The number of double bonds of unsaturated lipid components in the five species of Cordyceps were all between 2-5, and the difference in the number of double bonds was not obvious, but there was a significant difference in the content of the unsaturated lipid components, especially the difference was significant in the number of carbon chains of 2 and 5. When the unsaturated carbon chain element was 2, the unsaturated lipid content was the highest among the TG-like lipids of the five samples; when the number of unsaturated carbon bonds was 5, the lipid content of YS and RG, DS, LS, and YXB was decreasing. Six lipid components unique to ys were found, Cer(t18:0/25:1(2OH)) (C43H85NO5), HexCer(d16:2/22:0(2OH))(C44H83NO9), PI(18:1_22:3)(C49H87O13P), PMeOH(16:0_18:2)(C38H71O8P), TG(24:0_18:3_18:3)(C63H110O6), and TG(18:2_20:0_20:1)(C61H112O6).

Conclusion: It is found that the detection of ldgts and dgga lipid components is expected to become the key lipid type components for the identification of CS. Six lipid components unique to YS were screened; can be used as the relevant marker lipid identity for distinguishing YS from other CS, especially for distinguishing RG from YS. The main metabolic pathway that differed among different groups was the metabolic pathways, suggesting that this pathway can be used as one of the main metabolic pathways to explore the quality differences and identify the differences between different kinds of CS.

Sphingolipid Remodeling in the Plasma Membrane Is Essential for Osmotic and Insectic Stress Tolerance in Arabidopsis

Yong-kang Li[1], Yu-meng Zhang[1], Guang-yi Dai[1,2], Yi-li Chen[1], Ding-kang Chen[1], Nan Yao[1]

[1] State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Shenzhen, China

[2] South China National Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

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ABSTRACT

Osmotic stress caused by drought, salinity, or cold stress is an important abiotic factor that decreases membrane integrity and causes cell death, thus decreasing plant growth and productivity. Remodeling cell membrane composition via lipid turnover can counter the loss of membrane integrity and cell death caused by osmotic stress. Sphingolipids are important components of eukaryotic membrane systems. A growing body of evidence has recently shown that sphingolipids participate in plant responses to biotic and abiotic responses. Here, we characterized the role of AtGCD1 (encoded by At1g33700) in sphingolipid remodeling and acclimation to osmotic stress in Arabidopsis (Arabidopsis thaliana). We determined that AtGCD1 functions as a glucosylceramidase that localizes to the plasma membrane. AtGCD1AtGCD4 are Arabidopsis homologs of human nonlysosomal glucosylceramidase. We show that recombinant AtGCD1 has no substrate preference for acyl chain length and that AtGCD1 and AtGCD3 (At4g10060) are essential for osmotic stress tolerance in Arabidopsis. In cells treated with mannitol, AtGCD1 and AtGCD3 hydrolyzed glucosylceramides to ceramides, leading to a decrease in glucosylceramide contents and an increase in glycosyl inositol phosphoceramide contents. We observed a substantial change in the molecular order of lipids and membrane tension at the plasma membrane of the Arabidopsis gcd1 gcd3 double mutant, indicating that glucosylceramidases compensate for changes in membrane properties to stabilize the membrane during osmotic stress. Moreover, loss of GCD1 and GCD3 enhances plant resistance to S. exigua. Our results suggest that sphingolipid remodeling regulates the physicochemical properties of cellular membranes during plant stress responses.

Revealing the Hydrophobic Interactions Between Lipid Bilayers and Transmembrane Peptides via Photo-Tagging and Mass Spectrometry

Jing Zhao[1,2], Xuewei Dong[3], Lipeng Qiao[1], Kai Yang[3], Yu Xia[1]

[1] MOE Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology Department of Chemistry, Tsinghua University, Beijing, China

[2] Beijing Life Science Academy, Beijing, China

[3] Center for Soft Condensed Matter Physics and Interdisciplinary Research & School of Physical Science and Technology, Soochow University, Suzhou, China

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ABSTRACT

Annular lipids play a pivotal role in maintaining membrane protein structure and function. Structural biology tools have offered valuable insights into the membrane protein conformation and closely-binding lipids. However, due to the complexity and flexibility of the annular lipids, pinpointing the precise site of interaction on the fatty acyl chains of lipids has remained a challenging endeavor. In this study, we designed a cleavable photo-tagging probe (NHS-c-BPA) based on p-benzoyl-l-phenylalanine (BPA) to staple the model peptide and surrounding annular lipids in artificial vesicles upon photoactivation. Specifically, MS/MS via collision-induced dissociation (CID) was developed to provide specific cleavages around the tagging site, offering a valuable tool to indicate the site of interaction. Firstly, BPA itself was chosen to test photo-tagging of saturated fatty acid, FA 18:0. The products resulting from BPA tagging at different carbons on FA 18:0 were separated by reversed-phase liquid chromatograph (RPLC). Collision-induced dissociation (CID) of these products offered distinct fragment ions at the tagging site. The fragmentation rule was corroborated by replacing the FA 18:0 with [D4]FA 18:0 or [13C18]FA 18:0, which was triggered by the phenyl transfer around the tagging site. Then, the developed cleavable probe BPA-c-NHS was incorporated into the lysine side chain in model transmembrane peptides WALP, such as P23-K17 (Ac-GWWLALALALALALALKLALWWA-NH2) and P23-K12 (Ac-GWWLALALALAKALALALALWWA-NH2). For both peptides, tagging products were observed from C4-C17, though their relative distributions differed. *P23-K17 favored tagging more toward the C5-C9 region of the fatty acyl chains, whereas *P23-K12 showed a bimodal distribution, with a noticeable increase in tagging toward C13-17. Consistent with molecular dynamic simulations, the distribution of the photo-tagging product can reflect changes in the buried depth of the modified residues or the relative position of the residue within the lipid bilayers. Additionally, by comparing the photo-tagging results of *P23-K17 and *P16-K11 in PC 18:0/18:0 vesicles, the established approach demonstrated its potential to probe variations in the lipid microenvironment influenced by transmembrane peptides of different length. In the future, by varying the composition of lipid vesicles, our methods could potentially investigate the stoichiometry of annular lipid and identify the preferred lipid surrounding the model transmembrane peptide.

Deep Profiling of Gangliosides Enabled by Selective Enrichment and Liquid Chromatography- Trapped Ion Mobility-Mass Spectrometry

Yichun Wang[1], Yu Xia[1]

[1] Department of Chemistry, Tsinghua University, Beijing, China

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ABSTRACT

Gangliosides are a class of acidic glycosphingolipids containing one or multiple sialic acid modifications on the glycans. They are typically found on the outer leaflet of the plasma membrane, playing roles in neuronal development, cellular signaling, and immune modulation. Mapping the molecular profile of gangliosides is crucial for investigating their biological functions. However, profiling of gangliosides is challenging due to the existence of interfering lipids and limited capability in resolving isomers and isobars. To reduce the matrix effect from interfering lipid species, we developed a TiO2 magnetic nanoparticle (MNP)-based procedure for the selective enrichment of gangliosides. Firstly, gangliosides in crude extracts were captured on TiO2 MNPs using an ACN/NH4OH (96/6, v/v) mixture. The selective capture of gangliosides relies on the coordination between Ti(IV) and the cis-diol group on the sugar moiety. Simultaneously, PI and PS are also captured by TiO2 MNPs through coordination between the Ti(IV) ions and the cis-diol groups in PI, as well as the serine residues in PS. Interfering lipids, such as SHexCer, with its C3-sulfation disrupting the cis-diol configuration, and PG, with its more flexible cis-diol, only exhibit weak coordination with Ti(IV) during the loading step. Subsequently, gangliosides were released from TiO2 MNPs using DMF, while most PS and PI remained on the TiO2 MNPs. It is hypothesized that higher polarity lipids, like gangliosides, tend to dissolve in DMF and disrupt their coordination, whereas lower polarity lipids, such as PI and PS, maintain coordination with Ti(IV). This procedure achieved over 96% recovery for standard GD1a/b and approximately 70% recovery for standard GM3, with over 80% removal of interfering lipids. In the analysis of porcine brain total lipids, the loading capacity on Amide-TIMS-MS increased from 0.5 μg to 6 μg per injection due to the removal of interfering lipids.

A workflow was established for deep profiling of gangliosides from porcine brain total lipids utilizing Amide hydrophilic interaction lipid chromatography HILIC separation-Trapped Ion Mobility-Mass Spectrometry and offline PaternòBüchi derivatization. Ultimately, 33 subclasses and 235 molecular structures of gangliosides were identified from porcine brain total lipid extracts, far exceeding previous reports. Further profiling of gangliosides at the chain composition level and C=C location level will be conducted to investigate structural alterations of gangliosides in disease.

Identification of Unsaturated Fatty Acids Derivatized by Dimethyl Disulfide by Chemical Ionization Tandem Mass Spectrometry with High Sensitivity and Specificity

Dong Hao Wang[1], Tingxiang Yang[1]

[1] School of Agriculture, Shenzhen Campus of Sun Yat-sen University, Shenzhen, China

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ABSTRACT

Derivatization of unsaturated fatty acids with dimethyl disulfide (DMDS) and analysis by electron ionization mass spectrometry (EIMS) represents a convenient offline method for the identification of double bond positions. However, the presence of overlapping mass spectra from multiple compounds poses significant challenges for spectral interpretation and library matching, leading to ambiguous molecular information and low sensitivity. A state-of-the-art method, covalent adduct chemical ionization (CACI) MS has been developed by our lab and represents an effective gas chromatography (GC) technique for elucidating double bond positions. This method, however, requires a special solvent introduction system. Here, we establish a novel chemical ionization (CI) tandem mass spectrometry method involving the pre-derivatization by DMDS. It overcomes the issue of mass spectral interference caused by co-elution, is specific to the analysis of targeted fatty acids, and do not require any customized device. Further, a multiple reaction monitoring (MRM) version of the method is developed by screening all the diagnostic ions of possible double bond positional isomers, which significantly boosts sensitivity. Compared to the traditional EIMS method, the new method exhibits a lower limit of detection (LLOD) that is one-tenth or lower. Employing the new method, unusual isomer 18:2(5Z,8Z) was co-analyzed with 18:2(9Z,12Z), and a novel 20:2(7Z,10Z) was characterized in human sebum. Additionally, minor 16:2(9Z,12Z) and polymethylene-interrupted isomers, 22:2(7Z,13Z) and 22:2(7Z,15Z) were identified in seafood and related products. Our method can be readily applied to any GC instrument equipped with tandem MS and is expected to facilitate the discovery and identification of unknown fatty acids from food, clinical, and environmental sources.

Comprehensive Profiling of Human Milk Gangliosides across Lactation Stages via Liquid Chromatography-Cyclic Ion Mobility-Mass Spectrometry System

Haiyue Hou[1], Shuya Yang[1], Zhenxia Du[1]

[1] Beijing University of Chemical Technology, Beijing, China

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ABSTRACT

Gangliosides (GAS), a subtype of glycosphingolipids, are complex lipids found in human milk and participate in diverse biological processes, including neural development, pathogen binding, and activation of the immune system. However, the comprehensive characterization of milk GAS is ever-challenging due to their structural complexity and low abundance, especially when authentic standards are unavailable. Thus, we utilized high-resolution liquid chromatography-cyclic ion mobility-mass spectrometry system (LC-cIM-MS) to characterize and evaluate the molecular distribution of GM1, GM3, and GD1 in human milk samples collected at distinct lactation stages. Arrival-time alignment technology of cIM-MS enables the generation of structurally specific MS/MS spectra, facilitating the accurate elucidation of GAS isomers at the level of sialic acid position. Using this approach, a remarkably diverse milk GAS molecular ion pattern was discovered, regarding abundant adduct forms and clear isotopic distribution pattern resolved by ion mobility. The collision cross section (CCS) measurements for GAS exhibited high reproducibility, and the unique CCS-m/z correlation trend lines related to species and charge were further characterized, demonstrate the potential for discovering new structures. Ultimately, 24 gangliosides were identified in human milk with an average mass error of 0.74 ppm, and the divergence of GAS profiles across the lactation period were revealed for the first time. These findings offer valuable insights for the precise optimization of infant formula.

