ICBL 2024

The 64th International Conference on the Bioscience of Lipids

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Welcome Message from The 64th International Conference on the Bioscience of Lipids

Following more than three years of planning, it is our honor and great pleasure to welcome you all to The 64th ICBL in Taipei, Taiwan, at the vibrant heart of East Asia.

The conference this year seeks to present a diverse range of topics and perspectives in basic, applicational, translational, and clinical lipid research. The six main sessions cover Advances in Lipidomics, From Lipids to Biomarkers, Disorders in Lipid Metabolism: Mechanisms and Solutions, Lipid Diversity and Evolution, Lipids in Clinical and Translational Research, and Lipids in Immunology and Aging. In addition, we have a special workshop showcasing lipid insights and an exciting new field of study, Lipidomics in Nutrition, which was organized in collaboration with the International Life Sciences Institute (ILSI) Southeast Asia Region.

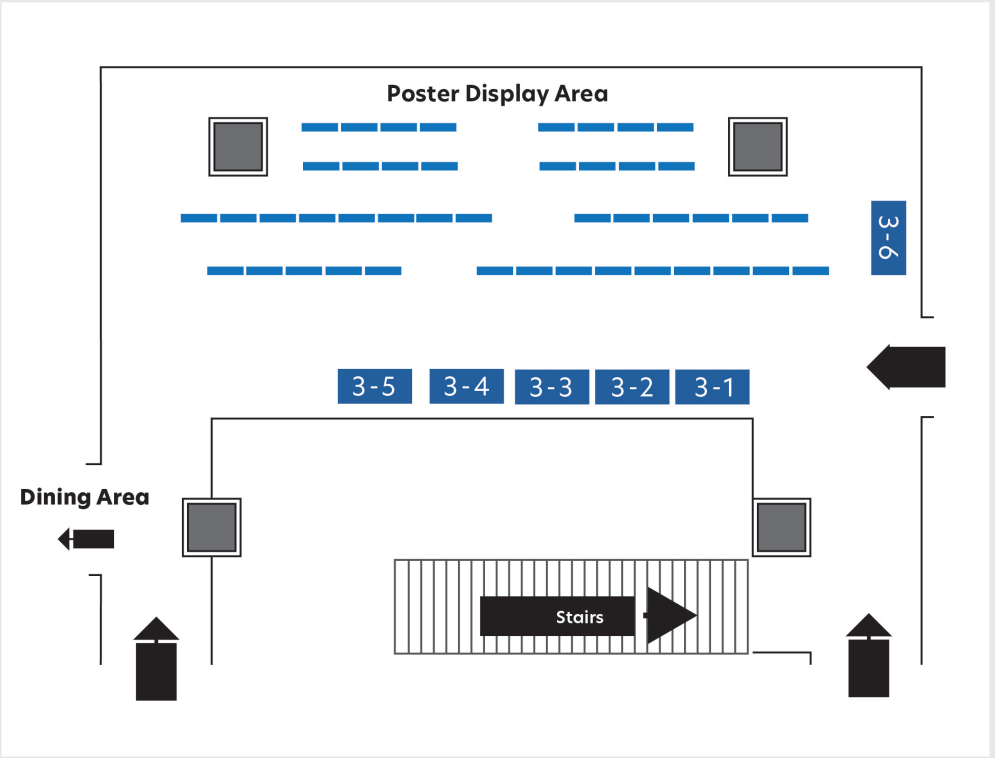
The Van Deenen Lecture this year will be presented by Dr. Jennifer Lippincott-Schwartz, in recognition of lifetime outstanding contributions in the field of lipid dynamics. The Van Deenen Lecturer from the previous year, Prof. Edward A. Dennis, will also present on his latest research findings, and we also have three other distinguished keynote speakers, Prof. Mendel Chu-Huang Chen of the Texas Heart Institute, Dr. Russell DeBose-Boyd of UT Southwestern Medical Center, and Prof. Kenneth K. Wu of the National Health Research Institutes in Taiwan. In addition, there are 11 invited talks and 24 other short talks by top local and international researchers, and we hope the scientific program will be stimulating and rewarding.

Finally, this conference could never have been possible without strong support from the international steering committee and local organizing committee; our co-organizers, the National Biotechnology Research Park, the National Health Research Institutes, and National Taiwan University Center of Biotechnology and College of Life Sciences; our generous sponsors; and our hardworking secretariat and event support partner, MedQCare. We hope that all of you enjoy the conference program and your stay in Taiwan, and may this be a wonderful start to many future collaborations and groundbreaking discoveries.

Chairperson, Dr. Hsinyu Lee

Co-Convener, Dr. Tang-Long Shen

3F Booths



3-1~2 HEART, Health Resource Technology, LLC
HEART & Heart Medical Laboratories

3-3 BIOTOOLS 圖爾思生物科技股份有限公司

3-4 MDPI

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3-6 國立臺灣大學 生物技術研究中心 Center for Biotechnology,NTU

2024-10-15 (TUE)

09:00-10:00	**Registration**	
10:00-10:10	**ICBL 2024 Opening**	
	Special Workshop: Emerging Insights in Lipid Research	
10:10-10:40	Evolution of LDL Research and Technology: From Bench to Industry	*Workshop Keynote :* ***Prof. Mendel Chu-Huang Chen,*** *Director, Vascular and Medicinal Research, The Texas Heart Institute, USA*
10:40-11:40	**Lipid Insights**	
	The Impact of Omega 3-6-9 Fatty Acids Treatment on Proteome of Lipid Metabolism-related Pathways in Fibroblasts from Patients with Neurodegeneration with Brain Iron Accumulation (NBIA)	***S3-16 : Prof. Mariusz R. Wieckowski,*** *Nencki Institute of Experimental Biology, Poland*
	The Role of Lipid Droplet Dynamics in Mediating CDK4/6 Inhibitor Resistance	***S3-19 : Chi-Hsun Yang,*** *National Cheng Kung University, Taiwan*
	Modulation of Fatty Acid Desaturases for the Study on the Roles of Polyunsaturated Fatty Acids (PUFAs) in Metabolic Syndrome	***S3-11: Prof. Hyeon-Cheol Lee-Okada,*** *Juntendo University, Japan*
	To Investigate the Expression of Triacylglycerols in Epididymal Adipose Tissue of Diabetic Mice	***S3-21: Yu-Ju Shih,*** *Chang Gung University, Taiwan*
	Lipidomics: Gut Microbes and Cancer Diagnosis	***S5-15: Prof. Richard Cheng-Chih Hsu,*** *National Taiwan University, Taiwan*
	National Biotechnology Research Park: Overview of Facilities and Services	***NBRP BioTReC, Taiwan***
11:40-12:30	**Luncheon**	
12:30-13:30	**Poster Session S1 & S4**	
	Lipidomics in Nutrition: Food Composition and Human Metabolism **Chair: Prof. Federico Torta,** National University of Singapore, Singapore	
13:30-13:40	Introduction	***Pres. Geoffry Smith,*** *ILSI SEA, Singapore*
13:40-14:05	An Overview of Food Lipids and Food Lipidomics	***Prof. M. Rosário Domingues,*** *University of Aveiro, Portugal*
14:05-14:30	Application of Lipidomics to Vegetable Oils for Classification, Quality Analysis, and Nutritional Value	***Prof. Marc Pignitter,*** *University of Vienna, Austria*
14:30-14:55	Expanding Lipid Nutrition Knowledge with Lipidomics: Fatty Acid Metabolism, Genetic Variant and Cancer Risk	***Prof. Thomas Brenna,*** *University of Texas at Austin, USA*
14:55-15:20	Lipidomic Analysis of Edible Oils, Microbiome Interactions and Human Health	***Prof. Jun Kunisawa,*** *National Institutes of Biomedical Innovation, Health and Nutrition, Japan*
15:30-16:00	**Refreshment Break**	
16:00-17:00	**Van Deenen Lecture**	
	Emerging imaging technologies to study subcellular architecture, dynamics and function	***Dr. Jennifer Lippincott-Schwartz,*** *Senior Group Leader and Head of 4D Cellular Physiology, Howard Hughes Medical Institute Janelia Research Campus, USA*
17:00-19:00	**Welcome Banquet**	

2024-10-16 (WED)

	Session 1: Advances in Lipidomics **Chair: Prof. Maverick Ma,** China Medical University, Taiwan	
09:30-11:00	Advances in Lipidomics leads to Biomarkers for Precision Medicine and Metabolism	*Lecture 1:* ***Prof. Edward A. Dennis,*** *UC-San Diego, USA*
	Integrating Lipid Characteristic Insights Into Advanced Lipidomics Data Analysis	*Lecture 2:* ***Prof. Wei-Chung Cheng,*** *China Medical University, Taiwan*
	Short Talks	
	Development of a quantitative method for phospholipid positional isomers in the brain	***S1-02 : Seiya Tanaka,*** *Tokyo University of Marine Science and Technology, Japan*
	Immune Function in Colon Cancer: Insights from Comparative Lipidomics	***S1-05 : Karim Pérez-Romero,*** *Health Research Institute of the Balearic Islands, Spain*
	Tissue-specific response of skin lipid profile to treatment of Atopic Dermatitis established by mass spectrometry imaging	***S1-12 : Prof. Gwendolyn Barceló-Coblijn,*** *Health Research Institute of the Balearic Islands, Spain*
11:00-12:00	**Luncheon**	
12:00-13:00	**Poster Session S3 & S6**	
13:00-13:30	**Keynote Lecture**	
	Proteostatic control of Cholesterol Synthesis	***Dr. Russell DeBose-Boyd,*** *Beatrice and Miguel Elias Distinguished Chair in Biomedical Science, University of Texas Southwestern Medical Center, USA*
	Session 2: From Lipids to Biomarkers **Chair: Prof. Maurice Liang-Yin Ke,** Kaohsiung Medical University, Taiwan	
13:30-15:00	The functional role of mitochondrial ARC enzymes in lipid metabolism in human cells	*Lecture 3:* ***Dr. Guenter Schwarz,*** *University of Cologne, Germany*
	Apolipoprotein E in Alzheimer's Disease: Intersection of Lipid Metabolism and Neurodegeneration	*Lecture 4:* ***Dr. Abdel Ali Belaidi,*** *University of Melbourne, Australia*
	Short Talks	
	Icosapent ethyl-supplementation in healthy individuals improves plasma and lipoprotein lipid profiles and reduces cardiovascular disease risk markers	***S2-07 : Lauri Äikäs,*** *University of Helsinki, Finland*
	Targeted Metabolomics Reveals Gender-Specific Biomarker Signatures for Identifying High-Risk Patients with MCI Rapidly Progressing to AD	***S2-10 : Dr. Yi-Long Huang,*** *National Yang Ming Chiao Tung University, Taiwan*
	Role of the group IIF phospholipase A2/lysoplasmalogen pathway in atopic dermatitis	***S6-08 : Kei Yamamoto,*** *Tokushima University, Japan*
15:00-15:30	**Refreshment Break**	
	Session 3: Disorders in Lipid Metabolism: Mechanisms and Solutions **Chair: Prof. I-Chen Peng**, National Cheng Kung University, Taiwan **Co-Chair: Prof. Chih-Chiang Chan**, National Taiwan University, Taiwan	
15:30-17:00	New Insights for SREBP Activation and Function: Intrinsic Connection with Glucose and Glutamine Metabolism	*Lecture 5:* ***Prof. Delian Guo,*** *Ohio State University, USA*
	Sphingolipids in Neurological Diseases	*Lecture 6:* ***Dr. Thorsten Hornemann,*** *University of Zurich, Switzerland*
	Short Talks	
	Oleate Promotes Triple-Negative Breast Cancer Cell Migration by Enhancing Filopodia Formation through a PLD/Cdc42-dependent Pathway	***S3-12 : Prof. Catherine Mounier,*** *UQAM, Canada*
	Tipping the switch between exosome secretion and autophagy by altered balance between ceramide and dihydroceramide	***S3-20 : Dr. Shu-Yi Huang,*** *National Taiwan University Hospital, Taiwan*
	Subclass-specific impact of ether lipid deficiency on murine rhizomelic chondrodysplasia punctata-like phenotypes	***S3-14 : Prof. Katrin Watschinger,*** *Medical University of Innsbruck, Austria*
17:00-18:00	**Steering Committee Regular Meeting**	**Cocktail Night**
17:00-19:00		

2024-10-17 (THU)

Session 4: Lipid Diversity and Evolution
Chair: Prof. Chau-Ti Ting, National Taiwan University, Taiwan

09:30-11:10	The Critical Role of Host Endoplasmic Reticulum in Lipid Body Biogenesis During the Coral-Symbiodinium Endosymbiosis	*Lecture 7:* ***Director General Chii-Shiarng Chen,*** *National Museum of Marine Biology and Aquarium, Taiwan*
	Illuminating neurosecretion: Optogenetic and click chemistry novel tools highlight the multiple roles of phosphatidic acid in neurotransmitter release	*Lecture 8 :* ***Dr. Nicolas Vitale,*** *Institut for Cellular and Molecular Integrated Neurosciences, France*
	Short Talks	
	Sterol metabolism and homeostasis in plants	***S4-09 : Prof. Hubert Schaller,*** *Strasbourg University, France*
	TRPV4-dependent Ca2+ influx determines cholesterol dynamics at the plasma membrane	***S4-11 : Yutaro Kuwashima,*** *Keio University, Japan*
	Discovery and Functional Analysis of a Novel Enzyme Phosphorylating Ceramide under Acidic Conditions	***S4-03 : Ami Nishino,*** *Chiba University, Japan*
	Impact of lipid composition on mitochondrial function and cell fate decisions	***S4-13 : Prof. Markus Keller,*** *Medical University of Innsbruck, Austria*
11:10-12:00	**Luncheon**	
12:00-13:00	**Poster Session S2 & S5**	

Session 5: Lipids in Clinical and Translational Research
Chair: Dr. Hsiang-Chun Lee, Kaohsiung Medical University Hospital, Taiwan

13:00-14:20	PIP2 Dependent Activation of a Cardiac K+ Channel	*Lecture 9:* ***Prof. Jianmin Cui,*** *Washington University in St. Louis, USA*
	Short-Chain Fatty Acids and Lipid Dysregulation in Childhood Asthma	*Lecture 10:* ***Dr. Chih-Yung Chiu,*** *Chang Gung Memorial Hospital at Linkou, Taiwan*
	Short Talks	
	The emerging role of lipids as drivers of systemic inflammation and organ failure in advanced liver disease	***S5-21 : Prof. Joan Clària,*** *University of Barcelona, Spain*
	10-second Lipidomic Analysis with Picosecond Infrared Laser Mass Spectrometry to Diagnose Brain Cancer Types	***S5-02 : Prof. Arash Zarrine-Afsar,*** *University of Toronto, Canada*
14:20-15:00	**Depart on City Tour**	
15:00-18:30	**City Tour: National Palace Museum** Experience 5,000 years of history in an ornate imperial setting with classic audio-guided tour	
18:30-21:00	**Gala Dinner** Grande Luxe Banquet Nangang	

2024-10-18 (FRI)

Session 6: Lipids in Immunology and Aging
Chair: Prof. Kuang-Tzu Huang, Chang Gung Memorial Hospital at Kaohsiung, Taiwan
Co-Chair: Prof. Hay-Yan J. Wang, National Sun Yat-sen University, Taiwan
Co-Chair: Dr. Kuan-Hung Lin, Academia Sinica, Taiwan

09:30-11:00	Autocrine/paracrine lysophosphatidylserine signaling suppresses B cell aggregation and ectopic lymphoid tissue formation.	*Lecture 11:* ***Prof. Junken Aoki,*** *The University of Tokyo, Japan*
	Specific Human Breast Milk Lipids Shape Neurobehavioral Development and Lifespan: A Study in Infants and Caenorhabditis elegans	*Lecture 12:* ***Dr. Wendy Wen-Li Hsu,*** *National Health Research Institutes, Taiwan*
	Short Talks	
	Targeting lipid peroxidation in protection against muscle wasting	***S6-10 : Prof. Katsuhiko Funai,*** *University of Utah, USA*
	Oncogene-Induced Senescence Elicits a Marked Lipid Metabolic Shift and Change in Cellular Morphology	***S6-05 : Stephen Ruiz,*** *Cornell University, USA*
	sPLA2-III recruits eosinophils by driving the paracrine LPA- eotaxin-2 axis in chitin-induced peritonitis	***S6-09 : Dr. Kyohei Horikiri,*** *The University of Tokyo, Japan*
11:00-11:30	**Closing Keynote**	
	Control of COX-2 expression and related inflammation and cancer metastasis by 5-methoxytryptophan	***Prof. Kenneth K. Wu*** *Honorary Investigator, National Health Research Institutes, Taiwan*
11:30-12:00	**Award Ceremony**	
12:00-12:10	**ICBL 2025 Introduction**	
12:10-12:20	**ICBL 2024 Closing**	
12:20-12:30	**Box lunch provided**	

Jennifer Lippincott-Schwartz

POSITION & AFFILIATION

- Senior Group Leader and Head of 4D Cellular Physiology, Howard Hughes Medical Institute Janelia Research Campus

SPEAKER BIO

Dr. Jennifer Lippincott-Schwartz is a Senior Group Leader at the Howard Hughes Medical Institute's Janelia Research Campus and Head of the Research Program on 4D Cellular Physiology. Lippincott-Schwartz has pioneered the use of green fluorescent protein technology for quantitative analysis and modelling of intracellular protein traffic and organelle dynamics in live cells. Her innovative techniques to label, image, quantify and model specific live cell protein populations and track their fate have provided vital tools used throughout the research community. Her findings using these techniques have reshaped thinking about the biogenesis, function, targeting, and maintenance of various subcellular organelles and macromolecular complexes and their crosstalk with regulators of the cell cycle, metabolism, aging, and cell fate determination. She is an elected member of the National Academy of Sciences, the National Academy of Medicine, the American Society of Arts and Sciences and the European Molecular Biology Organization. She is also a Fellow of The Biophysical Society, The Royal Microscopical Society and The American Society of Cell Biology. Her awards include the E.B. Wilson Medal of the American Society of Cell Biology, the Newcomb Cleveland Prize of the American Association for the Advancement of Science, the Van Deenen Medal, the Keith Porter Award of the American Society of Cell Biology, the Feodor Lynen Medal, and the Feulgen Prize of the Society of Histochemistry. She co-authored the textbook "Cell Biology" and was President of the American Society of Cell Biology. Dr. Lippincott-Schwartz attended Swarthmore College, received her MS from Stanford University, and obtained her PhD in Biochemistry from Johns Hopkins University.

Emerging imaging technologies to study subcellular architecture, dynamics and function

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In this talk, I will focus on how emerging fluorescent technologies are increasing spatio-temporal resolution dramatically, permitting simultaneous multispectral imaging of multiple cellular components and their specialized lipid/membrane organization. In addition, results will be discussed from whole cell milling using Focused Ion Beam Electron Microscopy (FIB-SEM), which reconstructs the entire cell volume at 4-voxel resolution. Using these tools, we seek to understand the interrelationships of different subcellular organelles as they carry out critical functions involving membrane and lipid trafficking in healthy and diseased conditions.

Russell DeBose-Boyd

POSITION & AFFILIATION

- Professor of Molecular Genetics, University of Texas Southwestern Medical Center

SPEAKER BIO

Dr. Russell DeBose-Boyd was born and raised in Oklahoma. He completed undergraduate studies at Southeastern Oklahoma State University and obtained a Bachelor of Science degree in Chemistry. He then joined the laboratory of Richard D. Cummings, Ph. D. in the Department of Biochemistry and Molecular Biology at the University of Oklahoma Health Sciences Center. Following his defense, Dr. DeBose-Boyd joined the laboratory of Joseph L. Goldstein, M.D. and Michael S. Brown, M.D. at UT Southwestern Medical Center as a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

After his postdoctoral fellowship, Drs. Goldstein and Brown invited Dr. DeBose-Boyd to join the faculty of the Molecular Genetics department as an Assistant Professor in 2003.

Dr. DeBose-Boyd was promoted to Associate Professor in 2007 and in 2009 he was appointed a Howard Hughes Medical Institute Early Career Scientist. In 2013, Dr. DeBose-Boyd was promoted to Professor and was named the Beatrice and Miguel Elias Distinguished Chair in Biomedical Science in 2016.

Dr. DeBose-Boyd's work has been recognized by several awards including the John Abel Award in Pharmacology (2010), Edwin Bierman Award (2021), Avanti Award in Lipids (2023), and the Hill Prize in Biological Sciences (2024). He was elected to the National Academy of Sciences in 2023.

Proteostatic control of Cholesterol Synthesis

UbiA prenyltransferase domain-containing protein-1 (UBIAD1) utilizes geranylgeranyl pyrophosphate (GGpp) to synthesize the vitamin K_2 subtype menaquinone-4 (MK-4). Mutations in *UBIAD1* cause Schnyder corneal dystrophy (SCD), which is characterized by corneal opacification owing to over-accumulation of cholesterol. Our studies disclosed a key role for UBIAD1 in regulating endoplasmic reticulum (ER)-localized HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol and nonsterol isoprenoids including GGpp. Feedback control of reductase involves sterol-induced ubiquitination, an obligatory reaction for its ER-associated degradation (ERAD) that is augmented by GGpp. Sterols also cause UBIAD1 to bind reductase, which inhibits ERAD and allows continued synthesis of nonsterol isoprenoids in sterol-replete cells. GGpp triggers release of reductase from UBIAD1, enhancing ERAD and stimulating translocation of UBIAD1 to Golgi. SCD-associated UBIAD1 resists GGpp-induced release from reductase and becomes sequestered in ER to inhibit ERAD.

Gene knockout studies in mice were attempted to explore the in vivo function of *UBIAD1*; however, homozygous germ-line deletion of Ubiad1 caused embryonic lethality. We generated homozygous deletion of Ubiad1 in knock-in mice expressing ubiquitination-resistant HMGCR, implying embryonic lethality results from enhanced ERAD of HMGCR. The study of Ubiad1-deficient mice offers the opportunity to determine the physiological significance of UBIAD1-mediated synthesis of MK-4.

Kenneth K. Wu

POSITION & AFFILIATION

- Academician, Academia Sinica
- Honorary Investigator, Institute of Cellular and System Medicine, National Health Research Institutes

SPEAKER BIO

Prof. Kenneth K. Wu MD, Ph.D., a graduate of National Taiwan University College of Medicine, obtained his M.S. from Yale University in New Haven, CT and Ph.D. in pharmacology from University of London, UK. He completed his medicine residency and Hematology-Oncology fellowship at the University of Iowa, Iowa City, IA. He spent most of his academic career (1983-2006) at University of Texas Medical School at Houston and UT MD Anderson Cancer Center in Houston, TX. He was professor and Director of the Vascular Biology Research Center as well as the NIH-supported Clinical Research Center. In 2006, he was elected as Distinguished Investigator and President, National Health Research Institutes (NHRI) in Taiwan. He is currently an Honorary Investigator at NHRI, Professor Emeritus at the University of Texas at Houston, and adjunct professor at Johns Hopkins University School of Medicine in Baltimore, MD. His research activities center around platelet and vascular biology, COX-2 transcriptional regulation and tryptophan mechanism. He has received international recognition for his research work on platelet aggregability and thrombosis, COX-2 transcriptional mechanism and control. He has received several awards, including the Sanofi Prize, and the Presidential Science Prize of Taiwan, and is a member of several prestigious scientific academies and societies.

Control of COX-2 expression and related inflammation and cancer metastasis by 5-methoxytryptophan

Cyclooxygenases (COX, also known as prostaglandin endoperoxide synthase, PGHS) occupy a pivotal role in the synthesis of a group of bioactive lipid mediators, i.e., prostaglandins, prostacyclin and thromboxane A2. Of the two COX isoforms, COX-2 expression is induced by diverse environmental stress factors, resulting in the production of abundant prostaglandins, which elicit inflammation and promote cancer growth and metastasis. COX-2 expression is controlled by intrinsic mechanisms. We discovered 5-methoxytryptophan (5-MTP), a tryptophan metabolite via a novel synthetic pathway, as a highly active molecule with COX-2 suppressing activity. It inhibits macrophage-mediated inflammation and blocks cancer cell migration, invasion, and EMT, as well as cancer metastasis. Unpublished results suggest that 5-MT controls cancer cell COX-2 expression and cancer cell migration by blocking Drp-1-mediated mitochondrial fission. This lecture will cover a brief overview of COX-2 discovery and actions, a review of 5-MTP and the mechanisms by which 5-MTP inhibits inflammation and tumorigenesis including unpublished results on mitochondrial dynamics and metabolism.

Mendel Chu-Huang Chen

POSITION & AFFILIATION

- Director, Vascular and Medicinal Research, The Texas Heart Institute

SPEAKER BIO

Professor Chu-Huang (Mendel) Chen, MD, PhD, serves as the Director of Vascular and Medicinal Research at The Texas Heart Institute in Houston, Texas, and as a Distinguished Visiting Professor at Shinshu University in Nagano, Japan. Previously, Dr. Chen held positions as Chair Professor of Medicine at Kaohsiung Medical University and Visiting Professor at China Medical University in Taiwan. Dr. Chen is a pioneer in the research of electronegative lipoproteins, specifically LDL (L5), VLDL (V5), and HDL (H5), which represent the most atherogenic entities in their respective lipoprotein classes. His work has significantly advanced the field of lipoprotein research, opening new avenues for discovery and potential treatment modalities. To further enhance research and clinical applications, Dr. Chen established HEART (Health Resource Technology, LLC) to facilitate advanced assay processes.

Dr. Chen's contributions to biological science and medicine are substantial in three major areas: **1. Biochemical Characterization of Electronegative Lipoproteins for Diagnostic Purposes:** Utilizing advanced omics technologies (lipidomics, proteomics, and transcriptomics), Dr. Chen's team has identified major differences in lipid and protein compositions between L5 and L1 (the least electronegative and health-beneficial LDL subfraction), as well as between V5/V1 and H5/H1. 2. **Translating Atherothrombogenic and Aging Properties into Clinical Settings:** His translational studies have established the in vivo relevance of L5/V5/H5 in the pathogenesis of atherosclerosis, thrombosis, systemic inflammation, and aging. **3. Defining L5/V5/H5 as Novel Causative Biomarkers in CAD for New Treatment Development:** Clinical trials are underway, and new treatment strategies are being developed based on these findings.

Throughout his career, Professor Chen has mentored numerous young scientists and physician-scientists, established a broad international collaborative network, and he welcomes further partnerships.

Evolution of LDL Research and Technology: From Bench to Industry

Traditionally labeled as "bad cholesterol" due to its role in atherogenesis, low-density lipoprotein (LDL) has emerged as a far more complex and multifaceted molecule than initially understood. This review delves into the evolution of LDL research, tracing its journey from early discoveries to current insights driven by advanced omics technologies and molecular biology. We explore the comprehensive history, structure, metabolism, and function of LDL in both cardiovascular health and disease.

Key developments in LDL research are outlined, from the elucidation of its structure to the application of modern imaging and analytical techniques that reveal the intricate heterogeneity of its three-dimensional architecture. Understanding LDL's metabolic pathways, from synthesis to clearance, provides critical insight into its physiological roles and clinical implications. Beyond cholesterol transport, LDL plays pivotal roles in inflammatory responses, immune modulation, and cellular signaling, reflecting its functional diversity and impact on cardiovascular health.

This lecture also addresses evolving clinical perspectives on LDL, discussing how these insights impact cardiovascular risk assessment, patient management, and potential therapeutic interventions. We emphasize the need for a more nuanced approach to understanding LDL biology, one that acknowledges its broader implications beyond cholesterol metabolism.

Furthermore, our research team has made significant strides in the study of electronegative lipoproteins, particularly LDL (L5), VLDL (V5), and HDL (H5). These subfractions are identified as highly atherogenic entities within their PAGE 3 OF 4 respective classes, representing critical factors in cardiovascular pathology. This groundbreaking work has expanded the horizons of lipoprotein research, paving the way for innovative treatment strategies. To support these advancements, Dr. Chen founded HEART (Health Resource Technology, LLC), enhancing research capabilities and translating findings into practical clinical applications.

In conclusion, this lecture highlights the dynamic and evolving nature of LDL research and its far-reaching implications for understanding cardiovascular health, offering new perspectives and directions for future research and therapeutic approaches.

ILSI SOUTHEAST ASIA REGION

Lipidomics in Nutrition: Food Composition and Human Metabolism

Federico Torta

POSITION & AFFILIATION

- Research Assistant Professor, Duke-NUS Medical School and Singapore Lipidomics Incubator (SLING), National University of Singapore

SPEAKER BIO

Prof. 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.

Geoffry Smith

POSITION & AFFILIATION

- President, International Life Sciences Institute (ILSI), Southeast Asia Region
- Chairman, Essential Micronutrients Foundation

SPEAKER BIO

Pres. Geoffry Smith is President of ILSI Southeast Asia Region based in Singapore, and a Representative of the institute to the ILSI Global Assembly. He is also the Chairman of the Essential Micronutrients Foundation, a non-profit organization which addresses micronutrient deficiencies globally as a public health issue. Mr. Smith serves as a member of the editorial board of the journal Food and Nutrition Bulletin. Prior to his current positions, Mr. Smith was the Global Director, Health Chelates for Akzo Nobel Functional Chemicals and directed the global business for these compounds in food and nutrition as well as pharmaceutical applications. He was responsible for the global project within Akzo Nobel addressing iron deficiency anemia.

M. Rosário Domingues

POSITION & AFFILIATION

- Associate Professor, Department of Chemistry at the University of Aveiro (UA).
- Director, Doctoral Program of Biochemistry at UA
- Leader, Lipidomic Laboratory of the Mass Spectrometry Centre of the UA

SPEAKER BIO

Prof. M. Rosário Domingues graduated with a degree in Pharmaceutical Sciences from the University of Coimbra (1990), received her Ph.D. degree in Chemistry (1998), and Habilitation in Biochemistry (2014) both at the University of Aveiro. Since 2016 she has held the position of Associate Professor with habilitation in the Mass Spectrometry Centre, Department of Chemistry at the University of Aveiro (UA). She is the Director of the Doctoral Program of Biochemistry at UA and the leader of the Lipidomic Laboratory of the Mass Spectrometry Centre of the UA and Marine Lipidomic lab at CESAM-UA.

She has over 25 years of research experience in the field of mass spectrometry, and is a well-established researcher in lipidomics, glycomics and changes in biomolecules associated with oxidative stress monitored by mass spectrometry. Her major research interests are focused in lipidomics, namely: Algae & Marine lipidomics, Oxidative lipidomics, Food & Microbial lipidomics and lipidomics in Health and Disease. She is the author of one book and ten book chapters with more than 390 articles published in international journals. She has coordinated and participated in several research projects funded by national and European programs (35 in total). Currently, she is the coordinator of the European project Cost Action CA19105 Pan-European Network in Lipidomics and EpiLipidomics.

An overview of food lipids and food lipidomics

Lipids are present in nearly all the food we consume, as they are essential components of cell membranes and molecules for energy storage. Despite misconceptions labeling fats as "good" or "bad" for health, lipids are vital nutrients, and are praised to contributing to 20-30% of our total energy intake. However, understanding the lipid composition of foods is challenging due to the immense diversity of lipid molecules, ranging from simple lipids like fatty acids and sterols to complex forms such as triglycerides, phospholipids, glycolipids, that include a large variety of molecules micronutrients like vitamins and modified lipids. Each food type, from lipid-rich sources like vegetable oils and nuts to low-fat foods like vegetables, has a unique lipid profile, making food lipidomics a highly diverse and complex field.
Mass spectrometry-based lipidomics has become essential for identifying this complexity. It not only helps in determining the nutritional and healthy value of foods but also ensures authenticity, traceability, and safety. By analyzing the specific lipid composition of food, lipidomics can verify origin, detect adulteration, and track products through the supply chain, safeguarding public health by identifying contaminants and spoilage. Thus lipidomics may allow food producers and regulators to track the movement and history of food products through the supply chain, ensuring transparency and safety for consumers.
Lipidomics is also crucial for developing functional food, with bioactive properties and health benefits beyond basic nutrition, or to support personalized nutrition, enabling the customization of diets based on individual lipid profiles to better manage or prevent chronic conditions such as obesity, diabetes, and cardiovascular diseases.
The increasing evidence highlights lipids' beneficial effects on human health, requires fully understanding and assessing the complete lipid profile of foods and improvement of analytical approaches and computational methods to increase consumer awareness of the significant role of lipids in food quality and food

Marc Pignitter

POSITION & AFFILIATION

- Associate Professor, University of Vienna

SPEAKER BIO

Prof. Marc Pignitter received his doctorate in pharmacy from the University of Graz, Austria. He completed postdoctoral stays at the University of Vienna, Austria, and in industry. He was a research associate at the National Biomedical EPR Center and the Free Radical Research Center at the Department of Biophysics, Medical College of Wisconsin in Milwaukee, USA. Another research stay took him to Australia, where he joined the group in Adelaide at Metabolomics Australia and participated in the Australian Endeavour Leadership Program. In 2017, he took up a tenure track position at the Institute of Physiological Chemistry at the University of Vienna. In 2021, he was promoted to the rank of Associate Professor at the same institution.

His research interests focus on the application of innovative approaches and technologies to control and better understand lipid oxidation in food and its health effects.

Application of lipidomics to vegetable oils for quality analysis, and nutritional value

This lecture will explore the use of lipidomics to track lipid oxidation progression in vegetable oils, and how the consumption of vegetable oils influences human health.
The application of lipidomics to vegetable oils provides a cutting-edge method for classifying, analyzing quality, and assessing nutritional value by examining lipid composition and oxidation products. Oxidative lipidomics (oxidomics) offers a detailed analysis of oxidation markers such as hydroperoxides and epoxides, which are often overlooked in conventional methods. Epoxides could be shown to be converted into proinflammatory diols during food preparation and digestion. In addition, chlorinated triacylglycerols were identified as novel products derived from in vitro digested epoxides. These compounds are crucial for understanding lipid degradation, which affects both food quality and human health.
By analyzing oxidation products like epoxidized triacylglycerols, lipidomics provides key insights into oil stability, shelf life, sensory properties, and nutritional value. Additionally, this approach helps optimize processing, storage, and packaging conditions to improve the oxidative stability of oils, making them more beneficial for human health. Ultimately, lipidomics offers valuable applications in the food and nutraceutical industries by improving oil quality, safety, and health benefits.

Thomas Brenna

POSITION & AFFILIATION

- Professor, University of Texas at Austin

SPEAKER BIO

Prof. Tom Brenna, PhD, is Professor of Pediatrics, of Human Nutrition, and of Chemistry at the University of Texas at Austin since 2017, after 28 years as a Professor of Human Nutrition, and of Food Science at Cornell University in Ithaca, NY. The major theme of his group's research is the chemical, biochemical, metabolic, genetic and ecological aspects of fatty acids. As an undergraduate he worked with the late Bob Jensen at the University of Connecticut and went to complete the PhD in Chemistry at Cornell. After four years as a staff engineer at IBM's Technology Campus in Endicott, NY, he was hired as Assistant Professor at Cornell.

His analytical chemistry research pioneered facile methods for the structure elucidation of fatty acid methyl esters, and independently developed some of the first molecular methods for high precision isotope ratio mass spectrometry, work that resulted in dozens of contributions to Analytical Chemistry and other leading chemistry journals. The research group was among those contributing to the recognition that dietary omega-3 fatty acids are required for proper brain and retinal function in developing infants and, more recently, has been interested in the nutrition and metabolism of branched-chain fatty acids. His work has contributed extensively to understanding of fatty acid biochemical pathways, and genetics. Notable international recognition are the American Oil Chemists Society's Supelco Research Award (2023) and the American Society for Nutrition's Osborne and Mendel (2017), and Robert Hermann Awards (2013).

Expanding lipid nutrition knowledge with lipidomics: fatty acid metabolism, genetic variant and cancer risk

Precision medicine is based on identifying biological factors that segregate individuals to improve disease risk, diagnosis, or treatment beyond a common treatment for all. Arachidonic acid (ARA) is the central precursor to eicosanoids, and it influences virtually every metabolic process via inflammation, thrombosis, angiogenesis, and other processes. ARA and associated PUFA are under genetic control, and all contribute to the lipid mediator signaling milieu that in part determines physiological responses. We recently showed that sustained exposure to the ARA antagonist omega-3 EPA reduces recurrent colorectal polyps in individuals with a fast ARA-synthesizing allele at the FADS Indel. We also show that carriers of the same allele have higher early preterm birth and respond to high DHA therapy. In animal studies, high dose ARA increases, and high dose EPA or DHA decreases, tumor size in neuroblastoma, and further that the effect may be mediated by altered eicosanomic signaling. Prevalence of the FADS indel genotypes varies widely across global populations, implying that the outcome of randomized trials related to any process influenced by ARA-derived signals will also vary based on population. No commonly measured SNPs in or near the FADS gene cluster are highly correlated (in high linkage disequilibrium). Thus, the strongest effects should be found with measuring the FADS Indel genotype itself. With existing and novel results from various studies, we will introduce novel results and integrate them with existing results to demonstrate how genotype and diet selectively alter lipidomic profile and the consequences for translation to human health.

Jun Kunisawa

POSITION & AFFILIATION

- Deputy Director General of the National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), and Director of the Microbial Research Center for Health and Medicine

SPEAKER BIO

Prof. Jun Kunisawa is Deputy Director General of National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), and serves as Director of Microbial Research Center for Health and Medicine, one of centers in NIBIOHN. He also serves as Adjunct/Visiting Professor at Osaka University, Kobe University, The University of Tokyo, Hiroshima University and Waseda University. He was awarded his Ph.D. from Osaka University at 2001, and received postdoctoral training at University of California, Berkeley. In 2004, he was recruited by The University of Tokyo, where he spent 9 years as Assistant and Associate Professor before moving to NIBIOHN to establish a new laboratory in 2013. In 2019, he was promoted to Director, and subsequently to Deputy Director General in 2024. His research has been focusing on the immune regulation by gut environment (e.g., diets and commensal bacteria) and its association with immune diseases and health. He is also doing translational research for the development of vaccines, medicines, and healthcare.

Lipidomic analysis of edible oils, microbiome interactions and human health

It is well known that dietary oils play an important role in maintaining health, which is considered not only in terms of quantity but also in terms of quality, which is determined by differences in fatty acid composition. We have focused on the quality of edible oils, particularly linseed oil (also known as flaxseed oil), which is rich in omega-3 fatty acids, and have demonstrated their anti-allergic and anti-inflammatory properties. The metabolism of these lipids involves not only the host's enzymes but also gut bacteria. Furthermore, analyses using human samples have shown individual differences in the patterns and amounts of metabolites produced from edible oils, which may account for the varying effects of these oils. We have also developed alternative methods using fermented foods for individuals who may not benefit from edible oils. In this presentation, we will introduce the immune regulatory mechanisms of these edible oils and explore the potential of personalized nutrition based on precision nutrition.

Note

Session Speakers

Edward A. Dennis

POSITION & AFFILIATION

- Distinguished Professor and Chancellor I Endowed Chair of Chemistry, Biochemistry and Pharmacology, University of California-San Diego, USA

SPEAKER BIO

Prof. Edward A. Dennis is Distinguished Professor of Chemistry, Biochemistry and Pharmacology and holds the Chancellor I Endowed Chair at the University of California at San Diego (UCSD). He received his B.A. from Yale University and his Ph.D. from Harvard University and carried out postdoctoral studies at Harvard Medical School. He received a Doctorate in Medicine (honorary) from Goethe University in Frankfurt, Germany, and a Doctorate (honorary) from the University of Lyon INSA in Lyon, France. Dr. Dennis started as Assistant Professor at UCSD and served two terms as Chair of the Department of Chemistry and Biochemistry. He received a Guggenheim Fellowship during which he served as a Visiting Professor at Harvard Medical School and a Visiting Scientist at Brandeis University. Dr. Dennis served as a Visiting Foreign Professor at the Collège de France in Paris, as an Adjunct Professor at The Scripps Research Institute, and as a Visiting Research Professor at the Université Pierre et Marie Curie. He also served as Chair and President of the Keystone Symposia, Editor-in-Chief of the Journal of Lipid Research, and Founder and Director of the LIPID MAPS Lipidomics Consortium. He is an inaugural Fellow of the American Association for the Advancement of Science (AAAS) and the American Society of Biochemistry and Molecular Biology (ASBMB). He was the recipient of ASBMB's Avanti Award in Lipid Enzymology, the European Federation for Lipid Science and Technology's European Lipid Science Award, the Yale Medal, the American Chemical Society San Diego Section, Distinguished Scientist Award, and the ASBMB Bert Vallee Award in Biomedical Science. Prof. Dennis' has over 430 publications (H-factor >100). His career research focus has been on the structure/function/mechanism/inhibition of the phospholipase A2 superfamily and their role in lipid metabolism, inflammation, and especially developing novel lipidomics based enzyme assays and biomarkers for human metabolic and infectious diseases.

Advances in Lipidomics leads to Biomarkers for Precision Medicine and Metabolism

Lipidomics is the newest omics and the number of publications has grown exponentially since **LIPID MAPS** started in 2003. Two main MS approaches linked to UPLC have evolved, one based on exact mass and one based on triple quadrupole fragmentation. A major limitation in lipidomics has been the automated identification of the multi-thousands of molecular species in each category of complex lipids, but now thousands of lipid molecular species can be identified with tools such as Lipid Data Analyzer (LDA). We have now determined the phospholipase A_2 (PLA_2) specificity in living macrophage cells including which specific phospholipid molecular species are hydrolyzed by each PLA_2 type at the molecular level **[Murawska *et al* (2024) J Lipid Res]**. These techniques have also enabled the determination of phospholipid molecular species in ocean species such as Ctenophores (Comb Jellies) at various ocean depths and the discovery using this same lipidomics platform that plasmalogens increase as the ocean depth increases from the surface to 4000 meters depth **[Winnikoff *et al* (2024) Science]**. This work explains how increased pressure is tolerated by increased plasmalogen content of the membranes and the important role of plasmalogens in resisting compression of the bilayer, which should help elucidate the role of plasmalogens in human metabolism. An example where quantitation in the picogram/femtomole range is essential is in the analysis of human blood plasma using triple-quadrupole technology. Blood plasma samples from patients with varying phenotypes of metabolic dysfunction-associated steatotic liver disease (MASLD) were used to identify a minimal set of lipid analytes reflective of underlying histologically confirmed MASLD. Plasma samples were utilized for targeted quantitation of circulating eicosanoids, related bioactive metabolites, and polyunsaturated fatty acids by UPLC-MS lipidomics analysis and bioinformatic approaches to identify MASLD and suggesting who needs further evaluation for MASH **[Quehenberger et *al* (2024) J Lipid Res]**.

Wei-Chung Cheng

POSITION & AFFILIATION

- Professor, Program for Cancer Biology and Drug Discovery, China Medical University, Taiwan

SPEAKER BIO

Prof. Wei-Chung Cheng specializes in employing bioinformatics strategies to dissect and understand omics data. His team has developed a range of databases and web tools designed to facilitate this analysis. Moreover, he actively collaborates with experimental biologists and medical scientists, engaging in research on pivotal topics such as cancer immunology, the discovery of cancer biomarkers, the study of cancer stem cells, and the development of novel bioinformatics tools.

In recent years, Prof. Cheng has shifted his research focus to lipidomic data analysis. This includes constructing a web tool for comprehensive lipidomic data analysis, developing algorithm for lipidomic tendency identification, and establishing a lipid function database.

Integrating lipid characteristic insights into advanced lipidomics data analysis

In the field of lipidomics, the complex nature of lipid structures and functions poses significant analytical challenges. To address this, we have developed LipidSig, the first web-based platform that provides integrated and comprehensive analysis for efficient data mining of lipidomic datasets. The tool simplifies the process and enables researchers to understand the complex nature of lipids and associate lipidomic data with specific characteristics and biological contexts.

LipidSig autonomously identifies lipid species and provides comprehensive characteristics upon data entry, accommodating different data processing methods and streamlining diverse lipidomic datasets. By automating complex analytical processes, including data pre-processing, lipid ID annotation, differential expression, enrichment analysis and network analysis, researchers can thoroughly investigate lipid properties and their biological implications.

The platform also includes innovative features such as the "Network" function, which provides a systems biology perspective on lipid interactions, and "Multiple Group" analysis, which helps investigate complex experimental designs. With its comprehensive suite of features for the analysis and visualization of lipid properties, LipidSig positions itself as an indispensable tool for advanced lipidomics research.

Guenter Schwarz

POSITION & AFFILIATION

- Professor, Biochemistry, University of Cologne, Germany

SPEAKER BIO

Prof. Guenter Schwarz graduated his studies in Plant Biology and Biochemistry and habilitated Venia legendi in Biochemistry and Molecular Biology at the Technical University of Braunschweig (TU), Germany. He is full Professor (W3) for Biochemistry at the University of Cologne, Germany. He and his team are interested to understand protein structure-function relations in different biological processes ranging from basic metabolism to molecular neuroscience. Their research is based on a variety of methods including molecular biology, protein biochemistry, enzymology, structural biology (protein crystallography), cell biology, bioinorganic chemistry and biotechnology.
Dr. Schwarz has worked in the field of molybdenum cofactor biosynthesis, enzymes and deficiency for more than 25 years. He has also studied the molecular mechanisms in the formation of inhibitory synapses in the central nervous system. Dr. Schwarz and his team have contributed to the understanding of eukaryotic molybdenum cofactor biosynthesis, with a focus on plants and humans, identifying critical steps and intermediates. They were able to develop a first treatment to cure molybdenum cofactor deficiency, which recently resulted in the regulatory approval of cPMP therapy by the FDA and EMA.

The functional role of mitochondrial ARC enzymes in lipid metabolism in human cells

Molybdenum cofactor enzymes are essential for life; in humans four of those enzymes can be found. A loss of molybdenum enzymes causes severe disease with early childhood death as usual outcome. The physiological role(s) of the molybdenum containing mitochondrial amidoxime reducing component (mARC1 and mARC2) enzymes remain unclear. It has been shown that recombinant mARC, with the help of cytochrome b_5 and cytochrome b_5 reductase, is able to reduce a variety of *N*-hydroxylated compounds. From studies in mice there is growing evidence that these enzymes are involved in lipid metabolism and the *MTARC1* p.A165T variant is associated with decreased risk of metabolic dysfunction-associated steatotic liver disease (MASLD). To further understand the physiological role of mARC enzymes, we searched for interaction partners in Flp-In™-293, a human kidney cell line, using co-immunoprecipitation, proximity labeling and complexome profiling. While the proposed redox partners were not consistently detected, proteins that localize to mitochondria-ER contact sites (MERCS) were enriched, suggesting that mARC enzymes localize to those sites within the outer mitochondrial membrane. Moreover, we used CRISPR/Cas9 to generate Flp-In™-293 cells devoid of mARC1 or mARC2 expression, resulting in a growth defect. Furthermore, mARC1- and mARC2-deficient cells were characterized using mass spectrometry to investigate their lipidome. We found dramatic changes with a loss in mARC1 resulting in strongly increased triglycerides and more lipid droplets stained by Oil Red O. In contrast, loss of mARC2 was accompanied by massively decreased lysophosphatidic acid and phosphatidic acid species. Preliminary experiments suggest that mARC2 KO provides protection against ferroptosis when induced by RSL3 inhibition of GPX4, suggesting alterations in PUFAs. Our data collectively suggest that mARC1 and mARC2 are both involved in lipid metabolism, however they are acting on distinct pathways and further work is needed to identify their respective substrates to understand the complex phenotypes discovered in this study.

Abdel Ali Belaidi

POSITION & AFFILIATION

- Senior Research Fellow, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia

SPEAKER BIO

Dr. Abdel Ali Belaidi (Licentiate, Diplom, PhD) is a Senior Research Fellow at the Florey Institute of Neuroscience and Mental Health at the University of Melbourne, Australia. He is an expert in brain iron metabolism, ferroptosis, apolipoprotein E and apolipoprotein E receptors and their association with neurodegeneration with a special focus on mechanism of neurodegeneration in Alzheimer's disease. He was awarded a PhD in Biochemistry at the University of Cologne in Germany in 2011 and continued his postdoctoral research in Germany in the field of molybdenum and sulphur amino acid metabolism and associated neuronal cell death. His achievements in this area included deciphering the last steps of the biosynthesis of the molybdenum cofactor in humans, the identification of excitotoxicity as a new mechanism of neuronal cell death in molybdenum cofactor deficiency. His research resulted in significant novel biochemical and clinical findings and has been translated to the development of the first successful therapy of human molybdenum cofactor deficiency type A. Dr. Belaidi was awarded an international research fellowship from the German Research foundation (DFG) and moved to Australia in 2015 where he undertook research in neuroscience with a special focus on mechanism of neuronal cell death associated with iron metabolism and was the recipient of a research fellowship and multiple research grants from the Alzheimer's Association and philanthropic grants. Dr. Belaidi's current research laboratory is specialized in investigating cell metabolism and cell death mechanisms implicated in neurodegeneration with particular interest in understanding the cross talk between cysteine, lipid and iron metabolism in determining neuronal susceptibility to cell death. His achievements in this area included the identification of apolipoprotein E as an inhibitor of cell death resulting from lipid peroxidation/ferroptosis in neurons, thus suggesting ferroptosis as a possible mechanism of neurodegeneration in Alzheimer's disease.

Apolipoprotein E in Alzheimer's disease: intersection of lipid metabolism and neurodegeneration

Lipoproteins are best known for their role in the homeostatic control of plasma and tissue lipid content. Brain lipoproteins play also a crucial role in repair by redistributing lipids to regenerating axons and to Schwann cells during remyelination. Apolipoprotein E (apoE) is the best studied lipoprotein in the brain, it is highly expressed in the brain and constitutes the principal lipid transport system in cerebrospinal fluid. However, apoE is most well-known for its association with Alzheimer's disease. In particular, the presence of the apoE ε4 isoform (one of 3 isoforms in humans) confers pronounced risk for Alzheimer's disease. While most research has concentrated on the pathologies associated with apoE allelic variations, the mechanisms of action of apoE, its neurochemical effect in the brain and the contribution of apoE receptors to the neurodegeneration process remain poorly understood. Our clinical and laboratory studies support a role of apoE in protecting neurons from a cell death pathway termed ferroptosis. Ferroptosis is a cell death pathway characterized by the accumulation of lethal lipid hydroperoxides resulting from iron-dependent oxidation of polyunsaturated fatty acids. Here, we present data supporting a function of apoE in tandem with apolipoprotein E receptor 2 (APOER2) in protecting neurons from ferroptosis. These findings support ferroptosis as a mechanism of neurodegeneration in AD and provide new insight into the role of apoE and apoE receptors in the pathology of AD, which may help develop new diagnostic and therapeutic approaches that can be explored to slow down neurodegeneration in AD.