Resolving the Geometry and Location of Lipid Unsaturation by Radical-Induced Isomerization and RPLC-MS/MS Coupled with Online Paternò-Büchi Reaction

Hengxue Shi[1], Yu Xia[1]

[1] Tsinghua University, Beijing, China

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ABSTRACT

Introduction: The location and geometry of a C=C bond impart distinct structural and functional properties to lipids. Recent progress in isomer-resolved tandem mass spectrometry (MS/MS) techniques has enabled profiling of lipids at the C=C bond location level. Identification of C=C geometric isomers often relies on the matching of the chromatographic retention behavior with lipid standards. However, the geometric and positional isomers of unsaturated lipids often co-elute, e.g. PC 18:1(Δ6Z)/18:1(Δ6Z) and PC 18:1(Δ9E)/18:1(Δ9E), causing challenges in their identification and quantitation in biological systems. Herein, we paired offline thiol radical-induced C=C isomerization with LC-MS/MS and online Paternò-Büchi (PB) derivatization. This approach enabled sensitive and accurate determination of both C=C geometry and position in unsaturated lipids.

Preliminary Data: The reactions of FA 18:1(Δ9Z) or FA 18:1(Δ9E) with 2-mercaptoethanol were used to optimize the efficiency of thiol radical-indued isomerization of C=C in lipids. The reaction solution was collected and subjected to RPLC-MS analysis, which provided a complete separation of C=C geometric isomers, with Z-isomers eluting earlier than E-isomers. Regardless of whether FA 18:1(Δ9Z) or FA 18:1(Δ9E) was used as the starting material, the isomerization reaction all led to a mixture of 20% FA 18:1(Δ9Z) and 80% FA 18:1(Δ9E). Additionally, no thiol addition products were detected. This preferential Z to E C=C isomerization was also observed in PE 18:0/18:1(Δ9Z) and DG 16:0/18:2(Δ9Z, Δ12Z). Therefore, the geometry of C=C could be identified based on the isomerization rule and the elution order from RPLC. Based on the LC peak area, quantitation of C=C geometric isomers was achieved with the linear dynamic range of isomer ratios being one order of magnitude.The acetone PB reaction was installed online right after LC separation and before MS analysis. Subsequent PB-MS/MS produced diagnostic ions for pinpointing C=C locations. By pairing the offline thiol radical-induced isomerization with RPLC-PB-MS/MS, both C=C geometric and position were determined, as exemplified by the analysis of a standard mixture of PC 18:1(Δ6Z)/18:1(Δ6Z) and PC 18:1(Δ9E)/18:1(Δ9E).Finally, we employed this workflow to investigate the alteration of membrane lipid compositions in P. Syringae after an exposure to a toxic organic solvent. Our findings revealed a significant increase of the trans C=C isomers of phospholipids after the addition of 2% toluene to the growth medium. Interestingly, the relative composition of lipid C=C location isomers remained constant, with a prevalence of n-7 C=C location.

Deep Profiling of Gut Bacteria Lipidome via Data-Dependent Acquisition and Radical-Directed Dissociation-Tandem Mass Spectrometry

Ruijun Jian[1], Xiaoqi Zhu[2], Hailiang Liu[2], Yu Xia[1]

[1] Department of Chemistry, Tsinghua University, Beijing, China

[1] State Key Laboratory of Cardiology and Medical Innovation Center, Shanghai East Hospital School of Medicine, Tongji University, Shanghai, China

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ABSTRACT

Introduction: An increasing amount of research reveals that lipids excreted by gut bacteria play a crucial role in maintaining host health homeostasis. For example, the branched phosphatidylethanolamine, PE a-15:0/i-15:0 in Akkermansia muciniphila induces beneficial immune responses. However, conventional lipid analysis methods do not provide detailed structural information of lipids. Herein, we introduce a comprehensive workflow that facilitates lipidomic profiling at the chain modification level for gut microbiota by harnessing data-dependent acquisition (DDA) and radical-directed dissociation-tandem mass spectrometry (RDD-MS/MS). This workflow enables the identification of fourteen lipid subclasses in Akkermansia muciniphila(Akk), including two new subclasses of ornithine-containing lipids unreported before.

Methods: TBN was in-lab synthesized. MeO-QN was purchased commercially. Akk was cultured under anaerobic conditions at 37 ° C in brain heart infusion. Lipids from 2 × 1010 bacteria were extracted via a modified Folch method, and then redissolved in 1 mL of MTBE/MeOH. For derivatization, 200 µL of the extract was reacted with TBN; another aliquot (200 µL) was subjected to saponification and then derivatized with MeO-QN. Lipidomic analysis was conducted on an X500R Q-TOF (Sciex) hyphenated with a 20AD HPLC (Shimadzu), employing a C18 column. Data were acquired by data-dependent acquisition and processed by MS-DIAL (version 5.1). RDD-MS/MS spectra were acquired by targeted MS/MS.

Preliminary data: We identified 14 lipid subclasses, including phosphatidylethanolamine (PE), lyso-phosphatidylethanolamine (LPE), cardiolipin, ornithine lipid, lyso-ornithine lipid (LOL). Importantly, we discovered two previously unreported subclasses of ornithine-containing lipids. The first subclass, named N-ornithine lipids, is an isomerized form of ornithine lipids with a second fatty acyl group linked to the δ-amine of LOL via an amide bond. The second subclass features a third fatty acyl group linked to the 3-OH of the N-ornithine lipids via an ester bond. These newly identified lipids were confirmed through a comparison to the LC retention times and MS/MS spectra of chemically synthesized lipids.

Total fatty acids were analyzed using MeO-QN-RDD-MS/MS, revealing seven fatty acids at the chain branched level, ranging from C13:0 to C18:0. Notably, FA a-15:0 constituted over half of the total fatty acid content. TBN-RDD MS/MS was employed to analyze chain modification of the amine-containing lysolipids, such as LPE and LOL. 12 unique structures of LPEs (LPE14:0-17:0) were identified with definitive sn-position and chain branching information. LPE a-15:0/0:0 accounted for over 50% of all LPEs. Seven distinct structures of LOLs (LOL15:0-18:0) were identified including several methyl branching isomers. As an example, LOL 17:0 was a mixture of the straight-chain (LOL n-17:0) and a branched isomer (LOL a-17:0).

Fine Structure Mass Spectrometric Characterisation of Triglyceride Positional Isomers Based on Electron-Activated Dissociation Technique

Ying Wang[1], Xiuqin Li[1], Qinghe Zhang[1]

[1] National Institute of Metrology of China, Beijing, China

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ABSTRACT

Triacylglycerols (TAGs) play an important role in living organisms, and there is a close relationship between their physiological functions and their structures. TAG is produced by esterification of three fatty acids and one molecule of glycerol. Its structure is influenced by the fatty acid species, the number of double bonds, the position of double bonds, the double bond configuration, and the connection position of the fatty acids in the glycerol backbone. The diversity and similarity of the structures pose a great challenge for the fine structure analysis of glycerol esters. Mass spectrometry (MS) is a powerful technique for chemical structure analysis, which can be used to analyse the molecular structure by using the characteristic fragment ions, and it has become the preferred analytical tool for the compositional analysis and structural identification of TAGs.

Collision-Induced Dissociation (CID) produces thermally excited ions that are insufficient for unambiguous identification or complete characterisation of triglyceride fatty acid chains. Electron Activated Dissociation (EAD) is an electron-mediated cleavage method based on the application of a 10 eV electron beam to positively charged lipid ions, which causes the EAD spectra to show abundant fragmentation ions, enabling the identification of fatty acyl positions in the lipid structure [1].

The aim of this study was to investigate the differences between the two dissociation methods CID and EAD in the structural resolution of triglycerides, as well as to probe the ability to discriminate between the identification of 10 isomeric compounds at the TAG position (Fig. 1). CID provides information on fatty acid chain composition and position through multistep dissociation and fragment ion ratio calculations. The EAD technique not only allows TAGs to undergo a loss of the diacyl chain, generating characteristic ion fragments with different peak sets in the 300-350 Da range, but also generates fragmentation peaks near 600 Da at the same cleavage site as in the CID mode. The EAD technique can better confirm the cleavage site of triglycerides and the positional isomerism of fatty acids, making it an effective strategy to distinguish TAG positional isomers with higher confidence. These results will provide a powerful tool for the in-depth study of lipid isomer structure.

Keywords: Triacylglycerols; positional isomers; structural characterisation; electron-activated dissociation

Reference:

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Structural Lipidomics Reveals Alterated Lipid Isomers in Drug-Induced Liver Injury

Jinling Lu[1], Yikun Liu[1], Zheng Ouyang[1], Wenpeng Zhang[1]

[1] Department of Precision Instrument, Tsinghua University, Beijing China

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ABSTRACT

Triptolide (TP), an active component of Tripterygium wilfordii Hook. F., used in the treatment of rheumatoid arthritis and lupus erythematosus, poses a risk of drug-induced liver injury (DILI). Investigating lipid metabolism can elucidate the pathogenesis of DILI, aid in its prevention, and inform treatment strategies. This study employs LC-MS/MS for lipidomics analysis to identify lipid structures in rat liver samples with TP-induced injury and to discover potential lipid biomarkers. To establish a TP-induced liver injury model, Sprague-Dawley rats were divided into control, low-dose, and high-dose groups (n=6 per group), receiving TP via gastric administration for 28 days. LC-MS/MS methods for identification of complex lipids beyond the species (headgroup information) and molecular species (chain composition) levels. These include identification at the levels of C=C location and sn-position. Significant alterations in lipid metabolism were observed in the rat model of TP-induced injury. The APC/APE ratio decreased, while the ALPC/APC ratio increased, potentially related to phosphatidyl-ethanolamine N-methyltransferase (PEMT) metabolism. The ratios of sn-isomer pairs PC 16:0_18:0, PC 16:0_18:1, PC 16:0_18:2, and PC 18:1_18:2 decreased, while the ratios of PC 18:0_18:1 and PC 16:0_20:4 sn-isomer pairs increased, likely reflecting changes in phospholipid acyltransferase (LPCAT) activity. Analysis revealed significant changes Δ9/Δ11 isomer pair in C18:1, including 9 pairs of PE and 10 pairs of PC isomers. The relative content of n-5 and n-9 isomers of FA 16:1 increased, while the n-7 isomers decreased. Additionally, the relative content of n-7 and n-9 isomers of FA 18:1, as well as n-9 isomers of FA 20:1 also increased. These changes in C=C isomers of lipids may be related to the activities of desaturation and elongation enzymes, as well as fatty acid oxidation. The study identified differential lipid metabolites, offering insights into potential biomarkers and mechanisms underlying TP-induced DILI.

Site-Specific Determination of Triacylglycerol Regioisomers by EIEIO Technique Combined with in Silico-Predicted Characteristic Fragmentation MS/MS Database

Zuojian Qin[1]

[1] Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan, China

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ABSTRACT

A novel strategy based on electron impact excitation of ions from organics (EIEIO) technique in conjunction with specialized characteristic fragmentation ions database was developed for analyzing different types (AAA/AAB/ABC) triacylglycerol (TG) regioisomers. Typical standards were used to validate the ability to structurally elucidate TG regioisomers and adequately determine the mass fragmentation behavior of TG species in EIEIO mode. Based on fragment pattern of TGs, the characteristic fragmentation ions for sn positional information of fatty acyl chains in TGs were automatically calculated with computational algorithm, and a silico-predicted CFID MS/MS database (including 57010 EIEIO-MS/MS spectral) was predicted to achieve a rapid and accurate regioisomeric assignment of TG regioisomers. This in silico-predicted CFID MS/MS database was first reported and applied in the TG regioisomeric assignment in plant oils with different source. The findings provide essential and innovative insights for the in-depth elucidation of TG regioisomers in plant oils from various sources.

Comprehensive Lipid Structural Identification in Single Cells with High Throughput

Chenxi Cao[1], Yao Qian[1], Zheng Ouyang[1], Xiaoxiao Ma[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

Single cell mass spectrometry (MS) is an essential technology for sensitive and multiplexed analysis of metabolites and lipids, facilitating cell phenotyping and pathway studies. However, due to the extremely small amount of sample available, the structural elucidation of lipids from individual cells remains a challenge, especially in high-throughput scenarios, where there is a need to balance the total analysis time per cell with the number of tandem MS (MS/MS) acquisitions.

In this work, we introduced a novel high-throughput approach for identifying the structural composition of lipids in individual cells, utilizing a Waters quadrupole-cyclic ion-mobility-time-of-flight mass spectrometer (Q-Cyclic IM-TOF-MS). Cells were introduced into the MS through a homemade three-layered capillary tubing (CyESI), and precursor ions from single cells were separated in the Cyclic IMS cell, followed by the acquisition of either full scan or co-fragmented spectra. Specifically, two sets of frames, acquired at low and high collision energies, were used to generate IM-MS spectra and IM-MS/MS spectra for a single cell. Based on the different lipid compositions at different IM elution time, an MS/MS deconvolution algorithm was implemented via nonnegative least square optimization, allowing for accurate identification and quantification of lipid species.

With this workflow, a cell analysis rate of 30 cells per minute was achieved, and a total of 15 and 36 lipids were identified from a single MDA-MB-468 cell using MS/MS information in positive and negative ion modes. Additionally, by further resuspending the cells in an ammonium bicarbonate aqueous solution, we successfully achieved the identification of the sn- positions of phosphatidylcholines in single cells, and the number of identified lipids from single cells in negative mode increased to 43 species.

In conclusion, our method provides a powerful platform for high throughput single-cell lipidomics analysis, with the capability of fine structure identification of lipids.

Intramolecular Paternò-Büchi Reaction for Highly Sensitive Profiling of Fatty Acid C=C Location Isomers

Lipeng Qiao[1], Jing Zhao[1], Hengxue Shi[1], Yichun Wang[1], Yu Xia[1]

[1] Tsinghua University, Beijing, China

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ABSTRACT

Introduction: The molecular distribution of fatty acids (FAs) reflects the metabolic status of a biological system and altered FA profiles are increasingly linked to disease conditions. Accurate profiling of FAs requires the identification and quantitation of each individual isomer, thus providing insights into their distinct metabolic pathways. The Paternò-Büchi (PB) reaction coupled with tandem mass spectrometry (MS/MS) has been applied for unsaturated lipid analysis. However, PB reaction is concentration-dependent, resulting in low yields for low concentration lipids (<1 μM). Herein, we have developed an intramolecular Paternò-Büchi (iPB) reaction-MS/MS to improve sensitivity and quantitation of low concentration lipids, overcoming the limitations of the conventional PB reaction.

Methods: The iPB reagent methyl 2-amino-3-(3-nicotinoylphenyl)propanoate (MePPA) was synthesized in lab. FAs were labeled by MePPA in acetonitrile. The derivatized FAs were subjected to reversed-phase liquid chromatography on a Micro-LC system. An online flow photo microreactor was installed between the LC and ESI source. The iPB reaction was initiated by 254 nm light irradiation from a low-pressure mercury lamp. MS data were collected on Orbitrap Exploris 240.

Preliminary Data: The iPB reaction was tested by MePPA-FA 18:1 (9Z), where the benzoylpyridine functional group in MePPA served as the PB reagent. Resulting from the two possible orientations during [2+2] cycloaddition, two regio-isomers of the iPB products were observed. Collision-induced dissociation (CID) of the protonated iPB products generated a pair of C=C diagnostic ions, one containing a macrocyclic structure and the other featuring an open-ring structure. The yield of the iPB reaction reached 40% after 4 s' UV irradiation. Compared with conventional PB reactions, the yield of iPB reactions was concentration independent across six orders of magnitude, greatly favoring the analysis of low concentration lipids around nM range. For polyunsaturated lipids, no sequential iPB reactions could proceed, allowing an improvement of sensitivity. More importantly, an RPLC-online iPB-MS/MS workflow was developed. Our results showed that MePPA-FAs exhibited high ionization efficiency in MS1 for relative quantitation of the sum composition level, achieving a limit of quantification (LOQ) of 0.5 nM at the sum composition level. By turning on the online iPB reaction, iPB-MS2 CID generated C=C diagnostic ions resulting from a neutral loss of the methyl end at the C=C bond, achieving a limit of identification (LOI) of sub-nM at the C=C location level. This workflow requires fewer than 103 cells to achieve deep profiling of total fatty acids (TFAs) from MCF-10A breast cell line, identifying 58 FAs at the sum composition level and 105 FAs at the C=C location level (C2-C26), including low concentration unusual polyunsaturated fatty acids FA 24:3 (n-6, 9, 15) and FA 26:3 (n-6, 9, 15).

Deep Profiling of Phosphoinositide Phosphates from Cells

Zidan Wang[1], Xue Jin[1], Shuaiting Yan[1], Yu Xia[1]

[1] Tsinghua university, Beijing, China

P28

ABSTRACT

Phosphoinositide phosphates (PIPn) belong to an important class of cellular signaling lipids, with estimated abundance less than 20 pmol/106 cells. PIPns are categorized into seven subclasses according to the number (n=1-3) and location of the phosphate groups on the inositol ring. In various signaling events, the molecular distribution of different PIPn are modulated. However, the low abundance of PIPn makes their analysis challenging. Herein, we have developed a one-pot PIPn enrichment method, allowing direct analysis of RAW and HEK293T cells. This method was paired with Chiral column separation and isomer-resolved MS/MS for profiling of PIPns in cells and organelles.
 Magnetic TiO2 nanoparticles of a diameter ~400 nm was synthesized in house. A flow microreactor consisting of UV transparently coated fused silica capillary (Polymicro Technologies/Molex, Phoenix, AZ, USA) was constructed for offline PaternòBüchi (PB) reactions. A low-pressure mercury lamp (254 nm, BHK, Inc., Ontario, CA, USA) was utilized to initiate the PB reactions. Lipid separations were performed on C18 Column (150 × 3.0 mm, 2.7 µm, Sigma-Aldrich, MO, USA) and CHIRALPAK IB-U (100 × 3mm, 1.6 µm, Daicel Corporation, Osaka, Japan). All MS data were collected on an X500R QTOF mass spectrometer (SCIEX, Toronto, CA).
 The one-pot enrichment method for PIPn harnessed the phosphate-Ti coordination. The PIPn species in standard mixtures or cell lysates were captured on TiO2 MNPs during the loading step, followed by a removal of interfering phospholipids during the washing step. Subsequently, phosphate methylation was performed on TiO2 MNPs using trimethylsilyl (TMS) diazomethane, which also disrupted the coordination and facilitated the elution of methylated PIPn from the TiO2 surface. This one-pot PIPn enrichment method achieved 80% recovery for standard PIPn from the 7 subclasses, demonstrating >99% removal of interfering phospholipids in cell lysates (RAW, HEK293T Cells). Benefiting from the above, the cell loading capacity on LC-MS can be increased from 10e5 to 10e7 per injection, resulting in a 102-fold improvement of PIPn sensitivity, allowing confident identification of PIPn at detailed structural level.
 A workflow was established for deep profiling of PIPn from RAW cells under lipopolysaccharide (LPS) stimulation. We detected over 110 distinct structures of PIPn at the sum composition level, 80 PIPn at the chain composition level, and 90 at the C=C location level in RAW cells (2e7 cell/injection). Using PIP 18:1_18:0 as an example, multiple C=C location isomers, including n-7, n-9, n-10, n-13 were identified and relatively quantified. We detected that the PIPn profile is characterized by saturated (1-2 double bonds) glycerol moieties after LPS stimulation instead of the polyunsaturated (3-4 double bonds) moieties in control cells. Further attempts at spatiotemporal PIPn analysis in organelles are also of interest.

DeepS: Accelerating 3D Mass Spectrometry Imaging via a Deep Neural Network

Dan Li[1], Yao Qian[1], Haiming Yao[1], Wenyong Yu[2], Xiaoxiao Ma[1]

[1] Department of Precision Instrument, Tsinghua University, Beijing, China

[2] School of Mechanical Science and Engineering, Huazhong University of Science and Technology, Wuhan, China

P29

ABSTRACT

Mass spectrometry imaging (MSI) is a powerful technique for visualizing and spatially mapping biomolecules in biological tissues and organs, with a particular emphasis on lipidomics. In lipidomics, MSI enables the direct analysis of lipids within their native spatial context, providing critical insights into lipid distribution, composition, and their role in biological processes. While two-dimensional (2D) MSI has achieved significant advancements in sensitivity, spatial resolution, and specificity, three-dimensional (3D) MSI offers an additional dimension, enabling depth profiling and comprehensive mapping of lipids and other biomolecules in complex biological structures. However, traditional 3D MSI techniques are time-consuming as they rely on the reconstruction of 3D images from 2D MSI analyses of numerous serial tissue sections. This highlights the need for optimized 3D MSI methodologies to enhance efficiency and accuracy in spatial lipidomics, allowing for more detailed and faster analyses of lipid distributions in tissues.

To address this challenge, we propose a novel workflow termed DeepS, designed to accelerate 3D MSI analyses. DeepS employs a 3D sparse sampling network (3D-SSNet) combined with a sparse sampling strategy to significantly expedite 3D MSI imaging without sacrificing image quality. Our method reconstructs sparsely sampled tissue sections using 3D-SSNet, achieving results comparable to those obtained from full sampling MSI, even at a reduced sampling ratio of 20-30%.

In this study, we demonstrate the effectiveness of DeepS by applying it to the 3D imaging of a mouse brain with Alzheimer's disease. For model training, we selected tissue sections equidistant in the dataset and used the depth information for enhanced reliability and accuracy. The workflow maintained high image quality while substantially reducing imaging time. Additionally, leveraging transfer learning, we extended the application of DeepS to more heterogeneous samples, such as a mouse brain with glioblastoma and a mouse kidney, further proving its versatility and robustness.

Our findings highlight that the DeepS workflow, through the integration of a sparse sampling strategy and advanced deep-generative modeling, offers a robust and efficient solution for 3D MSI. This methodology not only accelerates the imaging process but also ensures the maintenance of high-quality 3D reconstructions, thus holding significant promise for various biomedical research applications.

Comprehensive Lipid Profiling Using DIA and Multi-Dimensional Separation

Yao Qian[1], Xiaoxiao Ma[1], Zheng Ouyang[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

The complexity of the lipidome in biological systems is evident in the diversity of lipid structures, the wide range of lipid concentrations, and their dynamic variations in biological processes. Recent advancements in mass spectrometry (MS) have significantly enhanced lipidome characterization, particularly with high-resolution MS instruments and multi-dimensional separation techniques. However, achieving fast and accurate lipidome readouts from low sample inputs remains challenging due to the inherent trade-off in current mass spectrometers between rapid acquisition of tandem mass (MS/MS) spectra and maintaining high sensitivity.