Delian Guo

POSITION & AFFILIATION

- Professor, Department of Radiation Oncology, The Ohio State University James Comprehensive Cancer Center and Medical School, USA

SPEAKER BIO

Prof. Deliang Guo is currently Urban and Shelley Meyer Professor in Cancer at the Department of Radiation Oncology, Ohio State University. His research is focused on understanding metabolic reprogramming mechanisms in cancer, in order to identify novel therapeutic targets and strategies. Dr. Guo received his PhD in molecular cell biology from Beijing Normal University in China, and went on to complete postdoctoral training in biomedical research at the University of California at Riverside, after which he moved to the University of California at Los Angeles as a postdoctoral fellow in Dr. Paul Mischel's lab, where he made the novel discovery that EGFR/PI3K/Akt signaling drives the reprogramming of lipid metabolism in glioblastoma to promote rapid tumor growth. Over the past decade, Dr. Guo has made groundbreaking discoveries linking glucose to lipid metabolism activation in cancer cells, and identifying large amounts of lipid droplets in aggressive brain tumor cells. Dr. Guo has received numerous awards for his research, and actively contributes to the lipid research community as a speaker and reviewer.

New Insights for SREBP Activation and Function: Intrinsic Connection with Glucose and Glutamine Metabolism

SREBPs (sterol regulatory element-binding proteins) are master transcriptional regulators that maintain lipid homeostasis in the human body. Dysregulation of SREBP activation results in various diseases, including cardiovascular diseases, fatty liver, and cancer. However, the regulatory mechanism for their activation remains incomplete understood. SREBPs are synthesized as inactive precursors and bind to SCAP (SREBP cleavage-activating protein) to form the SCAP/SREBP complex across the ER (endoplasmic reticulum) membrane. Their activation requires SCAP to transport from the ER to the Golgi for proteolytic cleavage. However, this process is suppressed by Insig (insulin-induced gene), an ER-resident protein, which binds to SCAP to retain the SCAP/SREBP complex in the ER. Thus, SCAP dissociation from Insig is the first crucial step in the SREBP activation process. Whereas how this step is triggered remains an intriguing question. Nobel laureates Brown & Goldstein's group previously identified that cholesterol or hydroxylyzed cholesterol (sterols) bind to SCAP or Insig to enhance their association, preventing SREBP activation. However, our recent study revealed that sterol reduction alone is not sufficient to activate SREBPs. We unveiled that glucose maintains SCAP stability by promoting its N-glycosylation. Meantime, glutamine uptake leads to the generation of ammonia that unexpectedly binds to SCAP, triggering the sequential conformational changes of SCAP and inducing its dissociation from Insig for subsequent SREBP activation. We further unveiled that SREBPs transcriptionally upregulate the expression of glutamine transporter ASCT2, resulting in heightened glutamine uptake and consumption. Enhanced glutamine consumption raises ammonia level, which further activates SREBPs, resulting in a feedforward loop to promote both glutamine consumption and lipogenesis for rapid tumor growth. In summary, our study uncovered that ammonia acts as the key activator for triggering SCAP/Insig dissociation and SREBP activation, unveiling the intrinsic connection between glucose, glutamine, and lipid metabolism. This connection is crucial for regulating metabolic homeostasis and promoting rapid tumor growth.

Thorsten Hornemann

POSITION & AFFILIATION

- Head of Research, Institute for Clinical Chemistry, University of Zurich, Switzerland

SPEAKER BIO

Dr. Thorsten Hornemann is professor for Lipidology at the Institute of Clinical Chemistry, University of Zurich where he heads the research and the lipidomics facility. His primary interest is the sphingolipid metabolism and sphingolipid-related pathologies. His contributions encompass the development of advanced mass spectrometry based analytical methods and metabolic labeling techniques, which have been crucial for investigating the structure, function, and metabolism of sphingolipids.
His work also involves establishing novel lipid metabolites and lipid-based metabolic signatures as diagnostic and prospective biomarkers in context of metabolic diseases. One of his major achievements includes the discovery and characterization of 1-deoxySphinglipids a class of atypical and neurotoxic sphingolipids, which have been pivotal in understanding the pathogenesis of the Hereditary Sensory and Autonomic Neuropathy Type 1 (HSAN1) and the diabetic sensory neuropathy.
His research has significantly contributed to our understanding on the role of sphingolipid in neurological condition, highlighting the importance of these lipids on nerve damage and pain perception pathways.

Sphingolipids in Neurological Diseases

Sphingolipids play a critical role in the pathophysiology of nerve disorders including leukodystrophies, the Hereditary Sensory and Autonomic Neuropathy Type 1 (HSAN1), Amyotrophic Lateral Sclerosis (ALS) and the Diabetic Sensory Neuropathy. They modulate cellular signaling pathways, which are crucial in maintaining neurological functions. Sphingolipids are essential for myelin sheath integrity and nerve function and influence pain perception pathways and neuronal inflammation, exacerbating neuropathic symptoms. Alterations in their metabolism can result in demyelination and axonal degeneration.
HSAN1, a rare genetic disorder, directly links to mutations affecting sphingolipid metabolism, which disrupts nerve structure and function, leading to sensory loss and autonomic dysfunction. HSAN1 is clinically similar to the diabetic sensory neuropathy, a common complication of diabetes and as well associated with imbalances in the sphingolipid metabolism, which contributes to nerve damage and pain.
Targeting sphingolipid metabolism therefore offers a promising therapeutic approach to stabilizing myelin and mitigating neurodegeneration in peripheral nerve disorders.

Chii-Shiarng Chen

POSITION & AFFILIATION

- Director General, National Museum of Marine Biology and Aquarium, Taiwan

SPEAKER BIO

Dr. Chii-Shiarng Chen obtained his PhD in Cell and Developmental Biology from University of Texas at Austin, USA. Being trained as both an organic chemist and cell biologist, his research focused on the synthesis and application of fluorescent probes for the Ca+2- and phospholipid-dependent Protein Kinase C (PKC) during the signal transduction pathway in eukaryotic cells. During the postdoctoral training under his mentor Dr. Richard Pagano in Department of Embryology, Carnegie Institute of Washington (Baltimore, MD) and then Mayo Clinic (Rochester, MN), Dr. Chen switched his interests to lipid trafficking regulation in endocytosis and secretory pathways. From 1997 to 2001, he was the group leader of cell biology laboratory in Molecular Probes (Eugene, OR). Afterward, he worked as a senior research scientist in Pacific Northwestern National Laboratory (Richland, WA). His work during these periods were published in variety of high rank journals including *Journal of Biological Chemistry, PNAS, The Lancet, Chemistry & Biology, Biophysical Journal, Free Radical Biology & Medicine* et al. Dr. Chen returned to Taiwan to join the research faculty in National Museum of Marine Biology and Aquarium (NMMBA) in 2004, and has been working on the regulation of marine endosymbiosis by integrating various cellular, proteomic and genetic approaches. He has published over 70 research articles on Coral-Dinoflagellate endosymbiosis and related topics in *Coral Reefs, Marine Biotechnology, Molecular Evolution, Journal of Experimental Biology, PLoS ONE,* and *Scientific Reports* et al. He is currently the Director General of the National Museum of Marine Biology & Aquarium (NMMBA).

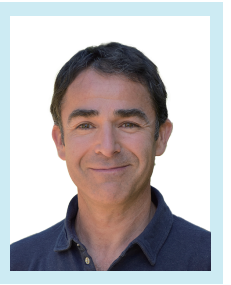
The critical role of host endoplasmic reticulum in lipid body biogenesis during the coral-*Symbiodinium* endosymbiosis

The coral-dinoflagellate endosymbiosis is generally defined as mutualistic because both partners benefit from the relationship. Despite decades of study the mechanism of this endosymbiosis has not been elucidated. Lipid bodies (LB) in the gastroderm of hermatypic corals have been identified as the key organelle involved in the endosymbiotic regulation between the host corals and their intracellular symbionts. Based on the dynamic LB formation in coral gastrodermal cells, recent studies have shown that this symbiosis is diel-regulated and an active and superior role of the coral host in symbiosis regulation was highlighted. The lipidome-scale data reveal that the dynamic, light-driven metabolism of lipogenesis in LBs is significantly regulated by coral hosts. Furthermore, proteomic and ultrastructural observations seem to suggest that host organelles, such as the endoplasmic reticulum (ER), are involved in LB biogenesis. According to the current consensus model of LB formation, neutral lipids are synthesized between the leaflets of the ER membranes and mature LBs later bud from the ER to form independent organelles containing a monolayer of phospholipids and a core of neutral lipids with unique fatty acid compositions. Nevertheless, the exact mechanism of LB biogenesis during the endosymbiosis is not elucidated, although the ER is likely involved. Herein, the diel rhythmicity of LB biogenesis was correlated with the expression of ER-specific chaperone BiP (immunoglobulin heavy chain binding protein or BiP; also termed 78-kDa glucose-regulated protein, GRP78). The BiP expression increased concurrently with LBs, suggesting the active involvement of chaperoning reaction for anti-stress and protein repair inside the host ER. Recently, transcriptomic investigation of the diel cycle in the stony coral *Euphyllia glabrescens* was performed to examine the underling genetic network of LB biogenesis. In hosts and symbionts, genes deferentially expressed during the diel cycle (i.e. DEGs, log2(FPKM ratio) >1 or <-1) are 43.63% and 16.59%, respectively. This also indicates that coral hosts, but not symbionts, are more responsive to the diel cycle. These data not only provide gene candidates responsible for LB biogenesis and endosymbiosis regulation, but also further confirm the pivotal role of ER in hosts.

Nicolas Vitale

POSITION & AFFILIATION

- Institut for Cellular and Molecular Integrated Neurosciences, France



SPEAKER BIO

Dr. Nicolas Vitale obtained his PhD in 1994 by working deciphering the role of trimeric G protein on regulated exocytosis at the University of Strasbourg (France). He then studied monomeric GTPases during a 4-year postdoc at the NIH (USA). Nicolas Vitale obtained a researcher position at Neurosciences Institute (CNRS) in Strasbourg. Since then, his team aims to decipher the molecular mechanisms of exocytosis and endocytosis. In particular, his research focuses on the role of lipids in hormone and neurotransmitter release processes under both healthy and pathological conditions. Over the years, his work has unraveled a pleiotropic role of phosphatidic acid produced by phospholipase D during neurosecretion. To gain a more detailed comprehension of the mechanics explaining these multiple functions and to overcome the current limitations to study the dynamics of phospholipids, he recently developed modern tools to study phosphatidic acid at the "speed of light".

Illuminating neurosecretion: Optogenetic and click chemistry novel tools highlight the multiple roles of phosphatidic acid in neurotransmitter release

The orchestrated release of neurotransmitters or hormones by secretory cells involves many vesicular trafficking steps for efficient and rapid release. In addition to key proteins, the contribution of lipids in these various steps along the secretory pathway has been recently postulated. Among them, phosphatidic acid (PA), the simplest glycerophospholipid, has been proposed to play pivotal roles in key trafficking steps, especially in membrane fusion and fission events, where lipid remodeling is deemed crucial.
For instance, using genetic knockdown, pharmacological inhibition of PA-producing enzymes, and PA sensors, we have highlighted the diverse contribution of this phospholipid across multiple stages of neurosecretion. Furthermore, lipidomic analysis of fractionated membranes has revealed the widespread presence of PA in numerous subcellular compartments and its active modulation during cellular stimulation. This sheds light on the complexity of PA signaling, with the existence of different PA pools defined not only in space, but also in time. However, establishing a functional link between these pools and the multiple functions attributed to PA has remained impossible using currently available tools. Hence, to overcome both spatial and temporal limitations, we developed a novel optogenetic strategy targeting lipid metabolism to specific organelles and new PA clickable PA analogues.
Hence, using light sensible PA metabolism enzymes to induce recruitment at specific subcellular membranes, we achieved by the minute modulation of PA levels within specific compartments. This precise control of PA levels coupled with confocal imaging to monitor exocytic sites enabled us, for the first time, to establish insights into the distinct pools of PA involved in specific steps of the secretory pathway. Furthermore, to preserve the biological properties of PA synthetic analogues, we developed a novel strategy for the synthesis of azide-based analogues allowing specific fatty acyl chain positioning. After functional validation of mono and poly-unsaturated forms of PA analogues in bovine chromaffin cells, we characterized their functional interactome during neurosecretion leading to the identification of known PA-interactors involved in exocytosis and many additional potential novel interactors. Altogether, these results validate the versality of these tools to study the biological activities of PA and could be extended to other glycerophospholipids.

Jianmin Cui

POSITION & AFFILIATION

- Professor, Department of Biomedical Engineering, Washington University in St. Louis, USA

SPEAKER BIO

Prof. Jianmin Cui is Professor on the Spencer T. Olin Endowment at Washington University in St. Louis, in the Department of Biomedical Engineering. He received Ph.D. in Physiology and Biophysics from State University of New York at Stony Brook and a post-doctoral training at Stanford University. He was an assistant professor of Biomedical Engineering at Case Western Reserve University before moving to St. Louis in 2004. His research interests are on membrane permeation to ions, drugs and genes, including 1) molecular mechanisms of ion channel function, molecular/cellular mechanism of disease-associated ion channel mutations, and small molecules that modulate ion channel function; and 2) ultrasound-mediated ion channel activation and drug/gene delivery. Prof. Cui received Established Investigator Award from the American Heart Association and is a fellow of the American Institute for Medical and Biological Engineering (AIMBE).

PIP_2 dependent activation of a cardiac K^+ channel

Voltage-gated ion channels generate dynamic ionic currents that are vital to the physiological functions of many tissues. These proteins contain separate voltage-sensing domains, which detect changes in transmembrane voltage, and pore domains, which conduct ions. Coupling of voltage sensing and pore opening is critical to the channel function and has been modeled as a protein-protein interaction between the two domains. I_{Ks} is a K^+ channel that is important to control the ventricular action potential duration and the heart rate. It is formed by the voltage gated K^+ channel KCNQ1 and regulatory subunit KCNE1. This talk shows that coupling in KCNQ1 channels requires the lipid phosphatidylinositol 4,5-bisphosphate (PIP2). Voltage-sensing domain activation failed to open the pore in the absence of PIP2. We identified a critical site for PIP_2-dependent coupling at the interface between the voltage-sensing domain and the pore domain, which is conserved in other KCNQ channels. Results also demonstrate that PIP2 binding to this site influences the interaction between KCNQ1 and KCNE1 to regulate the current amplitude of I_{Ks}.

Chih-Yung Chiu

POSITION & AFFILIATION

- Department of Pediatrics, Chang Gung Memorial Hospital at Linkou, and Chang Gung University, Taoyuan, Taiwan
- Clinical Metabolomics Core Laboratory, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan

SPEAKER BIO

Dr. Chih-Yung Chiu is a pediatric pulmonologist who completed his Ph.D. training at Imperial College London and is currently working at Linkou Chang Gung Memorial Hospital with over 20 years of experience in pediatric respiratory care. He is a co-principal investigator of a birth cohort study in the Prediction of Allergies in Taiwanese Children (PATCH) joint study initiating since 2007 to investigate the epidemiology and predictive factors of asthma and allergies in Taiwanese children. Many clinical studies regarding this birth cohort have been done and published. In addition, his research interests have long involved in genomics, proteomics, microbiomics, metabolomics, and biological sciences for pediatric diseases. The major study field of his research is now to apply metabolomics techniques by using 1H-NMR spectroscopy for further understanding the development of complex pediatric atopic diseases such as asthma and atopic dermatitis to provide protective or therapeutic strategies in clinics.
Dr. Chiu specializes in the study of gut microbiota and its impact on childhood asthma and allergies. Following the analysis of biomarkers related to allergic asthma, Dr. Chiu has advanced to investigate the correlation between metabolites and the occurrence of asthma in children using metabolomics. Further studies identified metabolites associated with the occurrence of asthma. Moreover, these metabolites were found to be closely related to gut microbiota within the body. Subsequent analysis of the relationship between respiratory tract and gut microbiota and allergic respiratory diseases revealed a significant correlation between respiratory and gut microbiota imbalance and dust mite sensitization, which contributes to allergic rhinitis and asthma. Beyond his clinical practice, Dr. Chiu is actively involved in research collaborations aimed at developing innovative solutions for respiratory health in children.

Short-Chain Fatty Acids and Lipid Dysregulation in Childhood Asthma

Asthma is a heterogeneous condition caused by the combined effects of multiple genetic factors interacting with environmental factors. Numerous risk factors for asthma and potential biomarkers such as vitamin D levels, allergen sensitization and serum IgE levels for asthma prediction have been studied widely. Metabolomics enables the discovery of small molecule metabolites by revealing any specific biomarker in human disease or certain metabolism pattern changes after genetic and environmental intervention. Several studies have been reported to reveal altered metabolic pathways associated with asthma pathogenesis. Nuclear Magnetic Resonance Spectroscopy (NMR) is extensively used in metabolomics, focusing on studying metabolites within organisms and their relation to health and disease. In our work, urinary metabolomic profiling reveals the evidence of microbe-environment interactions over time in the development of asthma in early childhood. Small molecule metabolites, 1-methylnicotinamide and allantoin, are related to house dust mite sensitization and could potentially be targets as protective against childhood asthma. On the other hand, gut microbial dysbiosis is increasingly identified with allergic diseases in children. A recent study in our laboratory reveals that the reduction of specific gut microbes in the phylum Firmicutes related to an increase in the fecal amino acids may contribute to childhood asthma. Furthermore, fecal butyrate, one of the three most important short-chain fatty acids, plays an important role in modulating allergic responses to allergens related to asthma. Simultaneously, the AbsoluteIDQ Q500 kit (Biocrates Life Sciences AG in Innsbruck, Austria) was also employed for targeted analysis, capable of assessing up to 630 metabolites spanning 26 biochemical classes. Sixteen aqueous metabolites and eight lipid metabolites were identified to be significantly associated with asthma. Among them, one lipid metabolite, diacylglycerol DG(16:0/16:0), which is associated with glycerolipid metabolism, was strongly linked to asthma. These findings strongly indicate the crucial roles of gut microbiome-derived short-chain fatty acids and lipid dysregulation in childhood asthma.

Junken Aoki

POSITION & AFFILIATION

- Professor, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan

SPEAKER BIO

Prof. Junken Aoki received his PhD in Pharmaceutical Sciences from the University of Tokyo, and has previously served as a researcher at the Tokyo Metropolitan Institute of Medical Science, as well as Research Professor at the Graduate School of Pharmaceutical Sciences of Tohoku University. He is currently Full Professor at the Graduate School of Pharmaceutical Sciences of the University of Tokyo. Prof. Aoki is renowned for his in-depth study of phospholipid biosynthesis, as well lysophospholipid mediators and their role in immune signaling. He has also developed many novel techniques for the study of lipid biology, including most recently publishing a method of visualizing biosynthesis by use of functional mass spectrometry imaging. He also participated in the research that identified aqueous alteration signatures in samples obtained from the Ryugu asteroid by the Hayabusa2 spacecraft. Prof. Aoki's work on phospholipids has deeply impacted both basic and clinical research, and he is a highly sought-after speaker at major lipid conferences.

Autocrine/paracrine lysophosphatidylserine signaling suppresses B cell aggregation and ectopic lymphoid tissue formation.

Accumulating evidence has suggested that $G\alpha_{13}$ signaling negatively regulates B cell functions. For example, $G\alpha_{13}$ deficiency resulted in an excessive proliferation of germinal center (GC) B cells, thus increasing the incidence of lymphoma. Body fluid-derived S1P signaling via Ga13-coupling $S1P_2$ receptor inhibits the outgrowth and migration of GC B cells and confines them within the GC, accounting partly for the phenotypes of $G\alpha_{13}$ deficiency. B cells express other $G\alpha_{13}$-coupled GPCRs, such as receptors for lysophosphatidylserine (LysoPS), which may regulate other B cell functions. We report here that LysoPS is produced upon B cell activation and suppresses its adhesive properties via its receptors LPS_2 and LPS_{2L}, thereby suppressing the formation of germinal center and tertiary lymphoid structures in mice. B cell activation markedly increased the cell-associated LysoPS, which was found to activate LPS_2 and LPS_{2L} expressed on HEK293 cells in a cell contact-dependent manner. When activated *in vitro*, B cells from $LPS_{2/2L}$ double knockout (DKO) mice formed larger cell aggregates than those from wild-type mice. In an induced GC B culture system, which resembles the B cell response in GC, $LPS_{2/2L}$ negatively regulated B cell adhesion and proliferation. In a T cell-dependent antigen immunization model, DKO mice showed lymphadenopathy with increased B cell proliferation and earlier appearance of the germinal center. In a mouse asthma model, DKO mice showed worsening asthma pathology, a marked increase in IgE levels, and numerous ectopic tertiary lymphoid structures with marked B cell aggregates in the lung. These results indicate that LysoPS produced upon B cell activation acts in an autocrine/paracrine fashion to suppress the formation of GC and tertiary lymphoid structures by inhibiting B-B or B-T interaction through $G\alpha_{13}$ signaling.

Wendy Wen-Li Hsu

POSITION & AFFILIATION

- Assistant Investigator, National Center for Geriatrics and Welfare Research, National Health Research Institutes

SPEAKER BIO

Dr. Wendy Wen-Li Hsu is an Assistant Investigator at the National Center for Geriatrics and Welfare Research, National Health Research Institutes, Taiwan. Her research focuses on the role of lipids in aging, disease, and cell function, particularly in the context of cardiovascular diseases, cancer, neurodevelopment and hair regeneration.

Dr. Hsu has extensive expertise in lipidomics, cell biology, and translational medicine. She holds a doctor degree from Waseda University, Japan, and has published numerous articles in prestigious journals like *Aging Cell*, *Life Sciences* and *Prostaglandins*, *Leukotrienes* and *Essential Fatty Acids*. She is also the inventor on several patents related to lipid-based approaches for neurodevelopmental disorders and hair regeneration.

Dr. Hsu is a passionate advocate for the advancement of lipid research and its potential to address critical health challenges. She is actively involved in several professional societies, including the Taiwan Society For Stem Cell Research, Taiwanese Society for Investigative Dermatology and Taiwan Association for Aerosol Research.

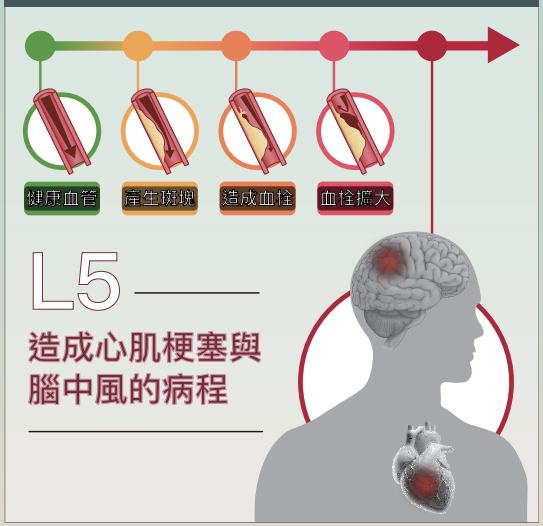
Specific Human Breast Milk Lipids Shape Neurobehavioral Development and Lifespan: A Study in Infants and *Caenorhabditis elegans*

The crucial role of human breast milk (HBM) lipids in infant neurodevelopment has been recognized, but a comprehensive understanding of the specific lipid components and their impact on cognitive development remains elusive. To investigate the influence of specific HBM lipids on infant neurodevelopment, we conducted a study combining quadrupole-time of flight mass spectrometry (LC/MS/MS, ESI-qTOF) for HBM lipid analysis with data from the Bayley-III cohort (a standardized developmental assessment of infants and toddlers, n=100). We clarified "good HBM lipid" and "bad HBM lipid" for infant neurodevelopment across the five domains of the Bayley-III, which include cognition, motor, social-emotional, and adaptive behavior. Interestingly, our study found that there was no significant correlation between the neurodevelopment of infants and the levels of omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are known to be crucial for central nervous system development. To further explore the impact of HBM lipids on neurodevelopment and lifespan, we employed *Caenorhabditis elegans (C. elegans)* as a model organism. Worms were exclusively supplemented with HBM lipids during their larval stage. Supplementation with "good HBM lipid" resulted in enhanced locomotor and foraging abilities, as well as increased expression of antioxidant defense genes. However, supplementation with "bad HBM lipid" led to impaired neurobehavioral development and a shortened lifespan, which were attributed to increased levels of reactive oxygen species (ROS) accumulation. Our study elucidated the biological functions and underlying mechanisms of HBM lipids in regulating neurodevelopment. The findings potentially demonstrate an adverse impact on neurobehavioral development across the lifespan, extending into adulthood.