In this study, we combined two-dimensional separation using liquid chromatography (LC) and ion mobility (IM) with data-independent acquisition (DIA) to theoretically fragment all ions for MS/MS analysis. Implemented on a Waters Cyclic-IMS mass spectrometer, our DIA method employs two frames with low and high collision energies to acquire both full scan spectra and MS/MS spectra across the entire m/z range. We developed a two-dimensional MS/MS deconvolution algorithm to accurately reconstruct high-quality MS/MS spectra for every detected three-dimensional feature. Using a spectrum-based target-decoy strategy, we annotated the deconvoluted MS/MS spectra with a low false discovery rate (2%).

The orthogonal separation by LC and IM enhances MS/MS sensitivity, enabling the annotation of 177 lipids from a single 0.5 L injection of human plasma in negative ion mode using a 26-minute chromatographic method. This number drops to 91 lipids at an injection volume of 32 nL and 40 lipids from 8 nL plasma. Our two-dimensional deconvolution algorithm increases the number of annotations by 36% compared to one-dimensional deconvolution of merged LC or IM data. Compared with commercially available software, our algorithm annotates 73% more lipids.

Spatially-Resolved Lipidomics and Proteomics Enabled by In-Gel Reactions in Gel-Assisted Mass Spectrometry Imaging

Shenghui Ye[1], Xiaoxiao Ma[1], Zheng Ouyang[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

Mass spectrometry imaging (MSI) is a powerful tool for spatial omics research, providing insights into the distributions of molecules within tissues. However, to enable precise molecule identification, untargeted spatial omics analysis on complex samples often requires preprocessing reactions, which can be challenging to perform on-tissue as they must balance reaction yields against image distortion caused by molecular diffusion. Recently, gel-assisted mass spectrometry imaging (GAMSI) has been developed to enhance MSI's spatial resolution by physically expanding sample slices using expansion microscopy techniques. In addition to anchoring molecules onto the expandable gel, we argue that GAMSI also provides a localized reaction environment within the gel matrix, which we may leverage to facilitate on-tissue reactions. We plan to demonstrate the integration of lipid Paterno-Buchi derivatization and protein digestion into GAMSI workflows, acquiring spatial lipidomics and proteomics data from consecutive tissue slices. This strategy should not only be applicable to other reaction-based MSI techniques but also have the potential to be extended for extracting multiomics information from a single tissue slice, enabling understanding of complex biological processes at the molecular level.

Cellular-Level Resolution Mass Spectrometry Imaging with Desorption Electrospray Ionization

Chengyi Xie[1,2], Jianing Wang[1], Zongwei Cai[1,2]

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

P32

ABSTRACT

Desorption electrospray ionization (DESI), introduced by Cooks and his team in 2004, stands out as an innovative ambient imaging technique. It is renowned for its minimal sample preparation, user-friendliness, and greater stability compared to other surface-based liquid droplet extraction and desorption imaging methods. Nevertheless, its spatial resolution, typically ranging from 50 to 150 μm, falls short of other soft ionization-based molecular mass spectrometry imaging (MSI) techniques, such as matrix-assisted laser desorption ionization (MALDI) and nanoDESI. This limitation stems from the inherent spray geometry of the ESI process, which leads to a larger projection diameter of the solvent plume on the tissue section. Although optimizing the spray head can enhance resolution to about 20 μm, the potential for improvement remains constrained. In this study, we introduced a cellular-level mass spectrometry imaging method termed 10X DESI MSI. A low-cost homebuilt DESI ion source was employed with a measured resolution of about 40-50 m for MSI. After implementing a nearly ten-fold expansion technique, DESI mass spectrometry imaging has achieved a lateral spatial resolution of approximately 5 m, making it suitable for cellular-level analysis. This improved resolution allowed for more detailed structural imaging of mouse brain tissue, surpassing the capabilities of standard DESI imaging, which is comparable to the upper limitation of commercial MALDI imaging system. Furthermore, the 10X DESI MSI technique was demonstrated with improved detection capabilities for specific lipids, such as ceramide and cerebroside. Importantly, the total lipid classes identified were not compromised compared to the control DESI MSI method. In summary, this study introduces a notable advancement in mass spectrometry imaging with the development of the 10X DESI MSI technique. This method achieves a nearly ten-fold enhancement in spatial resolution, facilitating detailed cellular-level analysis, and can be also be utilized for other imaging systems such as nanoDESI, LESA, etc. The cellular-level resolution expansion MSI method is applicable to various DESI ion sources, from home-built to commercial products, offering a cost-effective approach for high-resolution mass spectrometry imaging.

In Situ Free Radical Epoxidation with Laser Desorption Ionization for Mass Spectrometry Imaging of Fatty Acid Isomers

Huimin Ye[1], Ruijun Jian[2], Wenpeng Zhang[1], Yu Xia[2], Zheng Ouyang[1]

[1] Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instruments, Tsinghua University, Beijing, China
[2] Department of Chemistry, Tsinghua University, Beijing, China

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ABSTRACT

Lipids participate in various cell functions, playing important roles in building membrane structure, signal transmission, energy transmission, etc. Mass spectrometry imaging (MSI) provides spatial information on lipid isomers, which is essential for studying the function of lipids in metabolic processes. Epoxidation derivatizations were developed for unsaturated lipids isomer MSI. However, ex-situ derivative products are easy to change during the imaging process. Herein, we developed a novel method for fatty acid(FA) isomers MSI based on on-surface radical epoxidation during the LDI process using benzoin dimethyl ether (DMPA). It enabled in-situ LDI-MS imaging of several FA C=C isomers in tissue samples. The lipid structure and spatial distribution were correlated in real-time, reducing the impact of the environment on epoxidation products.

MS1/MS2 Fusion Sampling Enables Multiplexed Tandem Mass Spectrometry Imaging of Lipids with C=C Specificity

Aolei Tan[1], Zheng Ouyang[1], Xiaoxiao Ma[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

Tandem mass spectrometry imaging (MSI) can enable structural characterization of lipids by generating diagnostic fragments specific to varied levels of lipid structures, e.g. lipid class, fatty acyls, carbon-carbon double bond (C=C) locations and even sn-positions. However, as MS/MS analysis of precursors are commonly performed in a sequential approach, the limited sample quantity at each tissue pixel restricts the rounds of MS/MS that can be performed sensitively with high spatial resolution. To increase the multiplicity of MS/MS imaging without loss of detection sensitivity, in this work, we propose a MS1/MS2 fusion sampling workflow for spatial lipidomic that integrates MS1 and MS2 data acquired from the tissue section. Across the tissue section of interests, MS1 spectra are densely sampled to provide high spatial resolution imaging results, albeit without detailed lipid structural information. By contrast, MS2 spectra of different lipid precursors are sparsely acquired to improve MS/MS imaging speed with high structure specificity. Through an image fusion algorithm, the MS1 and MS2 imaging modalities are integrated to achieve high-resolution multiplexed MS/MS spatial imaging of lipids with high structural specificity. Tandem MSI analysis of at least 5 precursor ions was achieved without reducing spatial resolution (10 um). Besides, we demonstrated the power of this method for the imaging of phospholipid C=C location isomers by coupling it with on-tissue photochemical derivatization.

A Simple Signal Enhancement Strategy for Rapid Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging

Thomas Ka-Yam LAM[1], Jianing Wang[1], Zongwei Cai[1]

[1] State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

P35

ABSTRACT

Matrix-assisted laser desorption/ionization (MALDI) is the most utilized technique in mass spectrometry imaging (MSI). While powerful in mapping molecules of interest such as drugs, metabolites, lipids, and proteins with high spatial resolution (sub 10 m), MALDI-MSI requires a complex and time-intensive sample preparation process, necessitating well-trained professionals to achieve quality MS imaging. Researchers have proposed a matrix pre-coated approach to reduce the time and cost of MS imaging. However, this method has limitations due to suboptimal tissue-matrix co-crystallization and extraction, leading to low ionization yields and poor image quality. This study presents a one-step, rapid method as a signal enhancement strategy for the MALDI workflow, taking approximately 30 seconds. The process involves spraying a small amount of water or 70% methanol (MeOH) onto a tissue section mounted on a pre-coated ITO slide for 15 seconds, followed by a 4-second microwave treatment. This approach yielded a 3.8- to 4-fold signal increase in the lipid region (6001000 m/z) and enhanced the signal-to-noise ratio in both 2,4-Dihydroxybenzoic acid (DHB) pre-coated slides in positive mode and N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDC) pre-coated slides in negative mode after treatment. In conclusion, our rapid enhancement strategy increases ion yield in the lipid region, resulting in improved MS image quality with only 30 seconds of sample preparation time. We believe this strategy can improve the utility of pre-coated MALDI as well as to promote rapid MALDI workflow in clinical research.

Construction of Temporal and Spatial Lipid Fingerprint Profiles and Identification of Quality Characteristics of Eriocheir Sinensis

Yameng Li[1], Qinxiong Rao[1], Qicai Zhang[1], Xianli Wang[1], Chengbin Liu[1], Danhe Wang[1], Di Huang[1], Weiguo Song[1]

[1] Institute for Agri-food Standards and Testing Technology, Shanghai Academy of Agricultural Sciences, Shanghai, China

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ABSTRACT

Eriocheir sinensis, namely Chinese mitten crab, is widely distributed in China's coastal waters and has important economic value. There are nearly 20 geographical indication products in China. Different geographical indication products form unique quality characteristics because of their specific natural ecological environment and breeding mode. At present, the evaluation of quality grade of E. sinensis mainly focuses on the external sensory characteristics and internal physicochemical indexes, while the research on the rich lipid characteristic components of E. sinensis is relatively lacking, and the correlation between them and the environmental characteristics of origin is not clear. As a result, the good quality characteristics of E. sinensis cannot be reflected and controlled, and the consumer demand for high quality E. sinensis cannot be met. Therefore, in this study, the lipophilic components were first extracted from the edible sites of E. sinensis by dichloromethane/methanol system, and the lipophilic components were separated by X-bridge C18 column. Then, a comprehensive study on the lipids of E. sinensis based on liquid chromatography-high resolution Orbitrap mass spectrometry was established. Secondly, the lipid fingerprint of Chongming Qingshui Crab, a geographical indication product with spatial and temporal dimensions such as tissue sites, growth period and marketing period, was constructed. In combination with the stoichiometric method, the lipid components that characterized the quality characteristics of E. sinensis were excavated, and the quality characteristic recognition technology was established. The purpose of this study is to analyze the formation and variation of quality characteristic components of E. sinensis, which is of great significance for promoting the formulation of quality control standard system of E. sinensis characteristic quality whole chain.

Single-Deposition Dual-Polarity Mass Spectrometry Imaging Strategy for Comprehensive Cellular Resolution Lipid Profiling in Mammal Tissues and Single Cells

Yanyan Chen[1]

[1] Hong Kong Baptist University, Hong Kong, China

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ABSTRACT

Advancing the precision and depth of lipid profiling at cellular resolution is crucial for understanding complex biological processes. A significant challenge in current methods is the difficulty of improving spatial resolution while maintaining comprehensive molecular coverage. Here, we present a novel single-deposition dual-polarity mass spectrometry imaging (SDDP-MSI) strategy designed to achieve comprehensive lipid profiling in mammal tissues and single cells. This method combines dual-polarity ionization, enabling simultaneous detection of both positive and negative ions, with a single-deposition technique for uniform sample preparation and improved spatial resolution. The SDDP-MSI strategy not only accelerates the imaging process but also minimizes potential sample degradation, loss of resolution and analytes, and issues related to sample deformation and image registration or alignment that could occur with repeated matrix applications. Moreover, the ability to maintain comprehensive data acquisition across both ionization modes without reapplying the matrix ensures maximum retention of molecular information. This feature is especially critical in applications like cellular resolution lipid profiling, where capturing a comprehensive snapshot of lipidomic diversity at the cellular level can help clarify cell function, disease mechanisms, and the effects of treatments. The SDDP strategy enhances the efficiency and quality of mass spectrometry imaging, making it a robust tool for biomedical research and beyond, while improving the precision and reliability of imaging results at the cellular scale.