Note

陰電性低密度脂蛋白相對定量分析 (L5%)

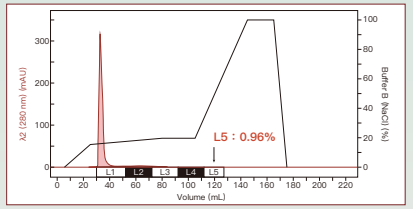
L5 % 與心血管疾病風險



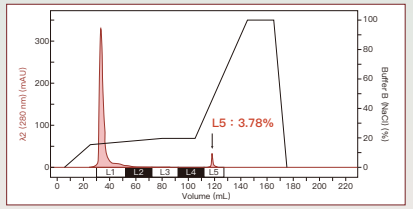
US & Global 專利審核中

L5 是最具陰電性並且有害健康的 LDL 亞群。藉由我們的專利技術，可以迅速並準確的分析出 LDL 中有多少百分比是 L5 (L5%)。

低風險

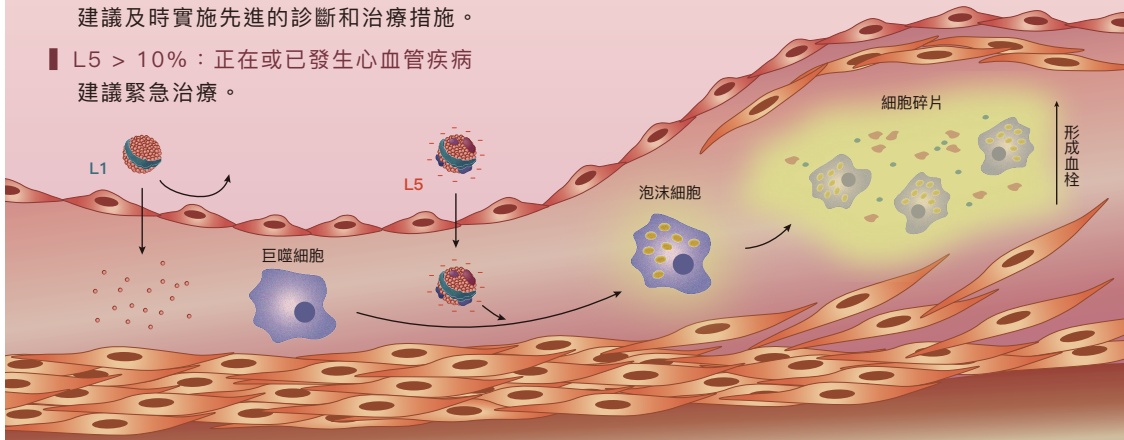


中高風險



L5 陰電性脂蛋白是低密度脂蛋白（LDL）的一個亞群，是真正的「壞膽固醇」。正常的 LDL 可被細胞分解利用，但 L5 攜帶的有害脂質會引起血管發炎反應，造成血管內皮細胞損傷；損傷的訊號吸引巨噬細胞形成粥狀斑塊囤積於血管壁內；損傷的訊號也會使血小板凝結形成血栓，阻塞血流通過，使供血量減少。若發生在心臟的冠狀動脈會造成心肌梗塞；若發生在腦血管則會造成腦中風。

- L5 < 2%：低風險
  若沒有心血管疾病與糖尿病病史，且 LDL-C ≦ 190 mg/dL，L5% < 2% 代表低風險。
- L5 ~ 2–5%：中高風險
  建議重新評估患者的整體危險因素，並在必要時提供適當的診斷檢查和治療。
- L5 > 5%：高風險
  建議進行進階診斷檢查。如跑步機測試、負荷超音波心動圖，必要時進行冠狀動脈造影。
- L5 > 8%：即將或正在發生心血管疾病
  建議及時實施先進的診斷和治療措施。
- L5 > 10%：正在或已發生心血管疾病
  建議緊急治療。



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- Microbial metabolites regulate social novelty via CaMKII neurons in the BNST. *Brain, Behavior, and Immunity.* (2023)
- Distinct protective effects of a novel *Akkermansia* sp. BCRC 18949 against DSS-Induced colitis in mice. *Journal of Functional Foods.* (2024)

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- Metabolomics facilitates differential diagnosis in inherited retinal degenerations via serum metabolite profiles. *Nature Communications.* (2024)

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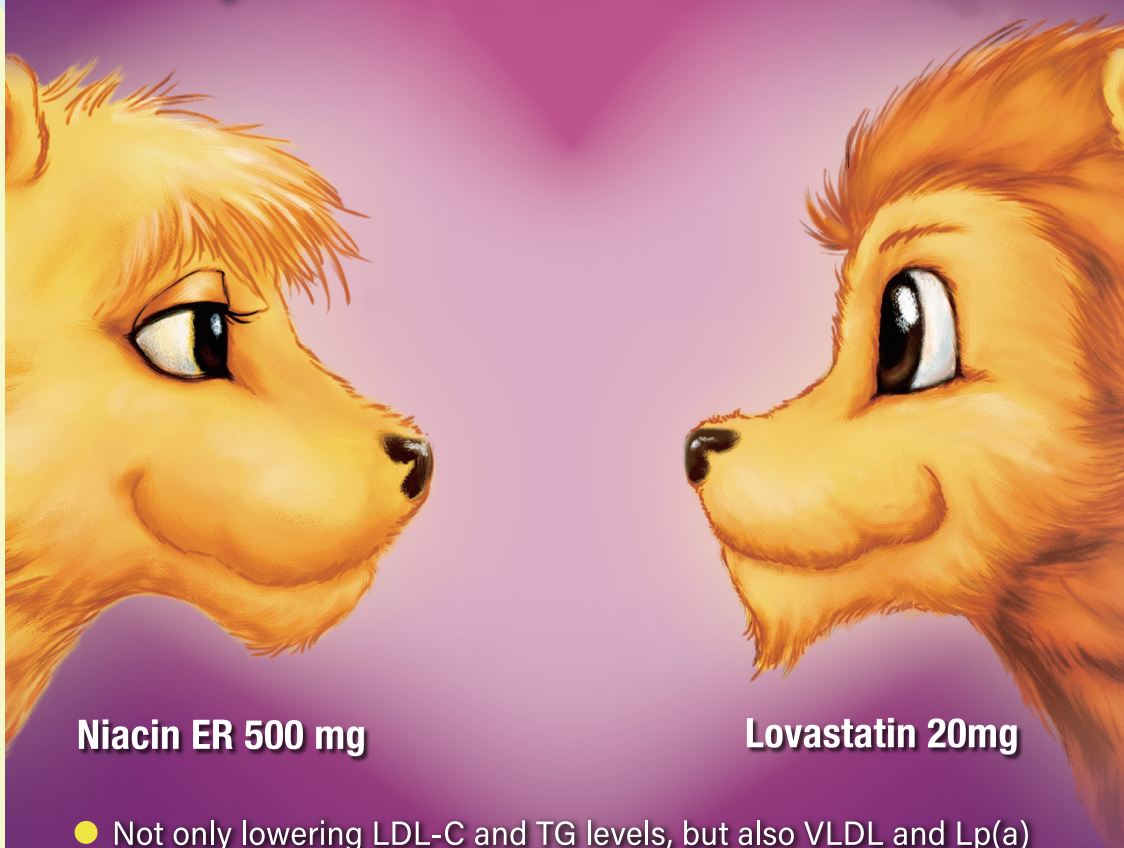

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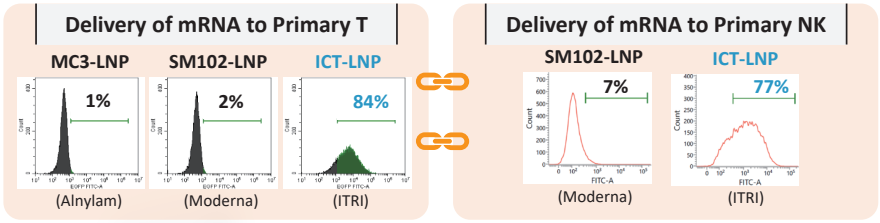
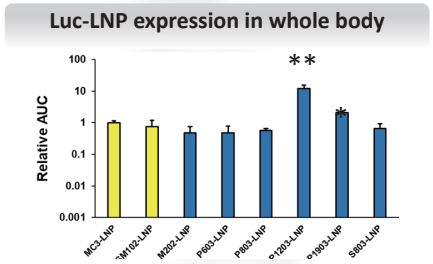
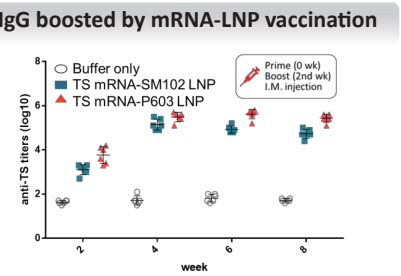
- ICT-LNPs are well tolerated based on cell viability (>95%) and proliferation data.



- *In-vivo* transfection efficiency in mice non-inferior to commercial LNP formulations.



AUC: hr x p/s 2-24hr，Total Area ± SEM, n=3~5
*: $p < 0.01$, **: $p < 0.001$; compared to MC3-LNP by one-way ANOVA followed by Dunnett's test



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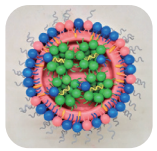

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S1-01 DESI Mass Spectrometry Imaging for Spatial Lipidomics Analysis of In Vitro 3D Cell Models

Chen-Yu Chang[1], Cheng-Hung Yang[2], Mei-Ling Cheng[1, 2]

[1]Graduate Institute of Biomedical Sciences, Chang Gung University
[2]Department of Biomedical Sciences, Chang Gung University

Lipids play a crucial role in tumor growth, not only providing energy for tumor cells but also participating in cell membrane construction and signal transduction, significantly influencing tumor cell proliferation, survival, and interaction with the microenvironment. Since these lipid functions are particularly prominent in a three-dimensional (3D) environment, traditional monolayer cell cultures are insufficient to fully simulate the real tumor microenvironment. To more accurately mimic in vivo biological processes, cell culture studies have evolved from traditional monolayer cultures to three-dimensional (3D) systems. By employing 3D models that replicate tissue structure and complexity, researchers can better understand the heterogeneity, architecture, and functionality of cancer cells during growth, thereby narrowing the gap between in vitro and in vivo systems. However, conventional mass spectrometry preprocessing often falls short in detecting metabolic changes in specific regions of samples. To address this, we utilized Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI), a technique that directly captures chemical information from various surfaces without preprocessing, to generate 2D mass spectra from cryosectioned spheroids. This approach allows for the analysis of metabolic changes in a 3D environment. In our study, HepG2 cells were cultured into spheroids and cryosectioned for DESI-MSI analysis to investigate lipid distribution differences between the inner and outer cell layers. Preliminary findings indicate that outer cells contain higher levels of triglycerides, diglycerides, monoglycerides, and cholesterol esters, while inner cells exhibit increased levels of phosphatidylcholine and phosphatidylethanolamine. Furthermore, phosphatidylcholine showed a distinct distribution pattern with varying total carbon numbers at a fixed level of unsaturation. Through imaging mass spectrometry, we established a lipidomic database of spheroids, providing insights into the small molecule changes in cancer cells during in vivo growth, ultimately aiming to bridge the gap between in vitro cell experiments and clinical drug applications.

"This abstract has been previously published at [The 20th Taiwan Society for Mass Spectrometry Annual Conference] held at [Tainan, Taiwan] on [June 26-28, 2024]."

Short Talks

S1-02 Development of a quantitative method for phospholipid positional isomers in the brain

Seiya Tanaka[1], Kana Fujiwara[1], Tomoyuki Koyama[1], Kazuaki Yoshinaga[2], Naohiro Gotoh[1]

[1] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan.
[1] Faculty of Food and Agricultural Sciences, Fukushima University, Fukushima, Japan.

Introduction

Phosphatidylcholine (PC), a key phospholipid, contains 2 fatty acids that can be bound at the *sn-1* and *sn-2* positions, resulting in positional isomers when different fatty acids are attached. It has been reported that PC positional isomers exist specifically in the brain. However, there is no established method for identifying phospholipid molecular species and quantifying individual isomers using authentic standards of each PC isomer. In this study, we aimed to prepare authentic analytical standards for PC positional isomers through chemical synthesis and preparative purification, and to quantify PC isomers in mouse brain.

Materials and Methods

PC isomers contain docosahexaenoic acid (DHA, 22:6) and palmitic acid (16:0) attached at the *sn-1* and *sn-2* positions was prepared by chemical synthesis. The mixture of PC isomers was purified by the preparative HPLC, and each fraction of PC isomers was quantified using quantitative NMR (qNMR). Standard solutions of PC(22:6/16:0) and PC(16:0/22:6) were analyzed using liquid chromatography-tandem mass spectrometry (LC/MS/MS) to generate calibration curves of PC positional isomers and quantify PC isomers in the mouse brain.

Results and Discussion

The ionization efficiency of PC(22:6/16:0) was 2.32 times higher than that of PC(16:0/22:6), indicating that the ionization efficiency depends on the binding position of the fatty acid such as docosahexaenoic acid and palmitic acid. It was confirmed that the development of this analytical method can contribute to the accurate quantification of the amounts of PC isomers in the brain.

References

1) K. Fujiwara, *S. Tanaka, T. Koyama, K. Yoshinaga, N. Gotoh, Evaluation of the ionization efficiency in phosphatidylcholine positional isomers with docosahexaenoic acid bound to the sn-1 or sn-2 position. *J. Chrom. B.*, under review.

S1-03 Development of ultraperformance liquid chromatography (UPLC) based methodology for assessing electronegative low-density lipoproteins in apolipoprotein E knockout mice

Hsiu-Chuan Chan[1], Liang- Yin Ke[2], Vinoth Kumar Ponnusamy[3*], Hua-Chen Chan[4*]

[1.] PhD program in Life Sciences, College of Life Sciences, Kaohsiung Medical University, Taiwan.
[2.] Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Taiwan.
[3.] Department of Medicinal and Applied Chemistry, Kaohsiung Medical University
[4.] Department of Medical Laboratory Science, College of Medicine, I-Shou University, Taiwan.

The elevation of electronegative low-density lipoproteins (LDL) plays a pivotal role in cardiovascular disease progression. Fast protein liquid chromatography-based electronegative lipoproteins quantification is time consuming and resource-intensive, demanding twenty milliliters of whole blood. Despite the common use of the apolipoprotein E knockout (*apoE-/-*) mouse model in cardiovascular research, evidence supporting the role of electronegative LDL in early atherosclerotic lesion development is lacking due to challenges in analyzing electronegative LDL levels in individual mice. To address these challenges, we aimed to enhance current methodologies by developing an ultraperformance liquid chromatography (UPLC)-based approach for quantifying electronegative lipoproteins. In our study, male C57BL/6 and *apoE-/-* mice, aged six weeks, were subjected to either a normal-chow diet or a high-fat diet for eight weeks. Submandibular blood samples were collected in EDTA tubes using a 20G needle on days 0 and 28. Lipid parameter measurements were conducted at the National Laboratory Animal Center. LDL from mice was isolated using the LipoSep kit and dialyzed against a 20 mM Tris buffer containing 0.5 mM EDTA. Electronegative LDL levels were quantified using an Ultra-performance liquid chromatography system. Our findings revealed a significant elevation in electronegative LDL levels in *apoE-/-* mice at six weeks of age, despite the absence of significant atherosclerotic lesions compared to C57BL/6 mice. Notably, feeding mice with a high-fat diet led to a dramatic increase in electronegative LDL levels, particularly in *apoE-/-* mice. Previous assessments of electronegative LDL levels in mouse models were limited to endpoint measurements, impeding the monitoring of dynamic changes. However, our novel UPLC-based methodology enables real-time detection of electronegative LDL in vivo within 24 hours, compared to the conventional 14-day process. Additionally, it requires only 0.5~0.7 mL of whole blood per mouse. This technology holds promise in establishing a platform for studying pathological mechanisms and developing therapeutic interventions.

S1-04 Extracting Potential Lipid Functions from Biomedical Literature Using Natural Language Processing Techniques

Wen-Jen Lin[1,2], Chia-Hsin Liu[3], Pei-Chun Shen[3], Hsiu Cheng Liu[3], Meng-Hsin Tsai[3], Wei-Chung Cheng[2,3,4]

[1]School of Medicine, China Medical University, Taichung 404333, Taiwan
[2]Graduate Institute of Biomedical Science, China Medical University, Taichung 404333, Taiwan
[3]Cancer Biology and Precision Therapeutics Center, China Medical University, Taichung 406040, Taiwan
[4]The Ph.D. program for Cancer Biology and Drug Discovery, China Medical University and Academia Sinica, Taichung 404328, Taiwan

Lipids are integral to a multitude of biological processes and pathologies. Notwithstanding their significance, there is a notable dearth of comprehensive databases that provide detailed information on lipid-related functions based on curated evidence. To address this deficiency, a new database has been developed as the inaugural repository of lipid functions, providing sentence-level evidence that links lipids to specific phenotypes and biological functions. This database employs natural language processing techniques to extract potential lipid functions from biomedical literature, with accuracy and reliability ensured through manual curation by a team of four domain experts. The resource employs classification systems for lipids, biological functions, and phenotypes, which are utilized for named entity recognition. Sentence-level evidence is then extracted to establish connections between lipids, biological processes, and potential diseases. By integrating these classification systems with a vast collection of sentence-level evidence, the database provides an overview of the associations between lipids, phenotypes, and biological functions through concise visualizations. Ultimately, this database reveals the complex relationships between lipids and biological mechanisms, highlighting their significant influence on physiological processes.

S1-05 Immune Function in Colon Cancer: Insights from Comparative Lipidomics

Karim Pérez-Romero[1], Ramón M Rodríguez[1], Albert Maimó-Barceló[1], Marco A. Martínez[2], Cristina Huergo Baños[3], José Andrés Fernández[3], Gwendolyn Barceló-Coblijn[1]

[1]Health Research Institute of the Balearic Islands (IdISBa); Palma, Spain;
[2]Anatomical Pathology Dpt., University Hospital Son Espases (HUSE), Palma, Spain;
[3]University of the Basque Country, Leioa, Spain;
[4]Gastroenterology Dpt., HUSE,
[5]Gastroenterology and General Surgery Dpt., HUSE.

Introduction. The tumor microenvironment (TME) is intimately related to tumor growth and metastasis. The understanding of the intricate network driving TME heterogeneity is essential for developing new tools for patient stratification and identifying biomarkers for immunotherapy. We aim to address this complexity by characterizing the lipidome of the immune compartment using Spatially Resolved techniques, in particular, matrix assisted laser desorption ionization (MALDI)- mass spectrometry imaging (MSI).

Methods. 10 patients with colon cancer (CC) and 7 healthy controls (HC) were recruited from the Gastroenterology Dpt. of the University Hospital Son Espases. Circulating immune cells were isolated from peripheral blood by *FACS*. For ex vivo experiments, we used buffy coats of 5 HC. Sorted cells were activated *ex vivo* using cell-type specific protocols. Last, 5 patients with CC undergoing colon surgical resection were enrolled from the *General Surgery Dpt*. to study immune infiltrates. Tumor-infiltrating lymphocytes (B Cells, $CD4^+$ and $CD8^+$ T Cells), tumor-associated macrophages (M1 and M2-like Mo) and tumor-associated neutrophils (TAN) were sorted after tumor enzymatic disaggregation. All isolated cells were applied onto poly-L-lysine-coated glass slides and analyzed using MALDI-IMS. Last, the spatial lipidome of CC tumor sections was imaged by MALDI-IMS.
Results and Discussion. The method developed enabled us to establish the lipidome on a relatively low number of isolated cells (~10^4 cells). The analysis showed a distinctive profile of the main membrane lipid species (phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and PE-plasmalogen (PE P) species), confirming the specificity of the immune lipid fingerprint. The comparison between HC and CC patients circulating populations revealed a significant impact of the clinical condition on arachidonic acid-containing phospholipids. Changes in phospholipid composition upon *ex vivo* activation were consistent with those observed in circulating immune cells of patients with CC, which were even more exacerbated in isolated tumor-infiltrating cells. Finally, we identified tumor lymphocyte infiltrates directly on the colon tumor section based on their specific lipid composition. Importantly, the lipid profiles of isolated infiltrated immune cell subsets were virtually identical to those of the tissue.
Conclusions. The lipid fingerprint is unique to each of the immune cell populations studied and sensitive to any alteration in the context of CC. These changes patients with CC mirrored those established upon the *ex vivo* activation of circulating immune cells and, notably, of tumor-infiltrating immune cells. The profiles of isolated cells matched those obtained from imaging tumor sections. All together, the cell-type specificity and metabolic sensitivity of the lipidome highlights the value of MALDI-IMS in the context of health research.

S1-06 Lipid Droplets Probed by Darkfield Images and Raman Spectroscopy

Huai-Shuo Chang[1], Wan-Ping Chan[1], Yu-Long Lin[1], Pin-I Chou[2], Xiang-Kai Huang[2], Tung-Hsuan Kang[2], Shih-En Huang[3,4], I-Chen Peng[2#], Yu-Min Yeh[3,4§], Shiuan-Yeh Chen[1*]

[1] Department of Photonics, National Cheng Kung University, Tainan, Taiwan
[2] Department of Life Sciences, National Cheng Kung University, Tainan
[3] Department of Oncology, National Cheng Kung University Hospital, Tainan,Taiwan
[4] College of Medicine, National Cheng Kung University, Tainan, Taiwan
#ipeng@ncku.edu.tw,§ s98031083@mail.ncku.edu.tw, *sychen72@nkcu.edu.tw

A lipid droplet is an organelle mainly containing triglyceride (TG) and sterol surrounded by a phospholipid monolayer. Cancer cells can re-program their metabolism to promote the growth and increase the resistance to chemotherapies. Through increasing the representation of fatty acid synthase (FAS) [1] or cluster of differentiation 36 (CD36) [2], the increasing amount of lipid droplets can be observed in cancer cells. Therefore, to investigate the quantity and composition of the lipid droplets may help to understand the lipid metabolism in cancer cells. The Oil red O (ORO) staining or fluorophore labelled antibodies are conventional methods to quantify the lipid in a cell. However, these methods may suffer from inaccuracy and photobleaching. Here, the darkfield microscopy is combined with Raman spectroscopy to identify the lipid droplets in a cancer cell.
Through tilted white light excitation, darkfield images from lipid droplets in a cell can be obtained due to the refractive index contrast. To boost the formation of lipid droplets, the liver cancer cells (HepG2) and colorectal cancer cells (Colo205, HCT116) are treated with cisplatin (CPT) and 5-FU, respectively. The cells attached to a cover slide are directly observed by a custom dark-field microscope equipped with Raman acquisition. The 632 nm laser spot is focused on the lipid droplets to obtain the Raman spectra and Raman images.
In the dark-field images, bright dots in the cells results from the scattering of the dots due to the contrast of refractive index. For HepG2, these bright spots also show clear lipid signatures in their Raman images collecting from 2848 cm^{-1} to 2864 cm^{-1} , which corresponds to the CH_2 vibration of lipid. The similar correlation can be found in Colo205. For HCT116, this correlation is less obvious since their dots is much smaller than HepG2. In addition, the line-scan of Raman spectroscopy is also utilized to accelerate the scanning speed of the Raman images. These results indicate that at least in HepG2 and Colo205, combining dark-field images and Raman spectroscopy may facilitate the quantification of lipid droplets and clarify the relationship between external stimulus (such as chemotherapies) and lipid droplets.

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S1-07 Lipid Effects of Coexposure to Bisphenol A and Alcohol in a Cell Model

Yi-Chia Chen[1, 2], Yi-Shiou Chiou[2], Sheng-Han Lee[3*]

[1]School of pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.
[2]Master Degree Program in Toxicology, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.
[3]School of Medicine, College of Medicine, National Sun Yat-Sen University, Kaohsiung, Taiwan.
*Corresponding author and presenting author

The toxic effects of individual bisphenol A (BPA) and alcohol (ALC) exposures were examined in previous studies. However, the coexposure effects and their related molecular mechanisms were still unclear. In this study, we examined the toxic effects in a cell model, especially in the lipid levels, using a lipidomic approach. An equal amount of HepG2 cells (n= 6) were conducted on individual exposures, including BPA only, ALC only, and BPA+ALC, whereas the control group received the same volume of vehicles (DMSO) without toxicants.

After nine days of exposure, the cells were collected for a liquid chromatography-mass spectrometry (LC-MS) based lipidomics. The partial least squares-discriminant analysis (PLS-DA) models showed that the whole lipid effects of ALC exposure and BPA+ALC coexposure were similar and then discriminated with BPA exposure and control exposure along with the componet1 axis. 39 critical lipids, including 16 sphingomyelins (SM), 20 phosphatidylcholines (PCs), and 3 lyso-PCs, were suggested among the group differences. These lipid alterations may play roles in maintaining membrane intact, energy homeostasis, and cellular signaling pathways. Our preliminary results suggest that the perturbed lipid effects were more evident in the ALC exposure than those in BPA exposure, whereas the BPA+ALC coexposure slightly increased the adverse effects.

S1-08 Molecular Events of Perfluorooctane Sulfonate Exposure Across Multiple Rat Organs: Insights from a Non-Targeted Lipidomic Approach

Tzu-Hsin Yen, Hao-Jan Liang, Ching-Yu Lin

Institute of Environmental and Occupational Health Sciences, National Taiwan University

The health effects of perfluorooctane sulfonate (PFOS) have been a significant public health concern. PFOS exposure has been associated with various organ toxicities, including hepatotoxicity, reproductive toxicity, cardiotoxicity, and nephrotoxicity. Recent metabolomics studies suggest a connection between PFOS exposure and lipids in human blood. However, the effects of PFOS across different organs are not fully understood. This study aims to employ a non-targeted lipidomic approach to identify the key events of PFOS exposure in multiple organs of rats, intending to elucidate the potential toxic mechanisms of PFOS exposure.

The liver and testis of male Sprague-Dawley rats were collected after exposure to a series of doses of PFOS by gavage for 21 days. MS (mass spectrometry)-based lipidomics with a non-targeted strategy was applied to analyze the lipidomes extracted from Folch's methods. Multivariate and univariate statistical analyses determined the critical lipid responses.

MS-based lipidomics identified significant responses in the liver and testis following PFOS exposure. In the liver, glycerophospholipids and sphingolipids were most notably affected by PFOS exposure. PFOS increased oxidized phosphatidylcholines, phosphatidylserine, and hexosylceramide levels while decreasing phosphatidylcholine and sphingomyelin levels. These lipid alterations may be linked to PFOS-related inflammation, apoptosis, and oxidative stress. In contrast, lipid changes in the testis differed markedly from those in the liver, with nearly 70% of the altered lipids being fatty acids. These findings suggest that the modes of action of PFOS toxicity may vary among organs, underscoring the importance of multi-organ analysis.

In conclusion, this study revealed significant lipid profile changes induced by PFOS in the liver and testis of rats. These findings may contribute to developing the modes of action for PFOS toxicity in risk assessments. In the future, we will further investigate PFOS-induced lipid responses in other organs, such as the heart and kidney, to enhance our understanding of the molecular events triggered by PFOS across multiple organs.

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S1-09 Probing Electrophilic Lipid Modifications in Phagocytic Macrophages using Chemical Biology

Kaiyuan Deng[1,2], Yosuke Isobe[1,2,3], Kazuya Tsumagari[4], Koshi Imami[4], Makoto Arita[1,2,3,5]

[1]Division of Physiological Chemistry and Metabolism, Faculty of Pharmacy, Keio University;
[2]Laboratory of Metabolomics, RIKEN center for Integrative Medical Sciences;
[3]Cellular and Molecular Epigenetics Laboratory, Graduate School of Medical Life Science, Yokohama City University;
[4]Proteome Homeostasis Research Unit, RIKEN center for Integrative Medical Sciences;
[5]Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q), Keio University.