High-Efficiency Mass Spectrometry Imaging Using a Single-Pixel Imaging Strategy

Aojie Zhang[1], Xiangyu Guo[1], Wenpeng Zhang[1], Zheng Ouyang[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

In high spatial resolution mass spectrometry imaging (MSI), the large number of sampling points and limitation in the number of desorbed ions for each small pixel represent major challenges. Herein, a novel approach is proposed by adopting a single-pixel imaging strategy and compressive sensing for MALDI MSI. The mass spectrometer is considered a "single-pixel camera" and a spatial light modulator is used to convert individual laser sampling point into laser matrix for a wide-field ionization. It is expected to resolve the sensitivity issue as well as to improve the speed of the imaging through compressive sensing. The single-pixel MSI method was validated using ink-printed samples. The red dye (m/z 443) was uniformly applied to a slide. Subsequently, a precisely shaped laser beam was focused onto the slide to ablate the dye to form a set of letters shapes 100 μm×100 μm in size and 40μm apart as a sampling sample. A hundred random Gaussian matrices (10×10) were used for laser sampling in subsequent MS analysis. By designing the phase diagram for the spatial light modulator and choosing the appropriate lens focal length, the focusing size of these laser matrices on the sample reached 100 μm×100 μm, yielding a theoretical resolution of 10 μm. The single-pixel MSI system was used to image an ablated area of 400 μm×400 μm, by which different signal intensities under each measurement matrix could be obtained. Using the OMP algorithm, the dye image in the area of 100 μm*100 μm could be reconstructed well with only 15-20 measurements. Imaging of a larger region could be achieved by repeating these steps. The MSI results were found to be consistent with the ablation shapes observed under the microscope. The time for MSI was reduced to 20% in comparison with the conventional sampling method for MSI. By adjusting the phase diagram and lens focal length, the spatial resolution of MSI can be readily designed to 5 μm -50 μm. We also evaluated this method with imaging of mouse brain sections. Phospholipid species were imaged at the spatial resolution of 10 μm. Currently, this method is being applied for imaging of phospholipids in diseased tissue samples.

A Comprehensive High-Resolution Imaging Metabolomics Workflow for Highly Heterogeneous Tissue

Xin Diao[1], Jianing Wang[1], Zongwei Cai[1]

[1] State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

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ABSTRACT

Mass spectrometry imaging (MSI) is a developing technique that spatially resolves the molecular composition of samples in a label-free manner. Matrix-assisted laser desorption/ionization technique is one of the most investigated ionization techniques in MSI. However, rigid samples usually cannot be easily analyzed due to challenging sample preparation. We developed a sample preparation method for the unfixed fresh frozen hard samples with minimal cracks by controlling sample thickness and drying method. A spin-flattening method was implemented to address the issues of surface flatness.. Using N-(1-Naphthyl) ethylenediamine dihydrochloride as the matrix, spatial information of organic metabolites and inorganic content was investigated in highly heterogeneous samples. Abundant metabolites, including small polar metabolites and lipids, were mainly detected imaged in the non-mineralized compartment of the sample, the calcium chloride cluster signal delineated the contours of the mineralized region of the sample. Worth noting that, MALDI simultaneously resolved the spatial distribution of various metal ions. To further explore the potential of our workflow, high lateral resolution MSI was performed on rat tibial growth plate cartilage at the different growth stages. 458.17 m/z and PE (34:1) are found to be complimentary expressed in growth plate cartilage, with 458.17 m/z having proximal spatial preference in cartilage and PE (34:1) more concentrated in the distal side of cartilage. The level of those metabolites also significantly altered at different growth stages, of which 458.17 m/z was diminished in older rats accompanied by a higher level of PE (34:1). Our finding suggested the potential involvement of those metabolites in bone development. Our method presents a comprehensive solution for mass spectrometry imaging (MSI) of highly heterogeneous tissues containing bones. By addressing the challenges of sample preparation, including surface flatness, bubbles, and severe cracking, our approach significantly improves the quality of MSI data. Additionally, this method offers a broad detection range that encompasses both metal ions and metabolites. This advancement enables detailed and accurate molecular characterization of rigid biological samples, enhancing the potential for applications in biomedical research.

Rapid and Highly Sensitive Analysis of Lipids by Capillary Electrophoresis and Miniature Mass Spectrometry System

Xiao Chen[1], Junhan Wu[1,2], Yikun Liu[1], Wenpeng Zhang[1], Zheng Ouyang[1]

[1] Department of Precision Instrument, Tsinghua University, Beijing, China

[2] PURSPEC Technology (China) Ltd., Suzhou, China

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ABSTRACT

Variations of lipid isomers are closely associated with dysregulation of metabolism in cancers and metabolic diseases. However, analysis of lipids in complex biological samples, especially rapid on-site analysis, is often affected by matrix effects. In this work, a novel capillary electrophoresis (CE) - miniature mass spectrometry system was developed to mitigate the matrix effect in the analysis of lipid biomarkers in biological samples. This system operated in an on-demand variable velocity electrophoresis mode, allowing seamless coupling of continuous CE flow with discontinuous atmospheric pressure interface. Compared with constant velocity mode, the variable velocity mode increased the sensitivity by threefold, and the RSD of quantification decreased from 51.4% to 11.5%. Besides, CE-MS enabled rapid separation of target analytes from the matrix prior to MS analysis, thereby enhancing the sensitivity for complex biological samples. The results indicated that, compared to direct-infusion nanoESI, this method enhanced the sensitivity for various analytes in complex matrices by 20-160 times. Notably, the limit of detection for cortisol could reach 18.9 pg/mL. Using this system, different subclasses of phospholipids, including PC, PE, PS, PI, and PG were well separated within 12 min. Furthermore, acetone-containing background buffer was developed for the online photochemical derivatization of C=C bonds of unsaturated phospholipids. It is possible to analyze lipid isomer biomarkers in raw biomedical samples and extremely small-volume samples.

Novel Reactive Matrix for Laser Desorption Ionization and Sensitive Mass Spectrometry Detection

Yaolu Ma[1,2,3], Zheyi Liu[1,2,3], Fangjun Wang[1,2,3]

[1] CAS Key Laboratory of Separation Sciences for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

[2] Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

[3] University of Chinese Academy of Sciences, Beijing, China

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ABSTRACT

Matrix-assisted laser desorption ionization (MALDI) is widely used in rapid mass spectrometry (MS) detection of a great variety of tissue biomolecules such as metabolites, lipids, proteins, and polysaccharides in a single experiment, but suffers several constraints including poor ionization properties, signal suppression, and matrix interference effect in low molecular region1. Traditional commercial matrices, such as DHB and CHCA, exhibit high matrix signal intensities in the low molecular weight region, leading to ion suppression effects and interference with the ionization of target molecules2. To enhance the sensitivity of analysis and achieve target-specific detection, numerous efforts were made in light of the inherent challenges associated with matrices. In this study, we developed a novel reactive matrix with a strong absorption peak in the UV region, which can be used both as a derivative reagent to react with peptide molecules and as a matrix to assist ionization. The reactive matrix reacts with the primary amine group at the N-terminal of the peptide to form a unique product, which increases the molecular weight of the original peptide to distinguish it from the matrix interference peaks, so as to achieve the purpose of improving the signal-to-noise ratio and analytical sensitivity.

In-Tip Microextraction Enables Fast Profiling of Phospholipid Isomers in Plasma by Mass Spectrometry

Qiqian Zhang[1], Zheng Ouyang[1], Wenpeng Zhang[1]

[1] State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing, China

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ABSTRACT

The fast and in situ mass spectrometry (MS) analysis of lipids from biofluids constitutes a significant advancement in the realm of point-of-care diagnostics. Disorder of lipid homeostasis is closely associated with the development of diseases such as cancer, diabetes, Alzheimer's disease, and cardiovascular disease. The detailed molecular configurations of lipids, notably the precise location of carbon-carbon double bonds (C=C) within the fatty acyl or alkyl chains, have been identified as a pivotal role in influencing various biological processes and functions.

However, the profiling of lipid isomers is still limited due to the complexities of the chemical deriviation methods or requisite instrument modification. In this work, we develop an in-tip microextraction method for fast profiling and relative quantitation of phospholipid isomers from biofluid samples. It enables high-throughput analysis of phospholipid C=C isomers from diseased samples through a simplified workflow.

A thin layer of polyporous organic polymer was modified inside a glass tip, which allows extraction of different classes of lipids from biofluid through a repeated enrichment strategy. A dip-in washing step was incorporated for clean-up of salts which can produce multiple adducts for lipids. And in-tip elution and nano-ESI were used for direct MS analysis. Through in-situ surface modification, the inner surface of the capillary tip was coated with a thin layer of polyporous polymer. The parameters and conditions were optimized, including the types of functional monomers, the composition of the pre-polymerization solution, the volume of the samples and elution solvent, and the extraction time. With optimal conditions, phospholipids, sphingolipids, fatty acids and glycerol lipids were profiled from biofluid samples within 1 min. Through incorporation of acetone or benzophenone into the elution solution, online photochemical derivatization was realized inside the polymer-coated capillary tip. This enabled characterization of lipid C=C and sn-isomer from plasma by PB-MS/MS method. An array was also developed based on the in-tip microextraction settings, by which more than 10 tips can be located and high-throughput microextraction can be performed automatedly. The device and workflow are now used for analyzing phospholipid C=C isomer biomarkers in the plasma samples of type 2 diabetes. Besides, the polymer-coated capillary was also used with a miniature MS system, with the disposable microsampling device and a simple workflow, the system has the potential to be applied into on-site analysis of phospholipid isomer biomarkers in clinical settings.

A Deep Lipidomics Analysis Method Applied to Serum

Jia Li[1]

[1] Guangdong University of Technology, Guangzhou, China

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ABSTRACT

Objectives: To develop an innovative DeepMarker LT lipid omics platform for comprehensive analysis of lipid metabolites in serum samples, aiming to offer effective guidance for differential diagnosis, intervention therapy, and mechanism research of lipid metabolism in diseases.

Methods: 6ul serum was absorbed, 15 kinds of deuterated lipids were added into the internal standard mixture, and the lipids of serum samples were extracted by dichloromethane-methanol-water system. Agilent 6546 UHPLC-Q-TOF was used to collect serum lipids. Lipid substances were identified through self-established three-level database. Before statistical analysis, all variations were corrected for by normalizing the intensity of each characteristic peak according to the internal standard and median intensity ratio.

Results: A total of 11831 unique chromatographic peaks were detected in the serum samples, of which 4078 peaks could be accurately identified or presumed to be matched. There were 911 characteristic peaks identified at level 1 (molecular species or species level) and 611 characteristic peaks identified at level 2 (species level). At the level 3 (species level), 2506 characteristic peaks were identified, and the Dai Xie peak type was better and had a good separation effect. The results show that the lipidomics platform can extract and identify serum lipids with maximum coverage and accuracy.

Conclusions: Compared with conventional methods, DeepMarker LT lipidomics platform embodies the all-round and multifaceted leading advantages of higher sensitivity, high coverage, high precision quantification, and high stability. It shows great potential to analyze the changes of lipid metabolism in organisms in disease states and search for potential lipid markers.