Macrophages remove apoptotic cells via phagocytosis during inflammation to maintain tissue homeostasis. This process is accompanied by various changes in the cellular metabolism, including the production of lipid metabolites by fatty acid oxygenases. Among these, highly reactive metabolites, called lipid-derived electrophiles (LDEs), modify cysteines and other nucleophilic amino acids in intracellular proteins. However, the landscape and functions of the modifications by these electrophilic metabolites are poorly characterized.
In this study, we used activity-based protein profiling (ABPP), a chemical biological approach, to quantitatively profile the cysteine reactivity landscape and identify the potential targets of endogenous LDE modification during macrophage phagocytosis. We adapted this methodology to mouse peritoneal macrophages, and identified multiple cysteine sites that are highly likely to be modified by LDEs generated by 12/15-lipoxygenase (12/15-LOX), a phagocytosis-related fatty acid oxygenase that is highly expressed in peritoneal macrophages. Among these, the actin-depolymerizing protein Cofilin-1 was found to be a target of 12/15-LOX-derived LDEs. In vitro Cofilin-1 activity was attenuated by 12/15-LOX-derived LDEs, and intracellular actin stabilization and phagocytosis were substantially enhanced by LDE treatment of mouse peritoneal macrophages. These results shed light on the new understanding of that intracellular LDE modification regulates macrophage phagocytosis, and further highlight the significance of using chemical biological techniques on lipid research.

S1-10 Tendency analysis of lipidome data

Chia-Hsin Liu[1], Wen-Jen Lin[2,3], Pei-Chun Shen[1], Hsiu-Cheng Liu[1], Meng-Hsin Tsai[1], Wei-Chung Cheng[1,3,4]

[1] School of Medicine, China Medical University, Taichung 404333, Taiwan
[2] Graduate Institute of Biomedical Science, China Medical University, Taichung 404333, Taiwan
[3] Cancer Biology and Precision Therapeutics Center, China Medical University, Taichung 406040, Taiwan.
[4] The Ph.D. program for Cancer Biology and Drug Discovery, China Medical University and Academia Sinica, Taichung 404328, Taiwan

Lipid species can be categorized into distinct functional subgroups based on unique lipid features such as side-chain length and double bond number. This is a significant departure from other omics fields. It is meaningful to identify the tendency of these lipid features to decipher lipidome remodeling, such as in response to various treatments. To address this, we are developing a novel tendency-analysis algorithm specifically designed to detect significant intervals of lipid characteristic alterations. This approach is based on the analysis of longitudinal data. However, we must overcome several challenges. First, the limited sample size and large variation in lipidomics data can diminish statistical power. Second, current algorithms typically examine single variables, despite the co-variation of lipid chain length and double bonds. Third, the inherent sparsity of lipidomics data further complicates the analysis. To overcome these obstacles, we employ techniques such as change point detection, Gaussian kernel smoothing, and permutation tests. Finally, we evaluated the performance of our tendency-analysis algorithm using real datasets, which demonstrated its potential to enhance the understanding of lipid characteristic alterations and improve lipidome data analysis.

S1-11 The Critical Role of Imputation and Normalization in Lipidomics Data Analysis

Hsiu-Cheng Liu[1], Chia-Hsin Liu [1], Pei-Chun Shen[1], Meng-Hsin Tsai [1], Wei-Chung Cheng [1,2,3]

[1] Cancer Biology and Precision Therapeutics Center, China Medical University, Taichung 404328, Taiwan.
[2] Graduate Institute of Biomedical Sciences, China Medical University, Taichung 404328, Taiwan.
[3] The Ph.D. program for Cancer Biology and Drug Discovery, China Medical University and Academia Sinica, Taichung 404328, Taiwan.

In the field of lipidomics, the quantification of lipids using mass spectrometry (MS) is beset with considerable challenges, including data variability, the presence of missing values, and the presence of noise. Pre-processing significantly influences subsequent analyses, and its impact is rarely explored in depth. In order to address these issues, our study examines the critical role of data pre-processing and its impact on the accuracy and reliability of subsequent analysis. The resaerch was structured around three key pre-processing steps: imputation, normalization, and the evaluation of the sequence in which these methods are applied. By analysing the effects of each step and their interactions, our objective was to determine the optimal pre-processing strategy for lipidomics data. The results demonstrated that proper pre-processing is essential for mitigating the inherent complexities of lipids quantification. Consequently, we propose a set of tailored pre-treatment recommendations based on different challenges encountered in lipidomics, providing researchers with a practical guide to enhance the quality and consistency of their analyses.

Short Talks

S1-12 Tissue-specific response of skin lipid profile to treatment of Atopic Dermatitis established by mass spectrometry imaging

Teresa Ximelis[1,2], M Magdalena Sansó[1,2], Rosa M. Perelló[1,2,3], Albert Maimó-Barceló[1,2], Ana Martín-Santiago[1,2,3], Ramón M Rodríguez[1,2], Gwendolyn Barceló-Coblijn[1,2],

[1]Health Research Institute of the Balearic Islands (IdISBa);
[2]Research Unit of the University Hospital Son Espases.(HUSE), Palma, [5]pain, 3Department of Dermatology Unit, HUSE, Palma, Spain;

Introduction. Monoclonal antibody (Mab) treatment of Atopic Dermatitis (AT) provides a promising new option. Further, only one Mab is the only biological drug approved for AT treatment. Hence, there is a clinical need to determine with greater precision the mechanisms of response to treatment and improve patient stratification. We used mass spectrometry imaging (MSI) to explore the changes in lipid profiles occurring during disease and treatment.

Methods. The study received approval from the Clinical Research Ethics Committee of the Balearic Islands (#IB 4815/22 PI). Physicians of the Dermatology Department performed the sample collection at 0 and 16 weeks after treatment. 4 mm punch biopsies were collected from the elbow flexure and a control area without AT and immediately snap frozen in liquid nitrogen and stored in a freezer at -80 ºC. Lipid MALDI-MSI analysis.15 µm thick histological sections (n=27) were prepared with a cryostat and analyzed by a MALDI timsTOF fleX (Bruker Daltonics) at a lateral resolution of 10 µm. Specialized software SCiLS Lab (v2024b Premium 3D, Bruker Daltonics) was used to run segmentation analysis. Lipid species assignment was performed using in-house developed software, Matlab R2020b, and the LIPIDMAPS database (https://lipidmaps.org).
Results and Discussion. MALDI-MSI lipidomics allowed us to associate a lipid cluster to each of the stratum layers present in the epidermis: corneum (SC), granulosum (SG), spinosum (SS), and basale (SB). Phosphatidylinositol (PI) 38:4 and 36:4 species were enriched in the SB layer, where adult stem cells resided, consistent with previous results in colon adult stem cells. Globally, SG was the most affected layer upon inflammation and treatment. Arachidonic acid (AA)-containing diacylglycerophospholipids levels were increased in the inflammed tissue. Intesteringly, their levels were not fully recovered after treatment.

Conclusions. MALDI-MSI analysis showed how sensitive the lipidome is to the and allows for establishing the bidimensional distribution of lipid species within a tissue section. The spatially resolved lipidome analysis allowed uncovering tissue-specific responses to chronic inflammation and TA treatment. In addition, results suggest that despite the positive clinical response to the treatment, the affected tissue retained some alterations at the molecular level.

S2-01 Apolipoprotein J Associates with the Switching of Bile Acid Biosynthetic Pathway in Patients with MAFLD

Hsin-Tzu Wang (王欣慈)[1], Chi-Jen Lo[4], Mei-Ling Cheng [4,5,6], Ya-Wen Chang[1], Yong Han Ke[1], Yu-Ting Hsu[1], Chi-Yao Chiu[3] and Hung-Yu Sun[1,2]

[1]Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
[2]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
[3]School of medicine, College of medicine, National Cheng Kung University, Tainan, Taiwan.
[4]Metabolomics Core Laboratory, Healthy Aging Research Center, Chang Gung University Taoyuan 333, Taiwan.
[5]Department of Biomedical Sciences, College of Medicine, Chang Gung University Taoyuan 333, Taiwan.
[6]Clinical Metabolomics Core Laboratory, Chang Gung Memorial Hospital Taoyuan, Taiwan.

Metabolic-associated fatty liver disease (MAFLD) has reached a global prevalence exceeding 35% of the population, with an increasingly younger demographic being affected. Recently, apolipoprotein J (ApoJ) has been identified as a pathogenic factor in the progression of MAFLD. ApoJ functions as a molecular chaperone by sustaining mTOR activation and inhibiting lipophagy. Interestingly, the activation of the bile acid (BA) alternative pathway was found to be enriched in the proteomic profile of hepatocytes from liver-specific ApoJ knockout mice. This study further investigates the association between ApoJ and BA profiles in patients at different stages of MAFLD. The results showed that serum ApoJ levels were significantly increased in patients with MAFLD and correlated with the severity of the disease (p = 0.012). BAs, including glycocholic acid (GCA), taurocholic acid (TCA), and taurodeoxycholic acid (TDCA), synthesized by the classical pathway, were significantly increased during the progression of MAFLD. Moreover, ApoJ correlated negatively with the BAs synthesized by the alternative pathway, suggesting a shift in bile acid synthesis pathways during MAFLD progression. Together, these findings suggest that rebalancing the BA synthesis pathway might be a potential therapeutic approach for alleviating MAFLD.

S2-02 Coding circular RNA succinate dehydrogenase complex assembly factor 2-mediated lipid metabolism enhances cellular fitness

Yu-Feng Xu[1], Yu-Chen Wu[1], Yi-Ren Huang[1], Hui-Hsuan Lin[2], Kuei-Yang Hsiao[1]

[1]National Chung Hsing University Institute of Biochemistry
[2]Doctoral Program in Tissue Engineering and Regenerative Medicine

Circular RNA (circRNA), formed through backsplicing, represents a class of single-stranded RNAs with a circular configuration. Our previous research identified a coding circRNA derived from the second and third exons of the succinate dehydrogenase complex assembly factor 2 (circSDHAF2), which produces a variant of the SDHAF2 protein. This circRNA is oncogenic, and its coding activity depends on the presence of an intron and is upregulated in breast tumor-derived cell lines, unlike those derived from normal mammary epithelial cells. This study explores the mechanism by which this SDHAF2 variant confers oncogenic properties, with metabolic profiling indicating alterations in fatty acid synthesis. To evaluate the impact of SDHAF2mh5 on fatty acid synthesis, we employed lipid staining using BODIPY, a lipophilic compound, on various cell lines overexpressing circSDHAF2, with fluorescent imaging revealing significant lipid accumulation in these cells. Constructing the open reading frame of circSDHAF2 in a linear fashion produced similar lipid accumulation, confirming that the protein product of circSDHAF2, and not merely its miRNA sponging activity, is responsible for causing lipid accumulation. The bioinfomatic analysis showed that the N-terminal of the protein shares a mitochondrial transit peptide with SDHAF2, while the fifth helix (h5) in the C-terminal is shorter, termed modified h5 (mh5). We hypothesized that SDHAF2mh5 may antagonize native SDHAF2 function. Fusion of green fluorescent protein with the mitochondrial transit peptide of SDHAF2 and subsequent transfection into breast and colorectal cancer cell lines confirmed the mitochondrial localization of SDHAF2mh5, suggesting a role in regulating succinate dehydrogenase activity in mitochondria. To determine if SDH deficiency-induced lipid accumulation is mediated by acetyl-CoA carboxylase (ACC), inhibitors such as 5-tetradecyloxy-2-furoic acid or CP-640186 were administered to circSDHAF2-overexpressing cells, reducing lipid droplets. Furthermore, depleting lipids with ACC inhibitors rendered these cells unable to resist stress induced by serum depletion. Collectively, this study unveils a novel role of coding circRNA in regulating lipid accumulation and cellular stress response through modulation of the tricarboxylic acid cycle in mitochondria, which may facilitate the development of alternative strategies to cope with various cancers.

S2-03 Decoding the Lipid-Differentiation Axis: Phosphatidylinositol Species in Health and Disease

Albert Maimó-Barceló[1], Karim Pérez-Romero[1], Teresa Malondra[1], Ramón M. Rodríguez[1], José A. Fernández[2], Gwendolyn Barceló-Coblijn[1]

[1]Lipids in Human Pathology, Institut d'Investigació Sanitària Illes Balears (IdISBa), Ctra. Valldemossa 79, E-07120 Palma, Balearic Islands, Spain; Research Unit, University Hospital Son Espases, Ctra. Valldemossa 79, E-07120 Palma, Balearic Islands, Spain.
[2]Dept. of Physical Chemistry, Fac. of Science and Technology, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940, Bilbao, Spain.

Cell differentiation is a critical process in biology where unspecialized cells, such as stem cells, become specialized in structure and function. This process is essential for the development, growth, and maintenance of multicellular organisms. Over recent years, we have identified several evidences pointing to a link between the lipid phenotype, particularly phosphatidylinositol (PI) species composition, and cell differentiation states. This lipid phenotype has been observed in several cellular contexts, including colonocyte differentiation, immune cell activation, and epidermis differentiation. Importantly, the PI profile modulation consists of changes in species containing AA that are replaced by monounsaturated species, involving different metabolic processes, converging at the same molecular species.

Consistent with its relevance, the PI lipid composition is significantly altered in pathological conditions such as cancer, inflammatory bowel disease, and dermatitis. These conditions are characterized by increased proliferative states and reduced cell differentiation, driven by multifactorial conditions that disrupt tissue homeostasis. In cancer, PI metabolism maintains a high level of PI 38:4 and PI 36:4, which supports uncontrolled cell proliferation via PI3K-PIP3-AKT, and hampers differentiation, contributing to tumorigenesis. Similarly, in IBD and dermatitis, the inflammatory processes disrupt normal PI metabolism, leading to cellular malfunctioning and impaired tissue integrity.

All together these findings highlight the potential of targeting lipid metabolic pathways as potential strategies for treating diseases characterized by altered cell proliferation and differentiation. Therefore, understanding the intricate relationship between lipid species and cellular states is critical and offers valuable insights into the mechanisms underlying tissue homeostasis and disease progression.

S2-04 Distinct Roles of Mitochondrial and Peroxisomal β-Oxidation in Breast Cancer Cells Overexpressing Coding circSDHAF2

Wan-Hua Wu[1], Yu-Feng Xu[1], Kuei-Yang Hsiao[1]

[1]National Chung Hsing University Institute of Biochemistry

Circular RNAs (circRNAs) are a novel class of endogenous non-coding RNAs that form continuous closed-loop structures through covalent linkage of downstream 5′ splice sites with upstream 3′ splice sites. Our previous research identified a coding circRNA derived from the second and third exons of the succinate dehydrogenase complex assembly factor 2 (circSDHAF2), which produces an oncogenic variant of the SDHAF2 protein and induces lipid droplet (LD) accumulation. However, the mechanism by which circSDHAF2-induced LD accumulation contributes to oncogenesis remains unclear. To explore the role of circSDHAF2-induced LD accumulation in oncogenesis, we established stable breast cancer cell lines (MCF-7, MDA-MB-231, and BT-549) overexpressing circSDHAF2. These cells exhibited significant LD accumulation compared to controls, suggesting that LDs might serve as alternative energy sources during nutrient deprivation. Upon serum starvation, LDs were significantly depleted after 16 hours, correlating with enhanced cell proliferation in circSDHAF2-overexpressing cells. Similar effects were observed under glucose deprivation and treatment with chemotherapeutic agents, including paclitaxel and doxorubicin. We hypothesize that circSDHAF2 overexpression leads to metabolic reprogramming that favors fatty acids as an energy source. Inhibiting mitochondrial and peroxisomal β-oxidation with etomoxir (targeting carnitine palmitoyltransferase 1) and 10,12-tricosadiynoic acid (targeting acyl-CoA oxidase-1) revealed that mitochondrial β-oxidation inhibition had minimal impact on cell growth, indicating a more critical role for peroxisomal β-oxidation in circSDHAF2-expressing tumor cells. These findings underscore the previously underappreciated importance of peroxisomal β-oxidation in supporting cellular fitness under nutrient deprivation and chemotherapeutic stress in circSDHAF2-overexpressing breast cancer cells.

S2-05 *Drosophila melanogaster* as a model for obesity by alteration of *Atf3*

Tzu-Min Hung[1], Chau-Ti Ting[1]

[1]Department of Life Science, National Taiwan University.

Obesity is a prevalent issue in contemporary society, with severe cases linked to chronic metabolic diseases and cardiovascular complications. *Atf3*, a stress-induced transcription factor, has been identified with lower expression levels in obese patients according to clinical data. *Atf3* gene knockout mice exhibit obesity-related traits and early signs of chronic metabolic diseases. In Drosophila, *Atf3* null mutant flies display enlarged lipid droplets, which can be rescued by expressing human *Atf3*, but this null mutant leads to a lethal phenotype during the larval stage. Given the conservation of *Atf3* between flies and mammals, we decided to establish an *Atf3*-induced obesity model using *Atf3* transposon element insertional mutation flies. In our study, we analyzed three strains: *Atf3[c04578]*, *Atf3[e00645]*, and *Atf3[MI01026]*, which have TE insertions in the noncoding region of *Atf3* and may influence its expression levels without causing lethality. To determine the obesity phenotype, we conducted a buoyancy assay in larvae, and measured body weight, starvation resistance, and triglyceride (TAG) levels in adults. Larvae consistently displayed a higher floating rate in buoyancy assays. In adults, *Atf3[c04578]* showed no significant differences in body weight and TAG levels, but lower starvation resistance in males. *Atf3[e00645]* exhibited higher body weight, starvation resistance, and TAG levels, except for males with no significant difference in starvation resistance. *Atf3[MI01026]* displayed both higher body weight and starvation resistance, but no significant difference in TAG levels. These results indicate that *Atf3[e00645]* and *Atf3[MI01026]* show consistent results across most assays. Next, we analyzed *Atf3* expression levels through RT-qPCR to determine if the obesity traits observed in mutant flies were caused by the lower expression levels of *Atf3*. Results showed that the mutant strain *Atf3[c04578]* exhibited lower expression levels in whole-fly analysis. To more specifically identify the influence of *Atf3*, we dissected flies into two parts: head and body (thorax and abdomen), and found that *Atf3[c04578]* and *Atf3[MI01026]* had lower *Atf3* expression levels specifically in the head, while *Atf3[e00645]* showed no significant differences. Our findings suggest that *Atf3* mutant flies exhibit lower expression levels in the head, indicating that *Atf3* expression in the brain region plays an important role in obesity progression. Additionally, our study highlights the significance of *Atf3* in obesity progression and identifies *Atf3[MI01026]* as consistently displaying obesity-related phenotypes, likely due to low expression levels in the head, underscoring its potential as an obesity model for further research.

S2-06 Exploring the molecular mechanism of MECR underlying obesity and associated neurodegenerative disorders

Jeng-Lin Li [1,2], Cheng-Li Hong[3], Chih-Chiang Chan[3], Wei-Shiung Yang[4]

[1]Internal Medicine, National Taiwan University Hospital-JinShan branch
[2]Graduate Program of Translational Medicine, National Taiwan University and Academia Sinica, Taiwan
[3]Graduate Institute of Physiology, National Taiwan University
[4]Internal Medicine, National Taiwan University Hospital

Obesity, a multifactorial disorder influenced by both genetic and environmental factors, is a growing global health issue with increased mortality in the general population. Obesity can lead to various comorbidities, such as neurodegenerative disorders. In the past decade, more and more genetic studies of monogenic obesity have indicated intense interactions between the adipose tissue and brain. However, the mechanism of monogenic obesity and the relationship between excess adipogenesis and neurodegeneration are ill-defined. We recently have identified a potential novel obesity-causing gene, *MECR*, associated with waist circumference via phenome-wide association study (PheWAS). MECR is an enzyme catalyzing the reduction of enoyl ACP to saturated acyl-ACP in mitochondrial fatty acid synthesis. Although studies have revealed MECR is strongly related to neurodegenerative diseases, there are few studies on the relationship between MECR and obesity. To investigate the pathogenic mechanisms of *MECR* in obesity, we initially used *Drosophila Mecr* knockdown model to examine obesity phenotypes and found loss of *Mecr* in fat tissues causes organismal obesity and insulin resistance.

In this study, we propose the following specific aims: (1) Clinical and cellular investigation of MECR in obesity to verify *Mecr* as a new biomarker; (2) Investigations of MECR-mediated obesity in animal models by genetic manipulating Mecr level in flies; (3) Mechanistic investigations of MECR in neurodegeneration to decipher the cell non-autonomous effects between fat tissues and nervous systems. By combining clinical studies, cellular models, and *Drosophila* genetics, we believe our findings will provide a better understanding of obesity pathogenesis and the fat-brain axis.

Key words: obesity, PheWAS, *Mecr, Drosophila*, insulin resistance, neurodegeneration.

Short Talks

S2-07 Icosapent ethyl -supplementation in healthy individuals improves plasma and lipoprotein lipid profiles and reduces cardiovascular disease risk markers

L. Äikäs[1,2], M. Hermansson[1], P.T. Kovanen[1], K. Öörni[1]

[1]Atherosclerosis Research Laboratory, Wihuri Research Institute, Helsinki, Finland
[2]Doctoral Programme of Biomedicine, University of Helsinki, Helsinki, Finland

Background and Aims: Icosapent ethyl (IPE) -supplementation serves as pharmacological intervention for secondary prevention of cardiovascular diseases (CVDs). We performed comprehensive metabolomics and lipidomics analyses to elucidate the impact of IPE-supplementation on lipoprotein composition and their proatherogenic potential.
Methods: Healthy, normolipidemic volunteers (n=72) were administered a daily dose of 4 g of IPE over a 28-day period. Plasma samples obtained before, during, at the conclusion, and post-supplementation were subjected to analysis using gas chromatography and NMR spectroscopy. Additionally, lipoproteins (HDL, LDL, and VLDL) were isolated and their lipidomes analysed by LC/MS. The binding of plasma lipoproteins to isolated human arterial proteoglycans was determined *ex vivo* as a surrogate marker of lipoprotein retention in the arterial wall.
Results: IPE-supplementation resulted in rapid reductions in multiple clinical risk markers, including triglyceride, remnant-cholesterol, and apoB-levels. The affinity of lipoproteins for human aortic proteoglycans and a 10-year CVD risk score (Coronary Event Risk Test) decreased as well. Metabolomic analysis highlighted a uniform shift induced by IPE across plasma and lipoprotein classes. However, intrinsic inter-individual differences in lipoprotein lipidomes outweighed temporal IPE-induced changes. Reduced proteoglycan-binding correlated with decreased apoB particles and cholesterol content, alongside changes in specific lipid species, including sphingomyelins and cholesterol esters. Notably, PC 38:3 levels, associated with obesity and CVD risk, also diminished with IPE-supplementation.
Conclusion: IPE-supplementation reduced markers of CVD risk by improving plasma/lipoprotein lipid profiles. This resulted in reduced binding of lipoproteins to arterial proteoglycans, a potential mechanism explaining the previously observed reduction in CVD after IPE-supplementation. Lipoprotein features that associated with increased proteoglycan-binding reflected characteristics of metabolic syndrome, but also included specific lipid molecular species. Importantly, our analyses underscored the presence of distinct lipoprotein signatures among individuals, likely influenced by genetic and lifestyle factors. These signatures persisted despite extensive remodelling of the metabolome induced by IPE.

S2-08 Machine learning-based identification of lipid biomarkers in lung tumor tissues

Chia-Ying Wu [1], Hua-Chen Chan[2], Liang-Yin Ke[3]

[1]The Master Program of AI Application in Health Industry, College of Health Sciences, Kaohsiung Medical University, Kaohsiung 807378, Taiwan;
[2]Department of Medical Laboratory Science, College of Medicine, I-Shou University, Kaohsiung 824005, Taiwan
[3]Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung 807378, Taiwan;

Lung cancer remains the leading cause of cancer-related deaths globally, with a five-year survival rate of only 7-28%. Targeted therapies are effective for specific genetic mutations, but nearly half of patients rely on traditional treatments, which have limited efficacy.
Dysregulated lipid metabolism often precedes disease onset, and mass spectrometry-based lipidomics may uncover therapeutic strategies. Machine learning, a powerful data analysis tool, was used in combination with lipidomics to identify key lipid components differentiating tumor from non-tumor tissues, focusing on those highly associated with cancer cells. Tumor and adjacent non-tumor tissues from 35 lung cancer patients at Mackay Memorial Hospital were analyzed. Lipids were extracted, separated by liquid chromatography, and analyzed for mass-to-charge ratio (m/z) and signal intensity. Key lipid components were identified using both criteria selection method and machine learning classification models. H&E staining was performed to determine the proportion of cancer cells, and its correlation with lipid components was analyzed. Using criteria selection method with four filters (p-value < 10^{-4}, fold change > 5, signal intensity > 3000, retention time > 1 min), 13 lipid components were identified. From these, the top five lipids with the highest AUC values were selected: m/z 1419.26 (retention time: 16.67 min), 1389.21 (16.36 min), 746.68 (16.98 min), 1367.23 (16.64 min), and 1411.2 (16.18 min). In the machine learning approach, a Random Forest (RF) model was developed based on all dataset and validated using the OOB (Out-of Bag) data, showing strong performance with an accuracy of 0.86 (95% CI = 0.78-0.94) and an AUC of 0.87. The top five lipid biomarkers identified by random forest were m/z 906.61 (retention time: 7.68 min), 1341.22 (16.59 min), 960.75 (14.04 min), 1024.68 (6.2 min), and 1367.23 (16.64 min), with m/z 906.61 notably showing a positive correlation with cancer cells. When combined with other markers, the random forest model's AUC improved to 0.92. This study combines lipidomics and machine learning to establish a predictive model for distinguishing lung cancer tumor from non-tumor tissues, suggesting that m/z 906.61 plays a crucial role in lung cancer pathogenesis.

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S2-09 MALDI-MSI Investigation of Cardiolipins Changes in Rat Organ Tissues by Drug-Induced Oxidative Stress

Chih Le Liu[1], Ping-Ju Tsai[2], Kuo-Chen Hung[3], Hay-Yan J. Wang[1*]

[1.] Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan
[2.] Department of Surgery, Yuan's General Hospital, Kaohsiung, Taiwan
[3.] Division of General and Digestive Surgery, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan

Cardiolipin (CL), an important phospholipid in the inner mitochondrial membrane, accounts for approximately 20% of the total mitochondrial lipids. Structurally, CL is composed of two phosphatidic acids connecting to the terminals of a central glycerol backbone. In Complex I, III, IV, and V of the electron transport chain, CLs serve as the proton donors, which also renders them susceptible to the assault of oxidative stress. Oxidation of CLs would release cytochrome C into cytoplasm, triggering the subsequent apoptotic events, and altering the CL distributions in situ. We therefore investigated the impact of oxidative stress on the alteration of in situ CLs in metabolically critical organs such as liver and kidney using MALDI-mass spectrometry imaging (MALDI-MSI).

In healthy rat liver, MALDI-MSI revealed the clusters of CLs containing a total of 70 acyl carbons (CL70), CL72, and CL74 species distributed along the hepatic acinus, and the renal CL clusters of CL70, CL72, and CL74 differentially expressed along the cortex and medulla. Oral acetaminophen (APAP)-induced acute oxidative stress drastically upregulated the expression of all hepatic CLs, yet cause no obvious changes in renal CLs. Chronic oxidative stress by oral APAP significantly upregulated the expression of hepatic CL72 group, yet differentially downregulated or upregulated the other two CL groups in different hepatic parenchyma. Same oxidative stress prominently downregulated the expression of CLs in outer renal medulla, and heterogeneously down-regulated the cortical CLs, suggesting a differential compromise of renal tissue functions.

Integrate the differential hepatic and renal CL expression with histological relevance further unveiled the lipidomic/metabolomic insights, providing additional omics annotation to the pathological process during oxidative insults in metabolically critical organ tissues.

(Part of the data in this abstract has been published in 72nd ASMS Conference on Mass Spectrometry and Allied Topics, Anaheim, CA, USA. Jun. 2nd - 6th, 2024)

Short Talks

S2-10 Targeted Metabolomics Reveals Gender-Specific Biomarker Signatures for Identifying High-Risk Patients with MCI Rapidly Progressing to AD

Yi-Long Huang[1] and Ting-Fen Tsai[1,2,3]

[1]Center for Healthy Longevity and Aging Sciences, and
[2]Department of Life Sciences and Institute of Genome Sciences, National Yang Ming Chiao Tung University, Taipei, Taiwan;
[3]Institute of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Taiwan

Mild cognitive impairment (MCI) represents an intermediate stage before progressing to Alzheimer's disease (AD). Identifying high-risk patients with MCI rapidly progressing to AD is crucial for early intervention; however, reliable blood-based biomarkers remain lacking. This study investigates metabolomic profiles using targeted metabolomics, namely AbsoluteIDQ p180 kit. We analyze plasma samples from the four groups: (1) Control group (n=43), subjects with cognitive un-impairment (CU); (2) stable MCI (S-MCI, n=39), patients maintained at MCI for more than 4 years; (3) progressive MCI (P-MCI, n=32), patients with MCI converted to AD within 3 years; (4) AD patients (n=44). Currently, several findings are summarized. **Firstly**, gender-specific difference of metabolomic profiles were identified in different comparison of S-MCI, P-MCI and AD patients. **Secondly**, comparing P-MCI versus S-MCI: (a) In males, two metabolites (lysoPC 18:1 and PC O-44:4) and sum of MUFA-LPCs are significantly increased in P-MCI; while one pathway (ω-Oxidation) is significantly decreased in P-MCI. (b) In females, six metabolites (PC O-34:3, PC O-32:2, PC O-32:1, PC O-36:5, PC O-34:2, and SM 34:1) are significantly increased in P-MCI. Interestingly, all of the six metabolites are lipid molecules; among them, 5/6 are phosphatidylcholine (PC) and 1/6 is sphingomyelin (SM). **Finally,** comparing AD versus P-MCI: (a) In males, two amino acids (Phenylalanine and Lysine), one lysoPC (LPC 20:3), one acylcarnitines (isovalerylcarnitine) and one biogenic amine (α-Aminoadipic acid) were significantly decreased in AD. Additionally, several indicators were significantly down-regulated in AD: the sum of essential amino acids, the sum of solely ketogenic amino acids, and the ratio of short-chain to medium-chain ACs. Notably, metabolite biomarker associated with the disorder of very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency was significantly increased in AD. (b) In females, one lipid metabolite (PC 36:0) was significantly down-regulated, while the activity of nitric oxide synthase (NOS) was significantly up-regulated in AD. Furthermore, metabolite biomarker associated with the disorder of carbamoyl phosphate synthase (CPS) deficiency was significantly increased in AD patients. Our results highlight the potential of targeted metabolomics in identifying high-risk MCI patients, and provide biomarkers for predicting AD progression, as well as underscore the importance of gender-specific metabolic profiling associated with dementia.

S3-01 Antioxidant defense system response in Neurodegeneration with Brain Iron Accumulation (NBIA) patients' fibroblasts following the administration of omega 3-6-9 fatty acids

Agata Wydrych[1*], Barbara Pakula[1*], Patrycja Jakubek-Olszewska[1*], Justyna Janikiewicz[1], Aneta M. Dobosz[1], Marta Skowrońska[2], Iwona Kurkowska-Jastrzębska[2], Werner J.H. Koopman[3], Agnieszka Dobrzyń[1], Magdalena Lebiedzinska-Arciszewska[1#], Mariusz R. Wieckowski[1#]

[1]Nencki Institute of Experimental Biology, PAS, Warsaw, Poland
[2]Institute of Psychiatry and Neurology, Warsaw, Poland
[3]Radboud University Medical Center, Nijmegen, The Netherlands

Neurodegeneration with Brain Iron Accumulation (NBIA) is a rare disease with diverse clinical symptoms, characterized by the deposition of the iron in the brain.
Among several NBIA subtypes, especially mitochondrial membrane protein-associated neurodegeneration (MPAN) caused by mutation in *C19orf12* gene is recently investigated due to the fact, that the molecular mechanism underlying MPAN is still not fully understood. Mutation in *C19orf12* gene of MPAN subtype, one of the most commonly diagnosed in Poland, is presumed to have an impact on the lipid metabolism in the cell. The goal of our research is to characterize redox homeostasis of NBIA fibroblasts (obtained in a cohort of 22 MPAN patients and 6 healthy donors) and to examine whether the intervention based on the administration of omega 3-6-9 fatty acids would have an influence on oxidative-stress related factors. Our experimental approach covers both, basal and OXPHOS promoting conditions, in order to better visualize mitochondrial metabolic defect in MPAN fibroblasts. Comparative proteomic analysis was based on liquid chromatography-MS3 spectrometry (LC-MS/MS) and the redox status was examined by fluorescent detection methods. Proteomic analysis of MPAN patients' fibroblasts showed several differences that refer a substantial number of proteins involved in redox homeostasis. Moreover, we evaluated changes in the proteomic profiles of patients' and control fibroblasts in the response to the omega 3-6-9 fatty acids treatment. Alterations observed in proteomes of MPAN fibroblasts and evaluation of the oxidative-stress related factors before and after administration of omega 3-6-9 fatty acids gives valuable insides of redox homeostasis of MPAN fibroblasts, contributing to a better understanding mechanisms underlying NBIA disease.
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S3-02 Deficiency of two lysophospholipases PNPLA6 and PNPLA7 leads to neurogenic muscle atrophy

Mai Kawaguchi[1,2], Tetsuya Hirabayashi[1], Kohji Kasahara[1], Makoto Murakami[2,3]

[1]Center for Basic Technology Research, Tokyo Metropolitan Institute of Medical Science.
[2]Laboratory of Microenvironmental Metabolic Health Sciences, Graduate School of Medicine, The University of Tokyo.
[3]AMED-CREST.

Autosomal recessive mutations in genes encoding several enzymes in the PNPLA(patatin-like phospholipase domain containing)/iPLA$_2$ (Ca^{2+}-independent phospholipase A_2) family are associated with neurodegenerative diseases such as hereditary spastic paraplegia, amyotrophic lateral sclerosis (ALS), parkinsonism, and neurodegeneration with brain iron accumulation (NBIA). PNPLA6 and its paralog PNPLA7 are structurally similar lysophosholipases, although it is unknown whether they are redundant or have distinct functions. To elucidate the physiological roles of these two lysophospholipases in the central nervous system (CNS), we generated CNS-specific single and double knockout mice for *Pnpla6* and *Pnpla7* under the Nestin promoter that drives recombination of the target genes in neurons, astrocytes, and oligodendrocytes. The double knockout *Pnpla6/7*$^{\Delta Nes}$ mice exhibited more severe phenotypes such as reduced weight gain, shortened lifespan and progressive neurodegenerative muscular atrophy than single knockouts. The neurological abnormalities in the double mutant mice included a reduced number of motor neurons, demyelination, progressive denervation at the neuromuscular junctions, cerebral and spinal inflammation with gliosis, and p62 accumulation in astrocytes. Lipidomics analysis of brain and spinal cord tissues revealed marked increases in various lysophospholipids, changes in fatty acid composition in phospholipids, and substantial reductions in myelin-related sphingolipids in *Pnpla6/7*$^{\Delta Nes}$ mice. Many of these phenotypes were also observed in astrocyte-specific *Pnpla6/7*-deficient mice. These results indicate that the regulation of phospholipid decomposition by lysophospholipases PNPLA6 and PNPLA7 in astrocytes is crucial for the maintenance of neuronal integrity.

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S3-03 Depletion of stearoyl-CoA desaturase 1 activity mediates mitochondrial failure through cardiolipin recalibrations in lipid-laden pancreatic β-cells

Justyna Janikiewicz[1], Maria E. Kendziorek[1], Ewelina Krogulec[1], Aneta M. Dobosz[1], Andrzej A. Szczepankiewicz[2], Katarzyna Kolczynska-Matysiak[3], Agnieszka Dobrzyn[1]

[1] Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland;
[2] Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland;
[3] Dioscuri Centre for Metabolic Diseases, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland.

Pandemics of obesity-linked type 2 diabetes (T2D) constitute substantial challenge for global health and a therapeutic conundrum. Elevated levels of free fatty acids (FA) became tightly correlated with insulin resistance of peripheral tissues and compromised pancreatic β-cell function in pathogenesis of T2D. Ultimately, the mitochondrial dysfunction and imbalanced dynamics occurring through accumulation of surplus lipids in the pancreas have been acknowledged as early signs of the disease. Moreover, insulin secretion is tuned by the bioenergetic state of mitochondria which undergo dynamic but frequently reversible remodeling sequels. The composition of mitochondrial signature phospholipids - cardiolipins (CL), is modulated by availability of the FA pool. Stearoyl-CoA desaturase 1 (SCD1) is the rate limiting enzyme catalyzing the biosynthesis of monounsaturated fatty acyl moieties and affects the overall rate of the β-cell survival. This study investigated the molecular effect of SCD1 depletion on the regulation of mitochondrial bioenergetics and architecture in pancreatic β-cells undergoing palmitotoxicity.

Ablation of SCD1 activity led to an impairment in bioenergetic function, indicated by more depolarized mitochondria and a drop in ATP production compared with INS-1E cells that were independently treated with palmitate. This effect occurred in parallel with higher amounts of compromised mitochondria, and alterations in I, III, IV and V OXPHOS complexes which directly bind with CL. The β-cells and pancreatic islets fraction of CL was enriched in C16:1, C18:1, C18:2, C20:4 and C20:3n-6 FA. Such lipid rearrangements coincided with abnormal CL synthesis, altered abundance of the effectors linked to CL remodeling/ fatty acyl moieties distribution (TAZ, LCLAT1, PLSCR3, ACSL5), and cristae shaping (Prohibitin1, MIGA2, Mic10). Increased hallmarks of lipid peroxidation upon SCD1 deficiency, complemented observations of palmitate-mediated collapse of cristae microarchitecture. The findings herein uncover a bridging role of Δ9 desaturation integrating the CL acyl side chain composition and mitochondrial lipid homeostasis to counteract lipotoxicity-derived mitochondrial damage in pancreatic β-cells.

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S3-04 Development of a new mouse model of fatty liver caused by polyunsaturated fatty acid deficiency

Misa Ando[1, 2], Hyeon-Cheol Lee-Okada[1], Takehiko Yokomizo[1]

[1]Department of Biochemistry, Juntendo University Graduate School of Medicine.
[2]Department of Breast Oncology, Juntendo University School of Medicine.

Background: In recent years, there has been an increase in the number of people with fatty liver due to the enhanced adoption of a Western sedentary lifestyle and a high-calorie diet. Although numerous studies have suggested the regulatory effects of polyunsaturated fatty acids (PUFAs) such as DHA on fatty liver, the precise mechanism remains unclear. PUFAs are synthesized from essential fatty acids by fatty acid desaturases FADS1 and FADS2. To establish PUFA-deficient animal models, we recently generated knockout mice with a deletion in *Fads2* gene alone (FADS2 KO) or both *Fads1* and *Fads2* genes (FADS1/2 DKO). In this study, we investigated whether these mice are useful animal models for studying the pathogenesis of fatty liver caused by PUFA deficiency.

Method: FADS2 KO and FADS1/2 DKO mice were obtained by heterozygous crossing. The littermate mice were weaned at 3 weeks of age and fed AIN-93G (7% soybean oil) or AIN-93G with 7% *Torreya nucifera* oil substituted for soybean oil for 8 weeks.

Result: FADS2 KO and FADS1/2 DKO mice exhibited mild and severe fatty liver, respectively. Lipidomic analyses revealed drastic accumulation of non-methylene- interrupted fatty acids (NMIFAs), especially sciadonic acid, in FADS2 KO liver but not in FADS1/2 DKO liver. Dietary supplementation of FADS1/2 DKO mice with *Torreya nucifera* oil, which contains sciadonic acid, significantly ameliorated the phenotype.

Discussion: This study revealed the protective effect of NMIFAs on the pathogenesis of fatty liver and proved that FADS1/2 DKO mice are a useful model for studying fatty liver caused by PUFA deficiency.

S3-05 DGAT1 and CD36-driven lipid droplet accumulation contributes to chemotherapy resistance in colorectal cancer

Pin-I Chou[1], Xiang-Kai Huang[1], Shih-En Huang[2], Wan-Ping Chan[3], Yu-Long Lin[3], Huai-Shuo Chang[3], Yu-Min Yeh[2], Shiuan-Yeh Chen[3], and I-Chen Peng[1]

[1]Department of Life Sciences, National Cheng Kung University, Tainan City 701, Taiwan
[2]Department of Oncology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City 701, Taiwan
[3]Department of Photonics, National Cheng Kung University, Tainan City 701, Taiwan

Chemotherapy is commonly used as an adjuvant treatment before or after surgery for colorectal cancer. However, chemotherapy resistance significantly impacts treatment efficacy and patient survival rates. Aberrant lipid metabolism has been found to affect the efficacy of chemotherapy in colorectal cancer. We firstly collected tumor samples from 48 stage IV colorectal cancer patients and observed lipid droplet accumulation within the tumor tissues. Based on the extent of lipid droplet presence, we categorized the patients into high and low lipid droplet groups using 10%, 15%, and 20% cutoffs. We found that overall survival was significantly longer in the low lipid droplet groups, indicating that higher lipid droplet levels are associated with a poorer prognosis. Interestingly, we found that lipid droplet accumulation and fatty acid uptake were increased in HCT116 and Colo205 cells surviving 5-fluorouracil (5-FU) treatment. To clarify whether lipid droplet formation and fatty acid uptake contribute to chemotherapy resistance in colorectal cancer cells, we used the diglyceride acyltransferase 1 (DGAT1) inhibitor A922500 and the fatty acid translocase CD36 inhibitor sulfosuccinimidyl oleate sodium (SSO) to reduce lipid droplet formation and fatty acid uptake following 5-FU treatment. Both A922500 and SSO decreased the IC50 (half maximal inhibitory concentration) of 5-FU in HCT116 and Colo205 cells, indicating that DGAT1 and CD36-driven lipid droplet accumulation contributes to chemotherapy resistance in colorectal cancer. Finally, we successfully obtained Raman signals of lipid droplets in colorectal cancer cells, facilitating rapid analysis of these cellular lipid droplets using Raman spectroscopy. The acquired Raman images and spectra will be used to further investigate the relationship between lipid droplets, chemotherapy resistance, and treatment efficacy.

S3-06 Glutamine Synthetase-mediated CD36 O-GlcNAcylation that Facilitates the Utilization of Fatty Acids Enhances Cisplatin Resistance in Hepatocellular Carcinoma

Tung-Hsuan Kang, Jia-Wei Chang, Zong-Chen Lin, and I-Chen Peng

Department of Life Sciences, National Cheng Kung University, Tainan City 701, Taiwan

Cisplatin (CPT) is a widely used chemotherapy drug for treating intermediate and advanced hepatocellular carcinoma (HCC). However, resistance to CPT is a major factor contributing to chemotherapy failure in HCC patients. Recent studies have highlighted the role of dysregulated lipid metabolism in chemotherapy resistance, revealing that cluster of differentiation 36 (CD36), a key mediator of fatty acid uptake, is involved in chemoresistance. Analysis of TCGA cancer datasets shows higher CD36 expression in HCC tissues compared to normal liver tissues. Data from the Kaplan-Meier Plotter database further indicate that stage III and IV HCC patients who typically received chemotherapy with high CD36 expression tend to have lower survival rates. In HepG2 and Huh7 cells surviving CPT treatment, we found an increase in CD36 expression and fatty acid uptake. Knockdown of CD36 reduced fatty acid uptake and lowered the IC50 (half maximal inhibitory concentration) of CPT in these cells. To explore the regulatory mechanism of CD36 expression upon CPT treatment, we observed increased expression of glutamine synthetase (GS), total protein O-linked-N-acetylglucosaminylation (O-GlcNAcylation), and O-GlcNAc transferase (OGT) in HepG2 cells surviving CPT treatment. Immunoprecipitation revealed an enhanced interaction between GS and glutamine-fructose-6-phosphate aminotransferase 1 (GFAT1), promoting CD36 O-GlcNAcylation. Furthermore, inhibition of GS and OGT reduced total protein O-GlcNAcylation, CD36 expression, and fatty acid uptake in these cells. To investigate the function of fatty acid uptake upon CPT treatment, we found enhanced fatty acid β-oxidation in HepG2 cells following CPT treatment, which was attenuated by inhibiting GS and OGT. Inhibition of GS, OGT, and fatty acid β-oxidation decreased the IC50 of CPT in these cells. Interestingly, supplying specific saturated and polyunsaturated fatty acids reduced CPT sensitivity, while inhibiting fatty acid β-oxidation counteracted this reduced sensitivity. Furthermore, HCC tissues exhibit higher GS and OGT expression compared to normal liver tissues. Analysis of GEO HCC datasets shows a positive correlation between GS and CD36 expression, as well as between OGT and CD36 expression. In patients with stage III and IV HCC, high GS expression is associated with lower survival rates. These results suggest that GS-mediated CD36 O-GlcNAcylation facilitates fatty acid uptake and the utilization of specific fatty acids, thereby enhancing CPT resistance in HCC. Our study provides a metabolic perspective on CPT resistance in HCC, which can be used to improve chemotherapy efficacy.

S3-07 Investigating the Role of 3-Ketodihydrosphingosine Reductase in PNS Neurodegeneration

Wan-Syuan Lin[1], Yu-Chi Tsai1, Yi Hsiao[1], Fei-Yang Tzou[1], Cheng-Li Hong[1], Kai-Hung Chen[1], Chia-Heng Hsu[1], Chih-Chiang Chan[1]

[1]Graduate Institute of Physiology, National Taiwan University.

Sphingolipids are abundant in the nervous system and play dual roles as structural components and signaling molecules. Sphingolipids are metabolized by a set of sphingolipid-metabolizing enzymes, mutations of which have been linked to neurological disorders. To understand the sphingolipid regulatory network, we generated a complete set of reporter flies to visualize the entire network of sphingolipid-metabolizing enzymes. We systemically profile their expression in the adult leg as a representative tissue of the peripheral nervous system (PNS) and found a subset of the network endogenously expressed in the adult PNS. For functional assessment, we performed negative geotaxis and adult thermal nociception assays in this subset of enzymes to identify the essential ones for neural function. Knockdown flies of 15 enzymes exhibited reduced function in the tests above. To verify the RNAi results, we also generated knock-out flies using CRISPR genome editing technique. Specifically, *Kdsr* (3-kytodihydrosphingosine reductase) KO flies exhibited progressive locomotor decline upon aging, indicating its role in PNS degeneration. At the cellular level, *Kdsr*-KO exhibited elevated DHE staining, suggesting an increase in oxidative stress. Since *Kdsr* is an ER-resident protein, we analyzed the transcripts of genes associated with ER stress response. The expression of these genes increased, indicating elevated ER stress in *Kdsr*-KO. Caspase-3 is a key proapoptotic factor that exhibits nuclear localization when activated. We observed an increase in caspase-3 immunofluorescences inside *Kdsr*-KO nuclei, indicating activation of apoptosis. In TEM, we observed an accumulation of lamellar bodies in the ventral nerve cord of *Kdsr*-KO fly, suggesting a defect in mitochondrial morphology. These findings underscore the importance of *Kdsr* in maintaining neural functions through mitochondria. Our future direction will focus on the role of *Kdsr* in ER-mitochondria contact sites for neurodegeneration.

S3-08 Lipid droplets-drived autophagy flux promote sorafenib resistance in hepatocellular carcinoma

Yuh-Harn Wu[1], Yi-Li Chen[1], Ting-You Yen[2], Cheng-Yi Chen[2]

[1]Insititute of Basic Medical Science, National Cheng Kung University, Tainan 70101, Taiwan.
[2]Department of Cell Biology and Anatomy, National Cheng Kung University, Tainan 70101, Taiwan

According to the global cancer statistics report, hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the third leading cause of cancer-related death worldwide. Sorafenib is the first certified multi-targeted tyrosine kinase inhibitor that inhibits tumor growth and angiogenesis by blocking protein phosphorylation. Although clinical data indicate that sorafenib can improve the survival rate of patients with advanced HCC, the acquired resistance still persists. Therefore, addressing drug resistance has become a crucial issue for HCC treatment. Lipid droplets (LDs) are lipid-rich organelles characterized by a neutral lipid core surrounded by a phospholipid monolayer. They participate in neutral lipid storage in eukaryotic cells. LDs accumulation has been associated with inflammation and tumorigenesis in various cancer types, which recent studies also indicate the involvement of LDs in mediating chemoresistance. Therefore, we aimed to investigate the role of LDs in HCC. Our preliminary results found that sorafenib resistance (SR) cells provide higher viability, migration and tumor growth compared to parental cells (PC), which also presence lower cell death ratio under sorafenib treatment. The results confirmed the acquired resistance abilities against sorafenib in these SR cells. By utilizing Oil red O and LipidTOX staining, we found that SR cells exist higher LDs than PC cells, which the levels of LDs can be reduced by LDs inhibitors. To further explore the role of LDs in sorafenib resistance, we treated HCC cells with sorafenib and noticed that sorafenib decreased LDs formation. There are two major pathways for LD catabolism regulation, including lipolysis and lipophagy. Considering that SR cells presence higher LDs and lower autophagy ratio, we hypothesized that sorafenib reduced LDs through autophagy. Suppression of autophagy by inhibitors showed an increment in LDs by western blot and immunofluorescence staining, suggesting that reduction of LDs was driven by sorafenib-induced autophagy. Taken together, our findings revealed that sorafenib decreased LDs in HCC PC cells but less effective in SR cells. LDs also provide malignancy phenotypes in SR cells, thus targeting LDs may be an additional approach in conquering target therapy resistance in HCC.

S3-09 Mechanistic Investigation of the Pathophysiological Role of Neutral Sphingomyelinase-3 in Microcephaly

Kai-Hung Chen, Fei-Yang Tzou, Yi-Jhan Li, Cheng-Li Hong, Wan-Syuan Lin, Hsin-Chun Yeh, Yi Hsiao, Chia-Heng Hsu, Jeng-Lin Li, Chih-Chiang Chan*

* Correspondent
Graduate Institute of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan

Sphingolipids are a class of bioactive lipids present in many organelles, with particularly high levels in neuronal cells. Sphingomyelin (SM), the most abundant sphingolipid in cellular membranes, undergoes hydrolysis by sphingomyelinase (SMase) enzymes, producing ceramide and influencing membrane integrity and signaling pathways. SMases are categorized into neutral and acidic SMases based on their pH optimum, thus regulating SM levels in different subcellular compartments. These enzymes are implicated in multiple neurological disorders, including microcephaly, Niemann-Pick disease type A/B, and Parkinson's disease. The human gene SMPD4 encodes a neutral sphingomyelinase-3, which localizes to the ER and nuclear envelope. Previous studies showed that mutations in SMPD4 caused microcephaly, a neurodevelopment disorder, yet the pathogenic mechanism remains largely unknown.