Keywords: Lipidomics; DeepMarker LT lipidomics platform; UHPLC-Q-TOF

High-Efficiency Dual-LIT Miniature Mass Spectrometer Enables Single-Cell Lipidomics at High Structural Specificity

Zhijun Cai[1], Ningxi Li[1], Siming Cheng[1], Xiaoxiao Ma[1], Zheng Ouyang[1]

[1] Tsinghua University, Beijing, China

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ABSTRACT

Introduction: With the development of lipidomics methods capable of acquiring comprehensive molecular structural information, there have been increased research interests to apply them to single-cell mass spectrometry (MS). It enables accurate cellular phenotyping and lipid metabolism pathway study. Large-scale lipidomic analysis for a single cell, however, still represents a significant challenge, due to the extremely small amount of samples available. In this work, we developed an effective strategy using a miniature mass spectrometer with dual linear ion traps (LITs) to significantly improve the efficiency in sample utilization, enabling MSn analyses of more than 100 lipid species in a single cell.

Results: To increase the coverage of lipids in a wide dynamic range, a two-step analysis procedure was developed. For the first step, high-abundance ions were ejected from LIT1 to minimize the space charge effect for MS/MS analysis of low-abundance ions. The second step was used for the analysis of high-abundance ions. Breast cancer cells (MDA-MB-468) were analyzed and 65 distinct lipid species were identified with high structural specificity, including 34 PCs, 19 PEs, 11 PSs and 1 SM. Importantly, a total of 28 lipid C=C location isomers were also identified. Compared to conventional shotgun analysis, this method extends the spectral dynamic range by about one order and doubles the number of lipid species analyzed.

For the comprehensive characterization of lipid structures from a single cell, a new strategy was used for the partial transfer of a selected lipid species from LIT1 to LIT2. For instance, ions of a selected lipid species could be transferred three times, for one MS3 and two MS4 analyses in LIT2. Structural elucidation was accomplished for five PCs, including [PB(PC 32:1)+Na]+, [PB(PC 34:2)+Na]+, [PB(PC 34:1)+Na]+, [PB(PC 36:2)+Na]+, [PB(PC 36:1)+Na]+. The MS3 analysis allowed the identification of sn-position or C=C location isomers, while MS4 analyses allowed the identification of C=C location with a specific sn-position for a lipid species. For breast cancer cells (MDA-MB-468), this strategy resulted in the identification of 15 lipid sn-position isomers and 14 C=C location isomers. Meanwhile, 20 lipid species were identified with both C=C location and sn-position, which has not been reported previously for single-cell analysis.

Lipidomic Analysis of Metabolic Dysfunction-Associated Fatty Liver Disease: Identification of Plasma and Liver Lipid Biomarkers

Yiwei Liu[1], Madhulika Singh[1], Floris A. Cohen[1], Amy C. Harms[1], Ahmed Ali[1], Thomas Hankemeier[1], Bruno Ramos- Molina[2]

[1] Research Metabolomics and Analytics Centre LACDR, Leiden University, Leiden, Netherlands

[2] Instituto Murciano de Investigación Biosanitaria

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ABSTRACT

Aims: The aim of this study is to identify lipid biomarkers in plasma and liver biopsy of patients diagnosed with different stages of Metabolic dysfunction-associated fatty liver disease (MAFLD), particularly focusing on Metabolic dysfunction-associated steatohepatitis (MASH). By correlating lipid profiles with disease status, we seek to enhance understanding of MAFLD/MASH pathophysiology, discover novel biomarkers reflecting disease severity, and develop less invasive diagnostic techniques for early detection and intervention.

Methods: Plasma and liver biopsy samples were obtained from obese patients diagnosed with MAFLD/MASH and obese patients with no signs of liver disease. Obese individuals without signs of liver disease served as the control group in this study. The samples were collected and delivered to us by Bruno Ramos-Molina from Instituto Murciano de Investigación Biosanitaria (IMIB).

Hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) was employed for comprehensive lipidomic analysis, allowing the detection of 1200 species across 18 lipid classes. For the HILIC-MS/MS method, the Exion LC AD (AB Sciex) coupled to a QTRAP6500+ mass spectrometer (AB Sciex) with an electrospray ion source was used.

Results: Analysis of plasma and liver samples revealed distinct alterations in lipid profiles between MAFLD/MASH patients compared to controls. Particularly, triglyceride levels exhibited significant elevation, predominantly in liver samples, indicative of substantial lipid accumulation in affected individuals. Additionally, levels of phosphatidylcholine, phosphatidylethanolamine, diacylglycerol, and sphingomyelin showed notable changes across different disease stages, underscoring dysregulated lipid metabolism associated with disease severity. Moreover, differentiation between MAFLD and MASH groups was discernible, suggesting the feasibility of stratification based on lipid biomarkers.

In conclusion, the utilization of hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) in this study enabled the identification of lipid biomarkers reflective of disease severity in MAFLD/MASH. These findings not only enhance our comprehension of the disease pathophysiology but also hold promise for the development of potential diagnostic tools in the future.

Cholesterol Promotes TMEM41B Mediated Phospholipid Scrambling

Yuanhang Yao[1], Lu Yan[1], Ning Gao[1], Xiao-Wei Chen[1]

[1] College of Future Technology Peking University, Beijing, China

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ABSTRACT

The phospholipid bilayer is a fundamental feature of cellular and subcellular structures, composed of two membrane lipid leaflets. Of note, the membrane lipids are synthesized asymmetrically at the cytosolic leaflet of the ER membrane, while the spontaneous cross-bilayer movement of phospholipids is prevented by their amphipathic nature and the bilayer organization of the membrane. This abundant phospholipid transmembrane movement is mediated by the ER scramblase. We previously identified that TMEM41B acts as an ER scramblase and mediates the phospholipids trans-bilayer transport at the ER membrane. Notably, TMEM41B serves as a key host factor of coronavirus, indicating viral hijacking of TMEM41B. Thus, exploring the regulation of TMEM41B may help develop new antiviral therapies. Here, we show that TMEM41B interacts with ERLINs, which creates a cholesterol-enriched environment for TMEM41B. Interestingly, cholesterol directly activates TMEM41B scramblase activity in vitro. In vivo, decreasing cholesterol levels surrounding TMEM41B by knocking out ERLINs, significantly inhibits viral infection. These findings suggest that targeting ER cholesterol, a key regulator of TMEM41B, could offer a safe and effective antiviral strategy. In conclusion, this study highlights the role of cholesterol in activating TMEM41B and proposes an antiviral therapeutic approach by modulating ER cholesterol levels.

LPPtiger2 Powered Epilipidomics Workflow: from Prediction to Identification of Modified Lipids

Zhixu Ni[1], Palina Nepachalovich[1], Maria Fedorova[1]

[1] Center of Membrane Biochemistry and Lipid Research, Faculty of Medicine Carl Gustav Carus of TU Dresden, Germany

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ABSTRACT

Within biological systems, lipids can undergo a range of enzymatic and nonenzymatic reactions which extends the complexity of lipidome to epilipidome including enzymatic and non-enzymatic modifications such as oxidation, nitration, sulfation, and halogenation.

Considering the extreme diversity of epilipid structures and their low abundance in the biological sample, it is a challenging task to identify epilipids from untargeted LC-MS/MS data.

The various fragmentation patterns of epilipids which often differ from the unmodified forms adds additional complexity for the software identification algorithms.

The original LPPtiger software provided a novel workflow based on the in silico prediction of customized epilipidome from known unmodified phospholipids, flowed by their in silico fragmentation and identification using multiple scoring systems.

Now LPPtiger 2 extends the workflow by covering oxidized neutral lipids (oxTG, oxDG, and oxCE), with improved in silico epilipidome predictions and fragmentation analysis. Optimized workflow provides tenfold speed improvement by merging the processing of different lipid subclasses into a single task. LPPtiger 2 also enables users to export in silico predicted epilipidome in a reusable library in JSON format, including structured information of elemental composition, fragmentation pattern, corresponding structures in SMILES format, fingerprint m/z lists, and other predicted information. The identification results can be exported in an interactive report with annotated six-panel figures in HTML format, a summary identification table in xlsx format, and an JSON file that includes all spectra assignments and scoring details. These JSON files provide complete access to all predictions and identification details, making it possible for data scientists to conduct further advanced post-processing. Among other new features, LPPtiger 2 provides new "Inclusion List Generator" module to support direct design of targeted (PRM/MRM) acquisition methods with automatically generated lists of epilipids precursor and fragment ion m/z. Furthermore, LPPtiger 2 supports mzML files converted from polarity switching mode for the identification of oxidized lipids. LipidLynxX is also integrated into LPPtiger 2 to perform lipid nomenclature conversions. LPPtiger 2 is open-source, cross-platform software released under AGPL license and is freely available for academic users on GitHub repository: https://github.com/LMAI-TUD/lpptiger2.

A Molecular Determinant for Tthe Association of Human Hormone-Sensitive Lipase with Lipid Droplets

Han peng[1]

[1] Westlake university, Hangzhou, Zhejiang, China

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ABSTRACT

Lipid droplets (LDs) are the main cellular storage sites for triacylglycerols (TAGs), playing a pivotal role in energy homeostasis and cell signaling. Hydrolysis of the stored TAGs begins with conversion of TAGs into diacylglycerols (DAGs) by adipose triglyceride lipase (ATGL), followed by hydrolysis of DAGs by hormone-sensitive lipase (HSL). Despite the central role of HSL in lipolysis, the molecular determinants of its LD association have remained elusive. Here, we resolved the cryo-EM structure of human HSL at 3.4 . Combining this with hydrogen-deuterium exchange mass spectrometry, in vitro and cellular assays, we identified residues 489-538 of HSL, referred to as the "H-motif", as an LD-binding motif mediating the direct interaction of HSL with LDs. LD binding mediated by the H-motif is independent of HSL phosphorylation catalyzed by the cAMP-dependent kinase PKA. Our findings unravel a previously unrecognized LD binding mechanism of HSL, advancing our understanding of the regulation of lipolysis.

Understanding the Genetic Basis of Lipid Variations in Clinical Strains of Klebsiella Pneumoniae

Zhen Xuan Mok[1], Shawn Vasoo[1, 2, 3], Indumathi Venkatachalam[4], Kalisvar Marimuthu[2,3], Michelle Ang[2], Douglas Chan[5], Benjamin Pei Zhi Cherng[4], Partha Pratim De[3], Rama Narayana Deepak[6], Li Yang Hsu[7], Karrie Kwan Ki Ko[4], Tse Hsien Koh[4], Raymond Tzer Pin Lin[2], Tong Yong Ng[8], Say Tat Ooi[6], Surinder Kaur Pada[5], Nares Smitasin[7], Louisa Sun[9], Thean Yen Tan[10], Cheong Hau Yiang[10], Nancy Wen Sim Tee[7], Koh Cheng Thoon[11], Jeanette Teo[7], Oon Tek Ng[1, 2, 3], Bernett Teck Kwong Lee[1, 6, 12, 13, 14], and Xue Li Guan[1]

[1] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
[2] National Centre for Infectious Diseases, Singapore
[3] Tan Tock Seng Hospital, Singapore
[4] Singapore General Hospital, Singapore
[5] Ng Teng Fong General Hospital, Singapore
[6] Khoo Teck Puat Hospital, Singapore
[7] National University of Singapore and National University Health System, Singapore
[8] Sengkang General Hospital, Singapore
[9] Alexandra Hospital, Singapore
[10] Changi General Hospital, Singapore
[11] KK Women's and Children's Hospital, Singapore
[12] Centre for Biomedical Informatics, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
[13] Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore
[14] Infectious Disease Labs (ID Labs), Agency for Science, Technology and Research (A*STAR), Singapore

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ABSTRACT

Klebsiella pneumoniae (K. pneumoniae) is an opportunistic pathogen from the family of Enterobacteriaceae. They commonly colonize the gastrointestinal tract of humans and can potentially lead to devastating infections with high mortality rates among elderly patients and patients with compromised immune system. Of even more urgent concern is the convergence of virulence and multi-drug resistance, resulting in fatal, morbid, and hard-to-treat infections. They are highly diverse both genetically and phenotypically, resulting in wide spectrum of antimicrobial resistance (AMR) and virulence. Lipids form a permeability barrier that bacterium possess to protect itself from the external environment and variations in lipids, particularly lipid A, have been known to be involved in AMR. However, there remains a gap how lipid variations arise in naturally. To facilitate the understanding of genetic basis behind these variations, we first used bioinformatics tools to annotate, before manually curating all lipid related genes, in a reference ATCC700721 strain, based on Kyoto Encyclopedia of Genes and Genomes or Uniprot records. Next, genomes from a collection of carbapenemase expressing K. pneumoniae from the European survey of carbapenemase-producing Enterobacteriaceae repository were analysed to establish the link between variations in lipid metabolism-related genes and association with resistance to carbapenems. Future works will include measuring the impact of these genetic differences on clinical phenotypes including outcome of infection and treatment.