To unveil the function of SMPD4, we use drosophila as a model and focus on the gene CG6962, a Drosophila homolog of SMPD4 in humans. We generated CG6962/SMPD4-reporter flies tagged with 3xHA epitope and Gal4 fragment by CRISPR-mediated genome editing. Expression profiling of the reporter fly showed that CG6962/SMPD4 is primarily expressed in neuroblast and neuron in the brain and the protein is localized to the nuclear envelope. Moreover, our comprehensive investigation, ranging from organelle-level characterization to functional testing, provides deeper insights into the underlying mechanisms governing CG6962/SMPD4 function. Our loss of function studies showed that CG6962-KO exhibited nuclear blebbing, decreased mature neuron numbers, and impaired cognitive function. These findings underscore the critical role of CG6962 in preserving nuclear envelope integrity and safeguarding learning and memory functions. Additionally, our observations of reduced larval brain size in CG6962-KO suggest the importance of CG6962 during neurodevelopment. Biochemically, we reveal that CG6962/SMPD4 regulates specific SM species. In summary, these results suggest the critical role of CG6962/SMPD4 in regulating specific SM species to maintain nuclear envelope structure and is essential for maintaining brain development and neuronal integrity in stabilizing cognitive functions.

This abstract has been previously published at Asia Pacific Drosophila Neurobiology Conference 3 (APDNC3) held at Tokyo, Japan on 2024.02.27.

S3-10 Metformin promotes association of lipid droplets with mitochondria, enhancing fatty acid oxidation and reducing lipid buildup in pancreatic β-cells exposed to lipotoxic stress

Aneta M. Dobosz, Ewelina Krogulec, Maria Kendziorek, Justyna Janikiewicz, Nataniel Stefanowski, Agnieszka Dobrzyn

Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

Pancreatic β-cell dysfunction arising from impaired lipid homeostasis is a major pathophysiological abnormality in type 2 diabetes. Chronic exposure to elevated levels of free fatty acids contributes to pancreatic β-cell failure through various mechanisms, including the impairment of mitochondrial dynamics and insulin release. Metformin (1,1-dimethylbiguanide hydrochloride) is currently the most widely used antidiabetic agent which reduces lipotoxicity-induced ectopic fat accumulation, apoptosis, and oxidative stress in insulin-targeted tissues. However, the mechanisms of metformin's action in pancreatic islets remain unclear. In this study, we aimed to investigate the direct effect of metformin on lipid storage and utilization in pancreatic β-cells and on the maintenance of β-cell function. Experiments were conducted using INS-1E pancreatic β-cell line, stimulated with metformin alone or in combination with palmitate. Our results showed that metformin decreased both the number and size of lipid droplets (LDs) in palmitate-treated INS-1E cells. This was accompanied by higher abundance of perilipin 5 (PLIN5), which decorates LDs surface and mediates LD interactions with other organelles. Consistently, metformin promoted the physical contact of mitochondria with LDs, increased mitochondria elongation and stimulated mitochondrial fatty acid β-oxidation in INS-1E cells subjected to lipotoxic insult. Additionally, under conditions of lipid overload, metformin limited the activity of adipose triglyceride lipase (ATGL) and lipogenic regulators such as stearoyl-CoA desaturase (SCD1). An assessment of the overall lipidome revealed that metformin alleviated excessive neutral lipid accumulation and partially reversed the palmitate-induced disruption of insulin secretion in INS-1E cells. Altogether, metformin may impact pancreatic β-cell susceptibility to palmitate by adjusting fatty acid supply and oxidation to prevent lipotoxic stress. These findings provide additional mechanistic insights toward better understanding the pleiotropic effects of metformin and its role in regulating β-cell function.

Short Talks

S3-11 Modulation of Fatty Acid Desaturases for the Study on the Roles of Polyunsaturated Fatty Acids (PUFAs) in Metabolic Syndrome

Hyeon-Cheol Lee-Okada[1], Misa Ando[1, 2], Takehiko Yokomizo[1]

[1]Department of Biochemistry, Juntendo University Graduate School of Medicine.
[2]Department of Breast Oncology, Juntendo University School of Medicine.

Polyunsaturated fatty acids (PUFAs) are essential components of cell membranes. The content and diversity of PUFA molecular species in each tissue and cell type are highly dependent on the daily diet. However, genetic variations in the genes responsible for PUFA biosynthesis also represent an important factor. Indeed, recent studies have indicated that the reduction in the activity of fatty acid desaturases FADS1 and FADS2, which are essential for PUFA biosynthesis, may contribute to the development of various diseases, including metabolic syndrome. The objective of this study was to ascertain whether mice with decreased FADS1 and FADS2 activities could serve as a useful model for investigating the role of PUFAs in the pathogenesis of metabolic syndrome. Mice heterozygously lacking both *Fads1* and *Fads2* genes (*Fads(Δ/+)* mice) fed a normal chow diet exhibited no discernible difference in body weight, liver weight, or triacylglycerol (TAG) and cholesterol content in the liver compared to the control wild-type (WT) mice. In contrast, when fed a high-fructose diet, female *Fads(Δ/+)* mice exhibited a significantly elevated hepatic TAG content compared to female WT mice. Notably, the exacerbation of the fatty liver phenotype was not observed in male *Fads(Δ/+)* mice. Furthermore, liver-specific FADS1/2 double knockout mice (LKO mice) were generated and the following was observed: 1) When fed a semi-purified diet AIN-93G, which contains essential fatty acids linoleic acid and alpha-linolenic acid but lacks downstream PUFAs such as arachidonic acid and docosahexaenoic acid, LKO mice exhibited a less severe fatty liver phenotype than global FADS1/2 knockout mice. 2) LKO dams conceived and gave birth without any discernible abnormality, but their milk contained less PUFAs than the control dams. This indicates that the liver is the primary but not exclusive organ responsible for supplying PUFAs to the body. In conclusion, these results suggest that modulating genes involved in PUFA biosynthesis in mice represents a promising approach for modeling various types of metabolic syndrome and investigating the roles of PUFAs in their pathogenesis.

Short Talks

S3-12 Oleate Promotes Triple Negative Breast Cancer Cell Migration by Enhancing Filopodia Formation through a PLD/Cdc42-dependent Pathway

Catherine Mounier. Zhiqiang Guo and Karl-Frédérik Bergeron

Biological Sciences Department, Université du Québec à Montréal (UQAM), Montréal, QC H2X 1Y4, Canada

Breast cancer, particularly triple-negative breast cancer (TNBC), poses a global health challenge. Emerging evidence has established a positive association between elevated levels of stearoyl-CoA desaturase 1 (SCD1) and its product oleate (OA) with cancer development and metastasis.
SCD1/OA leads to alterations in migration speed, direction, and cell morphology in TNBC cells, yet the underlying molecular mechanisms remain elusive. To address this gap, we aim to investigate the impact of OA on remodeling the actin structure in TNBC cell lines, and the underlying signaling. Using TNBC cell lines and bioinformatics tools, we show that OA stimulation induces rapid cell membrane ruffling and enhances filopodia formation. OA treatment triggers the subcellular translocation of Arp2/3 complex and Cdc42. Inhibiting Cdc42, not the Arp2/3 complex, effectively abolishes OA-induced filopodia formation and cell migration. Additionally, our findings suggest that phospholipase D is involved in Cdc42-dependent filopodia formation and cell migration. Lastly, the elevated expression of Cdc42 in breast tumor tissues is associated with a lower survival rate in TNBC patients.
Our study outlines a new signaling pathway in the OA-induced migration of TNBC cells, via the promotion of Cdc42-dependent filopodia formation, providing a novel insight for therapeutic strategies in TNBC treatment.

S3-13 Role of Bone Marrow Adipose Tissue in Uremic Toxin Indoxyl Sulfate-Related Chronic Kidney Disease-Minerals and Bone Diseases in a Mouse Model

鐘耀邦 Yao-Pang Chung[1], Yi-Hsin Lo[1], Rong-Sen Yang[2], Shing-Hwa Liu[1]

[1] Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan.
[2] Department of Orthopaedics, College of Medicine, National Taiwan University, Taipei, Taiwan.

Uremic toxin indoxyl sulfate (IS) is known to accumulate in patients with chronic kidney disease (CKD). The accumulation of IS and metabolic disorders caused by CKD will disrupt the bone metabolic balance, potentially leading to CKD-minerals and bone diseases (CKD-MBD). Although past studies have shown that IS induces osteoporosis through reduced osteoblast activity and increased osteoclast activity, the involvement of bone marrow adipose tissue (BMAT) has not been mentioned. Recently, more studies have suggested that BMAT is related to metabolic diseases such as aging, obesity, and diabetes. However, it is still unclear whether BMAT is involved in CKD-MBD.
AST120, an IS scavenger, has been shown to effectively improve CKD. Here, we investigated whether BMAT played a role in inducing CKD-MBD in an adenine-induced CKD mouse model, and explored whether AST120 could have a therapeutic effect. The C57BL/6 mice were divided into three groups: control group, adenine group, and adenine+AST120 group. Mice were administered by oral gavage with 50 mg/kg adenine daily for 28 days to induce CKD in the presence or absence of AST120 by oral gavage with 4 mg/kg daily for the last 14 days. First of all, the micro-CT results showed that adenine exposure significantly reduced bone mineral density (BMD) and altered the cortical bone and trabecular bone microstructures. The serum biochemical values showed that the concentrations of IS, calcium, and phosphorus in the adenine group were significantly higher than in the control group. In addition, the serum levels of osteocalcin (bone formation marker), CTX-1 (bone resorption marker), and intact parathyroid hormone (iPTH) also increased in the adenine group. Further analysis using H&E staining and Oil Red O staining revealed an increased number of bone tissue pores and a higher accumulation of lipid droplets in the adenine group. Analysis with Osmium tetroxide staining indicated a significant increase in both the quantity and area of BMAT in the adenine group. Results from osteoblast and adipocyte marker proteins showed that the expression levels of these proteins in the adenine group were significantly higher than in the control group. Additionally, immunofluorescence staining analysis demonstrated greater co-expression of RUNX2 and PPAR-γ in the adenine group. These changes in the bones of the adenine group were effectively reversed by treatment with AST120. Taken together, the above results indicate that IS is involved in CKD-MBD in an adenine-induced CKD mouse model, and BMAT may play an important role in this process.

Keywords
Chronic kidney disease, Indoxyl sulfate, CKD-MBD, bone marrow adipose tissue

Short Talks

S3-14 Subclass-specific impact of ether lipid deficiency on murine rhizomelic chondrodysplasia punctata-like phenotypes

Ilaria Dorigatti[1], Charlotte Brecx[2], Denise Kummer[1], Georg Golderer1, Ernst R. Werner[1], Fabian Dorninger[2], Johannes Berger[2], Katrin Watschinger[1]

[1]Institute of Molecular Biochemistry, Biocenter; Medical University of Innsbruck, Innsbruck, Austria;
[2]Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna, Vienna, Austria

Among the wide variety of lipids in the mammalian body, there is the class of ether lipids that fulfills many important functions including structuring the brain, protecting the eye from cataract, enabling male fertility and signaling. Ether lipids can be classified as plasmanyl and plasmenyl lipids (better known as plasmalogens), depending on the absence or presence of a vinyl ether double bond in the fatty alcohol attached to the sn-1 position of glycerol, which is introduced by plasmanylethanolamine desaturase (PEDS1). Ether lipid deficiency in humans, due to mutations in enzymes catalyzing the early enzymatic steps, e.g. glyceronephosphate O-acyltransferase (GNPAT), lead to rhizomelic chondrodysplasia punctata (RCDP) severely affecting many tissues including the brain, eye, and bone. Investigations to dissect the subclass-specific importance and impact of the two main ether lipid subclasses that ultimately lead to the defects mentioned above were long impeded by the fact that the genetic identity of PEDS1 was not known.

The recent discovery of the Peds1 gene and the availability of Peds1 knockout mice, selectively lacking plasmalogens, allow us now to compare the phenotypes of these mice, their tissues and cells with those of Gnpat knockout mice, which are devoid of all ether lipids. We have bred both mouse models on the same background and are currently conducting a head-to-head analysis evaluating osteogenesis, brain function, eye structure and function, fertility and adipogenesis.

First results clearly show that Peds1 knockout results in a milder overall phenotype than Gnpat knockout in the murine organism and also some of the so far investigated cellular functions remain intact if mice lack only plasmalogens, but can synthesize plasmanyl lipids. This reveals that the presence of plasmanyl lipids is more beneficial than originally thought for the mammalian body and at least some of the investigated cellular processes are not hampered by selective plasmalogen deficiency.

Our study sheds light on the importance of plasmalogens and will dissect their role from that of the plasmanyl lipids, which comprise important biologically active lipids like platelet-activating factor and seminolipid that have not received adequate attention so far in the field of ether lipid metabolism.

S3-15 The impact of lipid accumulation on the redox status in a cellular model depicting the progression from steatosis to steatosis with inflammation

Barbara Pakula[1], Agnieszka Karkucinska-Wieckowska[2], Olga Horakova[3], Magdalena Lebiedzinska Arciszewska[1], Isaiah Sabinari[3], Maciej Pronicki[2], M. Rossmeisl[3], Patrycja Jakubek-Olszewska[1#], Mariusz R. Wieckowski[1#]

[1]Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland
[2]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland
[3]Laboratory of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
[#]co-senior authorship

According to World Health Organization, 60% of adults are overweight or obese. As an adaptation to excessive food intake, hepatocytes accumulate triacylglycerols in a safe lipid droplet form and enhance fatty acid oxidation. When cells' capacity to safely store and remove excessive lipids is exceeded, metabolic dysfunction-associated steatotic liver disease (MASLD) can be developed.
MASLD has been estimated to affect roughly 30% of the global population. In 20% of these patients, the disease progresses to a more severe condition - metabolic dysfunction-associated steatohepatitis (MASH).
The aim of this study was to investigate the impact of progression from simple
steatosis to steatosis with inflammation on redox status parameters in HepG2/C3A cells. A cellular model mimicking the progressive steatotic condition in hepatocytes of MASLD patients is based on HepG2/C3A cells incubated with a mixture of free fatty acids (FFAs), reflecting the lipid composition in hepatocytes of patients with fatty liver, and FFAs in combination with tumor necrosis factor alpha (TNFa), mimicking steatosis with inflammation in MASH patients' liver. The investigated parameters included levels of superoxide and general ROS, as well as levels of proteins involved in the antioxidant response, such as NRF2, CAT, SOD1/2, and GPX4. We also examined the levels of oxidative damage markers, such as carbonylated proteins and 4-hydroxynonenal (HNE)-modified proteins (a marker of lipid peroxidation). The obtained results provide valuable insights into the impact of progression from simple steatosis to steatosis with inflammation on cellular redox status, contributing to a better understanding of the mechanisms underlying MASLD progression, which remains poorly understood.

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S3-16 The impact of omega 3-6-9 fatty acids treatment on proteome of lipid metabolism-related pathways in fibroblasts from patients with Neurodegeneration with Brain Iron Accumulation (NBIA)

Agata Wydrych[1], Barbara Pakuła[1], Patrycja Jakubek-Olszewska[1], Justyna Janikiewicz[2], Aneta M. Dobosz[2], Iwona Kurkowska-Jastrzębska[3], Paolo Pinton[4], Werner J.H. Koopman[2], Agnieszka Dobrzyń[2], Marta Skowrońska[3], Magdalena Lebiedzińska-Arciszewska[1], Mariusz R. Wieckowski[1]

[1]Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland
[2]Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, Warsaw, Poland
[3]IInd Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland
[4]Laboratory for Technologies of Advanced Therapies, Department of Medical Sciences, Section of Experimental Medicine, University of Ferrara, Ferrara, Italy
[5]Radboud University Medical Center, Nijmegen, The Netherlands.

Neurodegeneration with Brain Iron Accumulation (NBIA) is a heterogeneous group of inherited diseases expressed by an excessive iron accumulation in the brain with prevalence rate of 1-3 patients/1000000 people. This rare disease is usually associated with slowly progressive pyramidal and extrapyramidal symptoms, axonal motor neuropathy, optic nerve atrophy and cognitive impairment. Recently, eleven NBIA subtypes have been distinguished. The four most common NBIA subtypes include pantothenate kinase-associated neurodegeneration (PKAN), PLA2G6-associated neurodegeneration (PLAN), mitochondrial membrane protein-associated neurodegeneration (MPAN) and beta-propeller protein-associated neurodegeneration (BPAN). Mutations in the NBIA-associated genes are known to affect several metabolic pathways including lipid metabolism as coenzyme A synthesis (in PKAN), fatty acid metabolism (hypothesis) (in MPAN) or membrane phospholipid biosynthesis (in PLAN) including synthesis of cardiolipin.

Our study was focused on the revealing of alterations in the lipid metabolism pathways in two most common NBIA subtypes- PKAN and MPAN. We analyzed proteomes of fibroblast derived from 20 patients suffering from the MPAN and 5 patients suffering from the PKAN subtypes of NBIA focusing on proteins involved in the lipid metabolism pathways. Moreover, we have investigated how treatment with omega 3-6-9 fatty acids impacts patients' fibroblasts proteome. We intend to clarify whether changes observed in cellular proteomes could explain beneficial effect of omega 3-6-9 fatty in NBIA patients.

The study is co-financed from the state budget from the Education and Science Ministry program entitled "Science for Society". Project number: NdS/537386/2021/2022, the amount of co-financing 1 900 000 PLN, total value of the project 1 900 000 PLN. Poland

S3-17 The Role of Cachectic Fat Loss in The Progression of Pancreatic Ductal Adenocarcinoma

Yu-Chun Lin (林俞君)[1], Ya-Chin Hou (侯雅琴)[1, 2], Yan-Shen Shan (沈延盛)[1, 2, 3]

[1]Institute of Clinical Medicine, College of Medicine, National Cheng Kung University
[2]Department of Surgery, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University; Tainan, Taiwan.
[3]Center of Comparative Medicine and Research, Innovation Headquarters, National Cheng Kung University; Tainan, Taiwan.

Pancreatic cancer (PC) is one of the most lethal tumors, often associated with a high incidence of cancer cachexia, which may influence patient condition and cause limited treatment efficacy. Systemic inflammation during cancer progression increases catabolic reactions in various tissues. Notably, fat tissue loss has been linked to lower survival in PC patients. In this study, we aim to uncover the mechanisms behind PC-induced fat loss and its impact on tumor progression. We have identified that interleukin-8 secreted by PC cells activates lipolysis and the fat browning pathway in mature adipocytes. The free fatty acids released by adipocytes enter the surrounding environment, promoting PC cell migration. Additionally, we observed enlarged very-low-density lipoprotein (VLDL) particles in the plasma of PC patients at late cancer stages or those with cachexia. Increased hepatic fat accumulation was also found in mice bearing pancreatic tumors, linking abnormal systemic lipid metabolism to PC development. Understanding the role of VLDL in PC progression may offer novel diagnostic or therapeutic strategies for clinical application.

S3-18 The Role of Endothelial Cells in the Development of Metabolic Diseases

Chien-Cheng Lai[1], Haaglim Cho[1], Robert Gurke[2], Stefan Offermanns[1,3,4,5]

[1] Department of Pharmacology, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
[2] Fraunhofer Institute for Translational Medicine and Pharmacology and Fraunhofer Cluster of Excellence for Immune Mediated Diseases, Frankfurt am Main, Germany
[3] Cardiopulmonary Institute, Bad Nauheim, Germany
[4] Center for Molecular Medicine, Goethe University Frankfurt, Germany
[5] German Center for Cardiovascular Research, partner site Frankfurt / Rhine-Main, Bad Nauheim, Germany

Diet-induced obesity (DIO) induces insulin resistance and increases the risk of cardiovascular diseases. Recent studies suggest that pathological vascular process, such as endothelial dysfunction, also plays a critical role in regulating systemic metabolism. Previous studies showed that already short-term high-fat diet (HFD) feeding induced glucose intolerance and insulin resistance. However, the underlying mechanism is unclear. Here we show that 3 days of HFD caused endothelial insulin resistance indicated by impaired insulin-induced endothelial NO-synthase (eNOS) activation in adipose tissues and reduced insulin-induced skeletal muscle perfusion. Moreover, C16 ceramide, whose plasma levels were found to increase after 3 days of HFD feeding, downregulated insulin-induced signaling in the endothelium. Interestingly, C16 ceramide induced the formation of lipid droplets, and the inhibition of lipid droplet accumulation rescued ceramide-induced inhibition of endothelial insulin signaling. Our findings highlight the importance of lipid metabolism in endothelial insulin signaling. Further studies will focus on the role of endothelial lipid metabolism and insulin resistance in short-term HFD-induced impairment of systemic metabolism, and aim to identify diagnostic markers or therapeutic targets to prevent and treat metabolic diseases at an early stage.

Short Talks

S3-19 The Role of Lipid Droplet Dynamics in Mediating CDK4/6 Inhibitor Resistance

Chi-Hsun Yang[1,2], Jie-Ning Li[1,2,3,4], Yu-Jhen Lyu[2,5], Hung-Yu Sun[6], Pai-Sheng Chen[1,2,3,4]

[1]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [2]Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [3]Breast Medical Center, National Cheng Kung University Hospital, Tainan, Taiwan. [4]Research Center for Medical Laboratory Biotechnology, National Cheng Kung University, Tainan, Taiwan. [5]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan. [6]Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

Luminal-type (estrogen receptor positive (ER+)) breast cancer accounts for ~70% of all types of breast cancer patients. Palbociclib, ribociclib and abemaciclib, the cyclin-dependent kinases 4 and 6 inhibitors (CDKis), are used as targeted therapy for ER+ advanced breast cancer. Unfortunately, cancers often undergo evolutionary adaptation that leads to the development of metastasis or recurrence following long-term treatment. In this study, we found that CDK4/6 inhibitor-resistant (CDKiR) cells are strongly dependent on lipid metabolism for their growth advantage, as disrupting lipid supply or inhibiting lipid transport into mitochondria resulted in impaired cell growth and increased sensitivity to CDK4/6 inhibitors. We also observed a dramatic dispersion of lipid droplets (LDs), cellular organelles involved in lipid storage and metabolism, in CDKiR cells. Moreover, these dispersed LDs are distributed in close proximity to mitochondria and co-localized with lysosomes, which in turn promotes their degradation, enhances lipophagy, and enables the efficient transfer of free fatty acids to mitochondria for lipid metabolism. Mechanistically, the dispersion of LDs is closely linked to the acetylation of tubulin. CDKiR cells exhibited a decrease in tubulin acetylation, which is associated with LD dispersion, while in contrast, enhanced tubulin acetylation in CDKiR cells leads to the clustering and accumulation of LDs within the cells, along with the loss of interaction with both mitochondria and lysosomes. Here, we discovered that CDKi resistance is achieved through the coordinated interaction of three key cellular organelles—mitochondria, dispersed lipid droplets, and lysosomes—thereby accelerating lipid metabolism to promote cell growth and survival. Understanding the mechanisms governing LD dispersion and developing strategies to effectively control lipophagy could offer potential therapeutic approaches for overcoming CDKi resistance.

Short Talks

S3-20 Tipping the switch between exosome secretion and autophagy by altered balance between ceramide and dihydroceramide

Shu-Yi Huang

Department of Medical Research, National Taiwan University Hospital

Dihydroceramide desaturases (mainly DEGS1 in humans, Ifc in Drosophila) are highly conserved enzymes catalyzing the conversion of dihydroceramide to ceramide in the de novo sphingolipid biosynthesis pathway. DEGS1 or Ifc deficiencies cause dihydroceramide accumulation and autophagy induction, resulting in light-induced neurodegeneration in flies and hypomyelinating leukodystrophy in human, underscoring the critical role of DEGS1 in the nervous system. While ceramide is a crucial constituent of exosome membranes, the effect of elevated dihydroceramide levels on exosome biogenesis remained unexplored. Exosomes are important mediators of intercellular communication. Because of the small size, it has not been easy to study the regulation of exosome biogenesis in an in vivo setting. We developed a novel assay with spatially controlled expression of exosome markers in Drosophila, allowing the visualization of exosome production in vivo. We showed that Ifc promoted exosome biogenesis by facilitating intraluminal vesicle (ILV) formation in vivo and in vitro. Moreover, DEGS1/Ifc may also promote exosome production by inhibiting the autophagic degradation of ILV-containing multivesicular endosomes. We have further analyzed how DEGS1/Ifc influenced exosome production and autophagy to elucidate the consequences of altered equilibrium between dihydroceramide and ceramide.

Short Talks

S3-21 To Investigate the Expression of Triacylglycerols in Epididymal Adipose Tissue of Diabetic Mice

Yu-Ju Shih[1], Mei-Ling Cheng[1, 2]

[1]Department of Biomedical Sciences, Chang Gung University, Guishan, Taoyuan. [2]Metabolomics Core Laboratory, Healthy Aging Research Center, Chang Gung University, Taoyuan

One white adipose tissue functions as an energy storage organ; in animals, triglycerides are primarily the principal energy reservoir. The composition of triglycerides within visceral adipose tissue via lipolysis generates free fatty acids, which are pivotal in influencing metabolic outcomes in the organism. In this study, we employed supercritical chromatography-tandem mass spectrometry to identify the profiles of both triglyceride and fatty acid within the epididymal adipose tissue of mice. We investigated variations in triglyceride levels within adipose tissue concerning genotype and age, utilizing mice with type 2 diabetes as an animal model. Our findings indicate that mice with type 2 diabetes display reduced triglyceride and fatty acid levels per unit tissue weight compared to the control group. Notably, this research illustrates an increase in palmitoleic acid expression within adipose tissue with increased feeding weeks, a phenomenon correlated with aging. Our study has revealed novel insights when simulating type 2 diabetes in our animal model. We have observed that the rise in palmitoleic acid within adipose tissue was delayed in the db/db group compared to the db/m and m/m groups. Interestingly, alterations in feed composition did not induce significant changes in the fatty acid profile within the mice's epididymal adipose tissue. These unique variances contribute to understanding the specific fatty acids crucial for triglyceride synthesis in adipose tissue and identifying metabolic disparities under diabetic conditions. Moreover, these results suggest the presence of autonomous lipid synthesis systems within adipose tissue, a finding that opens up new avenues for research in this field.