Establishment of Lipidomes Reference for Medically Relevant Panel of Enterobacteriaceae

Elise Zi Qi Ng[1], Zhen Xuan Mok[1], Xue Li Guan[1]

[1] Lee Kong Chian School of Medicine Nanyang Technological University, Singapore

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ABSTRACT

Antimicrobial resistant (AMR) bacteria are a global concern as the infection could be persistent due to poor response to existing treatment. Despite continuous advancement of antimicrobials to fight against AMR bacteria, there is a lack of breakthrough in novel antibiotics classes. Enterobacteriaceae is a large family of Gram-negative bacteria, which includes pathogenic genera such as Escherichia, Klebsiella, Salmonella and Proteus, that are commonly found in human gastrointestinal tract as normal flora but also have the ability to cause urinary tract infections and diarrhoea. As Gram-negative bacteria, they are protected from the environment by a lipid bilayer, which acts as a permeability barrier to antimicrobials. Within each species, Enterobacteriaceae are genetically diverse and how this translates to metabolic differences remains unknown. Strain variation analysis of lipidome is currently hampered by a lack of references. Here, we established a panel of Klebsiella pneumoniae as reference for lipidome analysis, which represents the diversity of K. pneumoniae phenotypes. These include K. pneumoniae ATCC 700721, K. pneumoniae ATCC BAA2473, and K. pneumoniae VA360, and K. pneumoniae SGH10 to serve as reference for general laboratory strain, New Delhi metallo--lactamase-1 carriage, K. pneumoniae carbapenemase (KPC) carriage, and hypervirulence, respectively. Bacteria were cultured in two media, specifically M9 minimal media with glucose as sole carbon source which serves to establish baseline metabolism of bacteria and cationic-adjusted Mueller-Hinton broth, commonly used for quantitative susceptibility testing of clinical isolates. Their baseline lipidome profiles were established using liquid chromatography-mass spectrometry. The lipid classes found in K. pneumoniae mirror that of the model bacterium, Escherichia coli. The biochemical signatures are clearly distinct between strains, and as expected are dependent on the media. The establishment of this reference panel will serve as a resource for future works to understand strain variations in lipid profiles and phenotypic consequences, including virulence and antimicrobial resistance.

Tracing Fatty Acids and Cholesterol Metabolic Flux with Stable Isotope Labeling Coupled with LC-MS Measurement

Ruiping He[1], Jianwei You[1], Tianyu Zhou[1], Li Chen[1]

[1] Institute of Metabolism & Integrative Biology Fudan University, Shanghai, China

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ABSTRACT

Understanding the dynamic changes of fatty acids and cholesterol is crucial for comprehending lipid metabolism in health and disease. However, traditional methods for studying their metabolic fluxes often require hazardous radioactive materials, which limit their broader applications. Here, we developed a stable isotope tracing approach to study the metabolic fluxes of fatty acids and cholesterol. Using deuterium water as the stable isotope tracer, we observed that newly-synthesized lipids in Huh-7 cells showed increasing lipid labeling over time. Metabolic fluxes were then calculated using an integral modeling and a Bootstrap method. Inhibition of cholesterol biosynthesis by Lovastatin decreased cholesterol biosynthesis flux, while increasing oleic acid production flux, implying complex interactions between fatty acid and cholesterol. In summary, our method provides a robust tool to investigate the dynamic processes of fatty acid and cholesterol metabolism. Its potential applications in vivo, such as lipid biosynthesis, absorption and transport, will shed light on new insights of lipid metabolism.

Effects of Endocytosis, Llipid Metabolism, and Diminished Microenvironmental Immunoreactivity of Ascites Tumor Cells on Platinum Therapy in Patients with High-Grade Plasmacytoid Ovarian Cancer

Ruiqi Zheng[1], Xun Hu[2], Ying Cui[3], Huiqin Guo[3], Ting Xiao[1]

[1] State Key Laboratory of Molecular Oncology National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

[2] Department of Imaging Diagnosis National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

[3] Department of Pathology National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

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ABSTRACT

Background: Platinum-based drugs are the cornerstone of ovarian cancer treatment. In this study, we mined the platinum resistance-related features in the ascites cells of ovarian cancer patients before chemotherapy from the protein level, which may help patients to adjust their treatment regimen in time.

Materials and methods: Eighteen patients with high-grade plasmacytoid ovarian cancer were included in this study, including 10 platinum-sensitive and 8 drug-resistant patients. Cell precipitates obtained by centrifugation of pre-chemotherapy ascites were collected for proteomic analysis, and the results of analysis were validated by immunohistochemistry (IHC), Western Blotting (WB), CCK8, and oil red staining in expanded samples and cell lines (sensitive strain SKOV3/drug resistant strain SKOV3-DDP).

Results: Prior to treatment, patients who were platinum-resistant at the time of treatment already showed up-regulation of endocytosis with its surrogate molecule SH3YL1, up-regulation of lipolytic metabolism with its surrogate molecule MLYCD, down-regulation of immunoreactivity with its surrogate molecule CD44, and demonstrated higher blood triglyceride levels. Public single-cell data indicated less infiltration of immunoreactive cells in the ascites of drug-resistant ovarian cancers, decreased immunogenicity of tumor cells, and decreased activation of intrinsic and adaptive immunity.Survival analysis of TCGA ovarian cancer public data after substitution into a vector machine model prediction to obtain sensitive/resistant phenotypes revealed significant differences between the groups.WB validated the expression of characteristic molecules in the two groups in agreement with the analytical results as well as the up-regulated exhaustion markers and down-regulated activation markers of immune cells in a lipid-rich environment. Oil red staining revealed that cells of the resistant strain exhibited a stronger tendency to lipid uptake. CCK8 experiments revealed that the macropinocytosis inhibitor EIPA reduced the IC50 of the resistant strain while inhibiting platinum uptake in the sensitive strain and subsequently increasing the semi-inhibitory concentration (IC50) in the sensitive group.

Conclusions: The lipid-rich microenvironment allows ovarian cancer cells to take up abundant lipids (e.g., triglycerides) and nucleotide-rich necrotic cell debris from the outside through endocytosis without

having to synthesize them on their own, which saves a large amount of adenosine triphosphate, reducing substances, and repair raw materials to cope with platinum damage. The accumulation of lipids in immune cells leads to the exhaustion phenotype. These three features may suggest platinum resistance in ovarian cancer and predict prognosis, and are possible targets for reversing platinum resistance.

Keywords: ovarian cancer, platinum resistance, endocytosis, lipid metabolism, tumor microenvironment

PALP: A Rapid Imaging Technique for Stratifying Ferroptosis Sensitivity in Situ

Fengxiang Wang[1], Emily T. Graham[2], Zhennan Shi[1], Stuart L. Schreiber[2], Yilong Zou[1]

[1] Westlake University, Hangzhou, Zhejiang, China

[2] Broad Institute, Cambridge, Massachusetts

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ABSTRACT

Ferroptosis is a non-apoptotic, iron-dependent form of oxidative cell death characterized by uncontrolled peroxidation of polyunsaturated fatty acid (PUFA)-containing phospholipids. Ferroptosis induction has been implicated as a new therapeutic strategy for treating various cancers. However, rapidly stratifying cells or cancer patients for their sensitivity to ferroptosis induction remains a major challenge in developing ferroptosis-targeted anti-cancer treatment. Herein, we establish a technique termed photochemical activation of membrane lipid peroxidation (PALP) to detect polyunsaturated phospholipids as well as report ferroptosis sensitivity in live cells and tissues in situ.

iPLIMS: A Mass Spectrometry Technique for Identifying Protein-Lipid Interactions with High Signal Accuracy

Yang Lu[1]

[1] School of Life Sciences, Westlake University, Hangzhou, Zhejiang, China

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ABSTRACT

Protein-lipid interaction is a central component of the crosstalk between the central dogma and cellular metabolites, yet identifying lipids associated with specific proteins remains technically challenging. Current methods, such as native mass spectrometry, NMR, and Cryo-EM, can sometimes be hindered by complexity or noise, making the detection of true signals more challenging.

Here, we introduce the identification of protein-lipid interactions via mass spectrometry (iPLIMS), a technique offering a high signal-to-noise ratio for determining lipid species associated with target proteins.

Unlike conventional lipid extraction from purified protein samples, iPLIMS incorporates two key modifications: (1) a protease digestion step that releases lipids from protein samples, with a heat-inactivated protease as a control; and (2) an immunoprecipitation step to deplete intact protein-lipid complexes, retaining lipids in the degraded protein sample flow-through.

iPLIMS effectively identified known lipid substrates of various lipid transporter proteins and lipid metabolic enzymes, including the cholesterol-NPC1 interaction, CEPT1-PC/PE interactions, and TACAN-CoA. We anticipate that iPLIMS will become a valuable tool for studying protein-lipid interactions.

In Vivo Metastasis Modeling and CRISPR Screens Reveal ACSL4 and Unsaturated-Lipids Supporting Extravasation and Colonization in Metastasis

Yuqi Wang[1]

[1] Westlake University, Hangzhou, Zhejiang, China

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ABSTRACT

Metastatic dissemination to distant organs requires cancer cells to adapt morphologically and metabolically, yet the role of the cellular lipidome in metastasis is poorly understood. Here, we identify a correlation between metastatic potential and polyunsaturated fatty acid (PUFA) metabolism across multiple cancers. Cancer cells from metastases displayed higher ferroptosis sensitivity and increased PUFA-lipid content compared to primary tumor cells in ovarian cancer patients. Metabolism-focused CRISPR screens in a mouse model of ovarian cancer with distant metastasis, established through two rounds of in vivo selection, revealed the PUFA-lipid biosynthesis enzyme ACSL4 as a pro-extravasation factor during cancer cell metastasis, particularly in hematogenous dissemination. ACSL4 and the PUFA-lipidome facilitate metastatic extravasation by increasing membrane fluidity and enhancing the invasiveness of disseminated cancer cells. Our findings demonstrate the critical role for PUFA-lipids in tumor metastasis, presenting a potential target for therapeutic intervention.

The Role of Ferroptosis in Atherosclerosis

Luxiao Li[1]

[1] Westlake University, Hangzhou, Zhejiang, China

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ABSTRACT

Atherosclerosis, a prevalent chronic vascular disease, poses a significant global health burden due to its persistently high incidence and mortality rates. In the pathogenesis of atherosclerosis, disturbances in lipid peroxidation and iron ion dynamics are frequently observed. Prior research suggests a potential pivotal role for ferroptosis in the pathological cascade of atherosclerosis, yet the precise molecular mechanisms remain elusive. Notably, our preliminary investigations have revealed that the induction of ferroptosis via NCN2 (a GPX4 antagonist) accelerates atherosclerotic progression, whereas the inhibition of ferroptosis using liproxstatin-1 and Vitamin E attenuates disease advancement. This effect may be mediated through the modulation of systemic inflammatory responses.

By integrating in situ lipidomics techniques with single-cell sequencing analysis, we have identified a distinct subpopulation of smooth muscle cells with lipid-uptake capabilities that may be particularly susceptible to ferroptosis. During ferroptosis, the secretion of specific proteins or metabolites induces macrophage polarization toward the pro-inflammatory M1 phenotype. Moreover, the increased sensitivity of these smooth muscle cells to ferroptosis is likely linked to distinct lipid metabolic characteristics. Building upon these observations, our aim is to elucidate the mechanistic underpinnings of ferroptosis-sensitive cells and their regulatory role in plaque formation. Next, we would like to extend clinical validation, we aspire to provide novel theoretical insights into the diagnosis and therapeutic interventions for atherosclerosis.

Coronavirus NSP6 Hijacks Host SREBP1/TMEM41B Circuit

Yonglun Wang[1], Xiaowei Chen[1]

[1] Institute of Molecular Medicine, College of Future Technology, Peking University, Beijing, China

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ABSTRACT

Host-based antivirals could offer broad-spectrum therapeutics and prophylactics against the constantly-mutating viruses including coronavirus and flavivirus, yet must target cellular vulnerabilities of viruses without grossly endangering the host. A common requirement in the replication of RNA viruses, coronaviruses and flaviviruses included, is the induction of the replication organelle (ROs) which requires host ER phospholipid scramblase TMEM41B. While TMEM41B is an undruggable gene due to its critical physiological function, the TMEM41B pathway may be targetable. Here we show that the lipid metabolism master regulator SREBP1c directly regulates TMEM41B gene transcription. Combined with the result that TMEM41B deficiency leads to membrane alteration and uncontrolled SREBPs activation, the data thus suggests that TMEM41B-secured balanced membrane leaflet acts as an end-product suppressor of the SREBP1 pathway, likely to form a feedback circuit to maximize membrane supply on-demand. Furthermore, the membrane-perturbing βCoV NSP6 hijacks host TMEM41B and activates SREBP1 pathway. In turn, both SREBP pathway and TMEM41B are required for the biogenesis of membraneous βCoV NSP6 compartment. Collectively, our data suggests that the master lipid regulator SREBP1 couples the phospholipid scramblase TMEM41B to constitute a host membrane biogenesis feedback circuit which is hijacked βCoV NSP6, and the circuit mediates the biogenesis of the membraneous NSP6 compartment.

A New Dihydroceramide Desaturase Inhibitor Improves Steatosis and Inflammation in a Mouse Model of MASLD

Oscar Pastor[1,3], Karla Ceballos[1], Bohdan Babiy[1], Silvia Sacristán[1], Irene Chavarría[1], Rebeca Busto[1],Javier Martínez-Botas[1], Jordi Bujons[2], Gemma Fábrias[2], Jose Luis Abad[3]

[1] Servicio de Bioquímica Clínica. Hospital Ramón y Cajal, IRYCIS

[2] Instituto de Química Avanzada de Catalua (CSIC), Barcelona, Spain

[3] Dpto. de Biología de Sistemas (Fisiología), Universidad de Alcalá de Henares, Barcelona, Spain

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ABSTRACT

Background: Observational studies report a consistent association between the increase in circulating levels of ceramides (Cer) and worse outcomes in obesity, type 2 diabetes (T2D), and metabolic dysfunction-associated steatotic liver disease (MASLD) [1]. Blocking liver ceramide production by inhibiting dihydroceramide desaturase-1 (DEGS1), the enzyme responsible for the conversion of dihydroceramides (dhCer) into ceramides (Cer) in the liver, is a potential pharmacological target, but therapeutics to safely lower ceramides in humans have not been developed.

Objective: The objectives of this work were: 1) to design and select new small molecule inhibitors of DEGS1, and 2) to explore their effects in a mouse model of MASLD.

Methods: Small molecule candidates for DEGS1 inhibition were obtained by organic synthesis from vitamin A/E lead compounds. DEGS1 activity was screened in mouse liver microsomes and a hepatocyte-like cell line (Huh7). Inhibitors were subsequently tested for liver DEGS1 activity in vivo in C57BL/6J mice. Finally, the best candidate (CLOP1) was injected for 8 weeks into mice fed a high-fat diet (HFD) with high carbohydrate content. Animals were sacrificed at 16, 22, and 30 weeks to reproduce a wide spectrum of histological damage (steatosis, inflammation, and fibrosis). Lipidomic measurements were performed by LC-MS/MS (https://clipidomics.com). Steatosis, inflammation, and fibrosis were examined by liver histology. Expression analysis was measured by qRT-PCR.

Results: Small molecule candidates were screened using a structure-activity relationship approach (SAR). CLOP1 demonstrated better in vitro inhibitory activity in mouse liver and Huh7 cell cultures than known inhibitors of DEGS1 like GT-11 and 4-HPR. Intraperitoneal injection of CLOP1 for 8 weeks decreased the Cer/dhCer ratio (a surrogate marker of DEGS1 inhibition). Further analysis demonstrated the bioavailability of CLOP1 in the liver of animals. Administration of CLOP1 to HFD mice reduced steatosis at 16 and 22 weeks and triacylglycerols in the liver. The inflammatory activity was also reduced and correlated with changes in transaminase levels (ALT, AST) and liver mRNA gene expression (Tnfa, Mcp1, Timp1, Tgfβ1) at 16, 22, and 30 weeks. Fibrosis (Sirius-red and Col1a1) was reduced in the liver of treated animals.

Conclusions: We identified CLOP1, a new small molecule DEGS1 inhibitor with in vitro activity in the nanomolar range. Administration of CLOP1 in a MASLD mouse model reached the liver and improved liver steatosis, inflammation, and fibrosis.

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LC-MS Simultaneous Profiling of Acyl-CoA and Acyl-Carnitine in Dynamic Metabolic Status

Jiangang Zhang[1]

[1] Chongqing University Cancer Hospital, Chongqing, China

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ABSTRACT

Background: Acyl-coenzyme A (CoA) and acyl-carnitine (CAR) are vital components in the regulation of intracellular fatty acid balance and serve as essential substrates in lipid metabolism. Accurate monitoring of their fluctuations across various contexts is crucial for assessing dynamic metabolic status. Liquid chromatography coupled with mass spectrometry (LC-MS) is currently the preferred method for analyzing acyl-CoA and acyl-CAR due to its high sensitivity. However, the wide concentration range and varying compound polarity of these metabolites in biological samples present challenges for comprehensive analysis.

Results: To address these obstacles, we have developed an integrated ion pair reagent-assisted LC-MS workflow utilizing dihexylammonium acetate (DHAA) that enables simultaneous profiling of acyl-CoA and acyl-CAR within a single 20-minute run. This method offers an extensive linear range (5-1000 ng/mL), a low limit of quantitation (LOQ) (0.5-5 ng/mL), and high transferability across different LC-MS systems, facilitating both relative and absolute quantitation of acyl-CoA and acyl-CAR. By employing this workflow, we successfully monitored dynamic changes in acyl-CoA and acyl-CAR levels during macrophage polarization, revealing diverse fatty acid synthesis (FAS) and oxidation (FAO) pathways. Our findings were further validated through 13C metabolic tracing, confirming the incorporation of isotopes into acyl-CoA and acyl-CAR during FAS and FAO.

Significance: Collectively, our results highlight the markedly different energy utilization patterns that occur during macrophage phenotype transitions, as demonstrated by comprehensive profiling of acyl-CoA and acyl-CAR.

Mechanistic Interrogation on Wound Healing and Scar Removing by Mo4/3B2-x Nanoscaffold Revealed Regulated Amino Acid and Purine Metabolism

Dingkun Zhang[1]

[1] West China Hospital, Sichuan University, Sichuan, China

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ABSTRACT

Wound rehabilitation is always time-consuming, scar formation further weakens therapeutic efficacy, and detailed mechanisms at the molecular level still remain unclear. In this work, a Mo4/3B2-x nanoscaffold is fabricated and utilized for wound healing and scar removing in mice model while metabolomics was used to study the metabolic reprogramming of metabolome during therapy at the molecular level. The results showed that transition metal borides, called Mo4/3B2-x nanoscaffold, could mimic superoxide dismutase (SOD) and glutathione peroxidase (GPx) to eliminate excess ROS in the wound microenvironment. During the therapeutic process, Mo4/3B2-x nanoscaffold could facilitate regeneration of wound and removal of scar by regulating biosynthesis of collagen, fibers, and blood vessels at the pathological, imaging, and molecular levels. Subsequent metabolomics study revealed that the Mo4/3B2-x nanoscaffold effectively ameliorated metabolic disorders in both wound and scar microenvironment through regulating ROS-related pathways including amino acid metabolism (including glycine and serine metabolism and glutamate metabolism) and purine metabolism. It is believed that this work could shed light on the potential clinical application of Mo4/3B2-x nanoscaffold as an effective therapeutic agent in traumatic diseases, together with providing insights into the development of analytical methodology for interrogating wound healing and scar removing-related metabolic mechanisms.

Disturbed Membrane Lipids in Ischemic Stroke: Targeting on the Thrombosis and Inflammatory Processes

Qian Wang[1], Dandan Wang[1], Chunling Wan[1]

[1] Bio-X Institutes Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders Ministry of Education, Shanghai Jiao Tong University, Shanghai, China

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ABSTRACT

Objective: Multiple suggestive clinical and molecular findings have indicated that membrane lipids may be extensively involved in the pathological process of ischemic stroke (IS). However, a systematic study of the membrane lipids landscape and its role in IS pathogenesis is still lacking. This study aimed to comprehensively explore the membrane lipid profile and elucidate its potential mechanisms in patients with IS.

Methods: We analyzed the untargeted erythrocyte membrane lipidome of 56 IS patients and 55 healthy controls (HC) using ultra-performance liquid chromatography-mass spectrometry. Genes and lipids involved in lipid signaling pathways were detected by qPCR and Enzyme-linked immunosorbent assay (ELISA). The relationship between lipid and gene dysregulation and clinical indicators of inflammation and thrombosis was assessed by Weighted Gene Co-Expression Network Analysis (WGCNA) and Spearman correlation analysis

Results: Our findings demonstrated that membrane lipid homeostasis was impaired in IS patients, primarily characterized by a significant reduction in glycerophospholipids (GPLs) and lysophospholipids (LPLs). The impaired lipids were enriched in three disturbed membrane lipid signaling pathways, forming a highly interconnected network that synergistically contributing to thrombosis and inflammatory processes in IS. Specifically, the hydrolysis of GPLs to LPLs containing saturated fatty acid (SFA) or monounsaturated fatty acid (MUFA) was significantly reduced in IS, which may be regulated by downregulated phospholipase A2. Concurrently, the PLC-mediated PI-IP3/DG pathway was disturbed, primarily indicated by reduced phosphatidylinositol (PI) content, elevated levels of inositol 1,4,5-trisphosphate (IP3) and phosphatidic acid (PA), along with decreased expression of phospholipases C (PLCs) and IP3 receptors (IP3Rs). The impaired sphingolipid metabolism in IS centered around sphingosine-1-phosphates (S1P). The sphingosine kinase 2-mediated reduction in S1P production and downregulation of S1P receptors (S1PRs) expression led to weakened S1P/S1PRs signaling. Notably, there is a significant positive correlation between the key genes involved in regulating these lipid metabolism pathways, whether in IS or HC, indicating the synergy of these lipid metabolism pathways. The WGCNA results also revealed that these disturbed lipid metabolism pathways were deeply involved in the inflammation and thrombus formation process of IS.

Conclusion: This study elucidates the characteristics of impaired membrane lipid homeostasis and its underlying mechanism in IS patients. The findings provide new insights into the involvement of membrane lipids in IS pathogenesis, potentially guiding future monitoring and intervention strategies.

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