Notes:
Part or all of this poster presentation has been published in the following seminars:

1. American Society for Mass Spectrometry (ASMS) 2023 Annual Conference held at HOUSTON (USA) on June 5 to 8, 2023.
2. Taiwan Society for Mass Spectrometry (TSMS) 2023 Annual Conference held at Taichung (ROC) on July 5 to 6, 2023.
3. Taiwan Society for Mass Spectrometry (TSMS) 2024 Annual Conference held at Tainan (ROC) on June 26 to 28, 2024.

S3-22 Uremic Toxin Indoxyl Sulfate Disrupts the Balance Between Osteogenic and Adipogenic Differentiation and Induces Lipid Droplet Accumulation in Osteoblasts

Yi-Hsin Lo[1], Yao-Pang Chung[1], Rong-Sen Yang[2], Shing-Hwa Liu[1]

[1]Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan. [2]Department of Orthopaedics, College of Medicine, National Taiwan University, Taipei, Taiwan.

Chronic kidney disease (CKD)-mineral and bone disorder (CKD-MBD) is a systemic disturbance of mineral and bone metabolism caused by CKD, which may be associated with the uremic toxin indoxyl sulfate (IS). In clinical studies, there is an association between moderate to severe CKD and increased bone marrow adiposity. Recent studies have shown that Runx2+ cells produce lipid droplets during differentiation under osteogenic culture, which may be one of the reasons for bone marrow adiposity. Additionally, IS inhibits osteoblast mineralization and impairs bone health. However, the role of IS in bone marrow adiposity remains unclear. Therefore, the aim of this study is to investigate whether IS affects balance between osteogenic and adipogenic differentiation and induces lipid droplet accumulation. The murine preosteoblast 7F2 cells were induced for osteogenic and adipogenic differentiation using a 1:1 mixture of osteogenic and adipogenic differentiation medium in the presence or absence of IS (0.1-1 mM) exposure. The effects of IS on both osteogenic and adipogenic differentiation were investigated individually at days 3, 6, 10, 12, and 14 of differentiation. Oil red O staining revealed a significant increase in lipid accumulation in the IS-treated cells compared to the control cells on days 3, 6, and 10 ($p<0.05$, $n=6$). Additionally, Alizarin Red S staining showed a significant decrease in mineralization in the IS-treated cells compared to the control cells on day 14 ($p<0.05$, $n=6$). During osteogenic differentiation, IS significantly inhibited the expression of Runx family transcription factor 2 (Runx2) on day 3, and suppressed the expression of osteopontin and collagen 1A1 on days 12 and 14 ($p<0.05$, $n=6$). However, during adipogenic differentiation, IS significantly increased the expression of peroxisome proliferator-activated receptor-gamma (PPAR-gamma), CCAAT/enhancer binding protein-alpha, and adiponectin on day 3, and significantly increased the expression of adipsin on day 10 ($p<0.05$, $n=6$). Under co-differentiation/culture conditions, we also observed the presence of lipid droplets in osteoblasts (lipid+ osteoblasts; co-expressed Runx2 and PPAR-gamma proteins) by immunofluorescence staining, which could be significantly enhanced by IS exposure ($p<0.05$, $n=6$). Taken together, these findings suggest that IS promotes the adipogenic differentiation in the early stage and inhibits the osteogenic differentiation in the later stage under co-differentiation/culture conditions, leading to an increase in lipid+ osteoblasts. The IS-enhanced lipid+ osteoblasts may play an important role in CKD-MBD.

Key words
Indoxyl sulfate, Osteogenic differentiation, Adipogenic differentiation, Lipid droplet

S4-01 Alteration of Ceramide-1 Phosphate during S Phase

Kenta Nakamura[1], Takuya Honda[1], Miaki Uzu[1], Hiroyuki Nakamura[1]

[1]Department of Pharmacy, Chiba University.

Background DNA is replicated during the DNA synthesis phase (S phase) in the cell cycle. The DNA replication mechanism is essential for cells to proliferate normally, and S phase abnormalities cause carcinogenesis, cancer cell exacerbation, and drug resistance. Elucidation of the molecular mechanism of S phase leads to the prevention of carcinogenesis and pharmacological targets of anticancer drugs.Ceramide is a central lipid of sphingolipids, and ceramide and its metabolites are regulators of the cell cycle as second messengers in the cell, but most reports are related to the cell cycle outside of S phase. In our laboratory, We found that the production of ceramide-1-phosphate (C1P), a metabolite of ceramide phosphorylated by ceramide kinase (CerK), is greatly reduced duringS phase in HeLa cells. This may provide a clue to the link between lipids and S phase, which has not yet been reported.In this study, we focus on the decrease in C1P productivity during S phase and examine the mechanism of C1P decrease and the effect of C1P in S phase.

Methods Cell synchronization

HeLa cells are incubated with 4 mM Thymidine for 24 hours to synchronize cells in S phase.

Evaluation of Ceramide Metabolite Productivity

NBD-ceramide was added to the cells, metabolized, and the cells were lipid-extracted using the Bligh and Dyer method, developed using TLC plates, and detected using ChemiDoc-MP. Image J was used for quantification.

Result In S phase, C1P productivity is reduced due to a decrease in CerK.

Thymidine treatment synchronized the cells to early S phase and quantified for sphingolipid metabolism. The results showed that C1P productivity was decreased in the thymidine group compared to the untreated group. The results in Western Blotting showed a significant decrease in protein expression of CerK after thymidine treatment. This result indicates that the decrease in C1P production in S phase is due to the decrease in protein expression of CerK.

Degradation of CerK in S phase is accelerated.

We confirmed whether the decrease in protein expression of CerK in S phase was due to accelerated protein degradation by treating it with a proteolysis inhibitor. In this study, MG-132 was used as an inhibitor of the ubiquitin-proteasome pathway and Bafilomycin A1 was used as an inhibitor of the lysosome pathway. Western blotting results showed that thymidine treatment resulted in decreased CerK protein expression, which was recovered by treatment with the inhibitor. This result indicates that the decrease in CerK protein expression in S phase is due to accelerated proteolysis of CerK.

Discussion It was shown that CerK expression is downregulated in S phase of HeLa cells due to accelerated proteolysis of CerK. Based on these results, it is predicted that C1P・CerK affects the progression of S phase.

S4-02 Cross-Species Lipidomic Disruptions Induced by Silver Nanoparticles: Insights into Conserved Molecular Mechanisms

Sung Won Kwon[1]

[1]Department of Pharmacy, College of Pharmacy, Seoul National University

This study aims to elucidate the cross-species molecular mechanisms underlying the metabolic effects of silver nanoparticles, with a focus on conserved lipidomic disruptions. Silver nanoparticles, widely used for their antibacterial and antifungal properties, raise concerns about potential health and environmental risks. Despite numerous studies, inconsistent molecular responses across species have hindered clear conclusions on their metabolic impact. To address this, a cross-species analysis of transcriptomic data from various studies on silver nanoparticle exposure was conducted. Pathway enrichment meta-analysis identified conserved biological pathways, including oxidative stress, apoptosis, autophagy, and disruptions in metabolite and lipid metabolism, as potential key events across different organisms. These findings were validated through experiments on *Caenorhabditis elegans* and HepG2 cells, revealing that oxidative stress and mitochondrial impairment are early, conserved key events following silver nanoparticle exposure. Both models showed significant increases in mitochondrial reactive oxygen species levels and mitochondrial fragmentation, indicating similar stress responses across species. Moreover, untargeted lipidomic profiling revealed substantial disruptions in lipid metabolism, particularly affecting sulfur-containing amino acids and lipid homeostasis. These effects were size-dependent, with smaller nanoparticles causing more pronounced disruptions. In summary, this study highlights oxidative stress, mitochondrial dysfunction, and metabolic disruptions as conserved mechanisms induced by silver nanoparticles across species. These findings emphasize the importance of considering these pathways in regulatory assessments of nanoparticle metabolism, contributing to the development of safer and more sustainable nanomaterials. This abstract content has been previously published in the Journal of Hazardous Materials (2023 459: 132208).

Short Talks

S4-03 Discovery and Functional Analysis of a Novel Enzyme Phosphorylating Ceramide under Acidic Conditions

Ami Nishino[1], Ayako Yamazaki[1], Takafumi Kohama[1], Hiroyuki Nakamura[1]

[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University

Purpose: Ceramide is a centrally located sphingolipid and is metabolized by various metabolic enzymes. These metabolites have been reported to be involved in the composition of membranes and various other physiological functions. Ceramide kinase (CERK), one of the ceramide metabolizing enzymes, was identified in 2002 as an enzyme that phosphorylates ceramide to produce ceramide-1-phosphate (C1P). However, in cells and mice genetically deficient in CERK, the amount of C1P is reduced by only about 40%, suggesting the existence of a CERK-independent C1P generation pathway. In this study, we found the possibility of a novel enzyme that phosphorylates ceramide under acidic conditions and aimed to analyze the function of this enzyme.

Methods and results: Fluorescently labeled ceramide (NBD-Cer) was added to homogenate samples of HeLa cells genetically deficient in CERK (CERK-KO cells) and incubated at 37°C for 30 min. The generated NBD-C1P was separated by TLC and the fluorescence intensity was measured by an image analysis system. When NBD-Cer was metabolized at pH ranging from 2 to 7, NBD-C1P was significantly generated at pH 4-5 (acidic condition), while little NBD-C1P was generated at pH 7 (neutral condition), the optimal pH for conventional CERK. NBD-C1P formation under acidic conditions was no longer observed in the absence of ATP or by boiling (enzyme inactivation) the cell homogenate samples. NBD-C1P formation was also no longer observed by treatment with CIAP, alkaline phosphatase. Therefore, it was suggested that there is an enzyme (acidic CERK) that phosphorylates ceramide and produces C1P under acidic conditions. Next, the possibility that acidic CERK exists in lysosomes, an acidic organelle, was examined: lysosomes of CERK-KO cells were fractionated by density gradient centrifugation using OPTI-prep, and when NBD-Cer was metabolized at pH 4, NBD-C1P was observed to be produced.

Discussion: It was found that the optimal pH of conventional CERK (neutral CERK) is neutral and localizes to the neutral fraction, while acidic CERK has an acidic optimal pH and is found in the acidic fraction, lysosomes. This implies that neutral and acidic CERKs generate C1P in distinct cellular locations, and the C1P produced by each enzyme might have unique physiological functions. We will attempt to identify acidic CERKs and perform detailed functional analysis in the future.

S4-04 Dissecting the regulatory mechanisms of asynthetic fission

Yue Rong Tan, Keat Ying Chan, Tsai-Ming Lu, Chen-Hui Chen

Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Besides mitosis and meiosis, zebrafish skin superficial epithelial cells (SECs) can divide through a process termed 'asynthetic fission', which does not involve replicating their genomic DNA content. Since cell division is typically tightly controlled and coupled with DNA replication, this unexpected finding prompted us to investigate the underlying regulatory mechanism of the process. To identify genetic regulators that may participate in this atypical division process, we conducted bulk-RNA sequencing on the SECs collected at 2, 6, 14, and 21 dpf, covering the most prevalent developmental time period of the division. From the analysis, we identified a set of genes whose expression dynamics coincide with the prevalence of the division. We are currently examining these genes to determine their influence on the occurring of asynthetic fission and their potential impact on the DNA replication-competent skin basal stem cell population. Through both gain-of-function and loss-of-function assays, I aim to identify specific genes and regulatory networks essential for enabling asynthetic fission in vertebrate somatic cells.

S4-05 Illuminating neurosecretion: Optogenetic and click chemistry novel tools highlight the multiple roles of phosphatidic acid in neurotransmitter release.

Vitale N, Wolf A, Tanguy E, Ory S, and Gasman S

Institut for Cellular and Molecular Integrated Neurosciences, CNRS UPR3212, 8 Allée du Général Rouvillois, 67000 Strasbourg - France

The orchestrated release of neurotransmitters or hormones by secretory cells involves many vesicular trafficking steps for efficient and rapid release. In addition to key proteins, the contribution of lipids in these various steps along the secretory pathway has been recently postulated. Among them, phosphatidic acid (PA), the simplest glycerophospholipid, has been proposed to play pivotal roles in key trafficking steps, especially in membrane fusion and fission events, where lipid remodeling is deemed crucial.

For instance, using genetic knockdown, pharmacological inhibition of PA-producing enzymes, and PA sensors, we have highlighted the diverse contribution of this phospholipid across multiple stages of neurosecretion. Furthermore, lipidomic analysis of fractionated membranes has revealed the widespread presence of PA in numerous subcellular compartments and its active modulation during cellular stimulation. This sheds light on the complexity of PA signaling, with the existence of different PA pools defined not only in space, but also in time. However, establishing a functional link between these pools and the multiple functions attributed to PA has remained impossible using currently available tools. Hence, to overcome both spatial and temporal limitations, we developed a novel optogenetic strategy targeting lipid metabolism to specific organelles and new PA clickable PA analogues.

Hence, using light sensible PA metabolism enzymes to induce recruitment at specific subcellular membranes, we achieved by the minute modulation of PA levels within specific compartments. This precise control of PA levels coupled with confocal imaging to monitor exocytic sites enabled us, for the first time, to establish insights into the distinct pools of PA involved in specific steps of the secretory pathway. Furthermore, to preserve the biological properties of PA synthetic analogues, we developed a novel strategy for the synthesis of azide-based analogues allowing specific fatty acyl chain positioning. After functional validation of mono and poly-unsaturated forms of PA analogues in bovine chromaffin cells, we characterized their functional interactome during neurosecretion leading to the identification of known PA-interactors involved in exocytosis and many additional potential novel interactors. Altogether, these results validate the versality of these tools to study the biological activities of PA and could be extended to other glycerophospholipids.

S4-06 Primary Cilia-mediated Upregulation of CD36 and Fatty Acid Uptake Promote Chemoresistance in Pancreatic Ductal Adenocarcinoma

Ming-Yu Wu, I-Chen Peng

Department of Life Sciences, National Cheng Kung University, Tainan City 701, Taiwan.

Primary cilia, non-motile microtubule-based organelles extending from the basal body on the cell surface, play crucial roles in sensory perception and intracellular signaling. The loss of primary cilia is commonly observed in pancreatic ductal adenocarcinoma (PDAC), and the regeneration of primary cilia following cisplatin treatment suggests a potential involvement in chemoresistance mechanisms.
However, the regulatory mechanisms of ciliogenesis and their functions in chemotherapeutic sensitivity related to altered lipid metabolism in PDAC remain unclear. In this study, we observed an increased expression of glutamine synthetase (GS), the primary cilia-associated protein intraflagellar transporter 88 (IFT88), and cluster of differentiation 36 (CD36), along with enhanced fatty acid uptake in PANC-1 cells treated with cisplatin. Knockdown of GS reduced cilogenesis, CD36 expression, and fatty acid uptake in cells surviving cisplatin treatment. Similarly, knockdown of IFT88 led to decreased CD36 expression and fatty acid uptake. Furthermore, knockdown or inhibition of GS, IFT88, and CD36 resulted in a decreased IC50 (half maximal inhibitory concentration) of cisplatin in PANC-1 cells, indicating enhanced sensitivity to the drug. These findings suggest that GS promotes primary cilia formation, which in turn upregulates CD36 expression and fatty acid uptake in PANC-1 cells, thereby reducing the cytotoxic effects of cisplatin. This study provides preliminary insights into the role of GS in regulating primary cilia, lipid metabolism, and chemoresistance in PDAC, highlighting potential therapeutic targets.

S4-07 Role of Phospholipid Decomposition by the Lysophospholipase PNPLA7 in Hepatocytes

Tetsuya Hirabayashi[1], Mai Kawaguchi[1,2], Kohji Kasahara[1], Makoto Murakami[2]

[1] Biomembrane Group, Center for Basic Technology Research, Tokyo Metropolitan Institute of Medical Science
[2] Laboratory of Microenvironmental Metabolic Health Sciences, Graduate School of Medicine, The University of Tokyo

Choline is a vital nutrient used in the biosynthesis of phosphatidylcholine (PC), a major component of membrane phospholipids, and serves as an important source of methyl groups in the liver. Although the majority of choline is stored in membrane PC, how this endogenous choline is liberated and utilized has long been unknown. Focusing on patatin-like phospholipase domain containing 7 (PNPLA7), a lysophospholipase that is responsible for phospholipid decomposition of hepatocyte membranes, we investigated the physiological significance of this enzyme using phenotypic analyses of *Pnpla7*-deficient mice and primary cultured hepatocytes. Metabolomics analysis revealed significant decreases in glycerophosphocholine, choline, and other methyl donors betaine (*N,N,N*-trimethylglycine) and *S*-adenosylmethionine in the liver of global *Pnpla7*-deficient mice. These mutant mice exhibited growth defects and reduced body fat, probably due to methyl group deficiency. While feeding a methionine- and choline-deficient diet is a commonly used rodent model of steatotic liver disease, the triglyceride content was unexpectedly reduced in the liver of these deficient mice fed a normal diet. Since the expression of lipogenesis-related genes was strongly reduced in the global knockout of *Pnpla7*, further analyses were done using mice with hepatocyte-specific deletion of Pnpla7. In these liver-specific knockout mice, fasting-induced triglyceride accumulation in the liver was strongly suppressed despite the same expression levels of lipogenesis-related genes in the mutant mice and wild-type controls. Thus, PNPLA7-mediated phospholipid decomposition is crucial not only for endogenous choline mobilization from the membranes but also for triglyceride synthesis in the liver.

This abstract has been previously published in part as an abstract (Hirabayashi et al., *Cell* Reports, 42, 111940, 2023).

S4-08 Sphingolipids biosensor reveals cell type-specific mechanism of sphingolipid catabolism in the brain

Fei-Yang Tzou[1], Wan-Syuan Lin[1], Kai-Hung Chen[1], Chia-Heng Hsu[1], Cheng-Li Hong[1], Chih-Chiang Chan[1]

[1] Graduate Institute of Physiology, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Sphingolipids are critical components of the nervous system, influencing brain development and function. Neuronal cells produce a diverse array of sphingolipids with specific roles in neurobiology. Sphingolipid composition in the brain changes throughout development, aging, and disease progression. The role of lipid alterations in driving neurodevelopmental disorders and neurodegenerative diseases is an active area of research. Understanding sphingolipid dynamics in vivo remains challenging due to technical limitations in tracking lipids within and across tissues. Genetically encoded biosensors offer the potential to overcome this limitation by providing real-time, specific visualization of lipid molecules. We herein develop genetically encoded biosensors for tracking ceramide, glucosylceramide, and ceramide phosphoethanolamines (CerPE, the structure analog of sphingomyelin) in Drosophila melanogaster. These probes enable tissue/cell type-specific labeling of sphingolipids, visualizing their distribution, levels, and dynamics. By using the CerPE probe, we discovered how neurons and glia coordinate the CerPE degradation in the brain. The development of in vivo sphingolipid biosensors paves the way for understanding the pathophysiological role of sphingolipids ranging from subcellular to organismal levels.

S4-09 Sterol metabolism and homeostasis in plants

Hubert Schaller[1], Sylvain Darnet[1ε] , Andréa Hemmerlin[1]

[1]Institut de biologie moléculaire des plantes, IBMP-CNRS, Strasbourg University, Plant Isoprenoid Biology team, Strasbourg, France

Sterols are essential building blocks of membranes and precursors of hormones, both functions having specific molecular structural requirements in diverse eukaryotic organisms[1-3]. Astonishing variations in sterolomes in organisms are revealed as genomes are sequenced and functionally analyzed[4,5].

Master switches control the sterol biosynthetic pathway and interconversion of sterols to sterol conjugates to maintain a balanced membrane sterol level. This is achieved in plants by specific enzymes e.g. a phospholipid sterol acyltransferase[6]. Strikingly, genetic mechanisms implied in sterol homeostasis are different in mammals, fungi and plants. As a matter of fact, the well-known SREBP machinery is absent from yeast and plants. However, a common aspect between plants and other organisms is a key limiting role of HMGR, an upstream enzyme implied in the mevalonate/isoprenoid pathway[7-9]. Up- regulation of the biosynthetic flux in this metabolic segment results in plants in the accumulation of sterols in the form of sterol esters that are deposited in cytoplasmic lipid droplets. Genetic reprogramming in a tobacco mutant and the phenotypes of its hypersterolemic leaves and lipid droplets are investigated to determine processes and proteins associated with sterol ester formation and mobilization at the cellular level[10]. To identify major components of the regulatory circuits at play in isoprenoid and phytosterol homeostasis, we have characterized high sterol esters producers in several plant species using genetic approaches. We have designed genetic screens searching for second site suppressor mutations of loss-of-function mutants hampered in the production of C_5 isoprenic building blocks. One of these extragenic suppressor alleles encodes a protein that shares similarity with a mammalian membralin yet overlooked and most probably implied in the quality control of ER membrane proteins. We now describe a new component that regulates the production of sterols in plants and is conserved in various organisms.

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[ε]present address : Unité Interaction Arbres Microorganismes, INRA, Université de Nancy, Vandoeuvre-lès-Nancy, France

S4-10 Total synthesis of all stereoisomers of negative phospholipids

Masato Abe[1]

[1]Department of Bioscience, Graduate School of Agriculture, Ehime University

Negative phospholipids are not only involved in the function of biological membranes, but also influence biological phenomena through interactions with various membrane proteins. The homochirality of life is seen in these complex lipids, and the conformation of the acylated secondary hydroxyl groups is strictly controlled. However, stereoisomers of negative phospholipids not found in nature are expected to have potential bioactivity, but their details remain unknown due to lack of availability.

In this study, we aimed to synthesize all stereoisomers of various negative phospholipids using the total synthesis method. Specifically, phosphatidylserine (PS), phosphatidylglycerol (PG), and lysocardiolipin (Lyso-CL).

These have chiral carbons in both the polar head and hydrophobic tail, resulting in a total of four stereoisomers for PS and PG, and a total of eight stereoisomers for Lyso-CL.

We took advantage of the reverse synthesis analysis shown in Figure 1 and eventually achieved the synthesis of all stereoisomers. In this synthesis, we used the phosphoramidite method as the key reaction in a three-molecule condensation reaction, which resulted in a convergent and high-yield synthesis.

S4-11 TRPV4-dependent Ca^{2+} influx determines cholesterol dynamics at the plasma membrane

Yutaro Kuwashima[1,2], Masataka Yanagawa[3], Masashi Maekawa[1], Mitsuhiro Abe[4], Yasushi Sako[4], Makoto Arita[1,5,6,7]

[1]Division of Physiological Chemistry and Metabolism, Graduate School of Pharmaceutical Sciences, Keio University.
[2]Department of Integrated Medicine and Biochemistry, Keio University School of Medicine.
[3]Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University.
[4]Cellular Informatics Laboratory, RIKEN Cluster for Pioneering Research (CPR).
[5]Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences (IMS).
[6]Cellular and Molecular Epigenetics Laboratory, Graduate School of Medical Life Science.
[7]Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q), Keio University.

The function of the transient receptor potential vanilloid 4 (TRPV4), a Ca^{2+}-permeable non-selective cation channel, is regulated by surrounding membrane lipids, including cholesterol and phosphoinositides. The transmembrane region of TRPV4 contains a cholesterol recognition amino acid consensus (CRAC) motif and its inverted (CARC) motif in the cytosolic leaflet of the plasma membrane. Here, we visualized the spatiotemporal interactions between TRPV4 and cholesterol at the plasma membrane of living cells by dual-color single-molecule imaging with total internal reflection fluorescence microscopy (TIRFM). To achieve this, we labelled cholesterol in the cytosolic leaflet using a cholesterol biosensor, D4H. Single-molecule tracking analysis revealed that the TRPV4 molecules colocalize with D4H-accessible cholesterol molecules mainly in the low fluidity membrane domains, where both are highly clustered. Agonist-induced TRPV4 activation remarkably decreased colocalization probability and association rate between TRPV4 and D4H-accessible cholesterol molecules. Upon TRPV4 activation, the particle density of D4H-accessible cholesterol molecules was decreased, while those in the fast-diffusing state were increased at the plasma membrane. Introducing the skeletal dysplasia-associated R616Q mutation into the CRAC/CARC motif of TRPV4, which reduced its interaction with cholesterol clusters, did not affect the D4H-accessible cholesterol dynamics. Mechanistically, TRPV4-mediated Ca^{2+} influx and its C-terminal calmodulin-binding site are crucial for modulating the plasmalemmal D4H-accessible cholesterol dynamics. We propose that TRPV4 remodels its surrounding plasmalemmal environment by manipulating cholesterol dynamics through Ca^{2+} influx.

S4-12 Unsaturated Lipids Maintain Lipid Homeostasis for Nuclear Membrane Expansion and Mitotic Fidelity

You-Liang Cheng[1], Jung-En Kuan[1], Chao-Wen Wang[2], Rey-Huei Chen[1]

Institut for Cellular and Molecular Integrated Neurosciences, CNRS UPR3212, 8 Allée du Général Rouvillois, 67000 Strasbourg - France

Lipid composition determines biological membrane properties, including fluidity, thickness, and curvature, which underlie the structure and dynamics of membrane organelles. We explored how lipid saturation might affect the dynamics of nuclear membrane during mitosis in budding yeast, *Saccharomyces cerevisiae*. We used lipid desaturase mutant *ole1-20* to increase membrane saturation, and found that it upregulates many genes including *OLE1* through the membrane saturation sensor Mga2. *ole1-20 mga2Δ* cells display several phenotypes, including impaired nuclear expansion, spindle bending, prolonged anaphase, and uneven nuclear division. Both increasing membrane fluidity by adding glycerol and promoting de novo phospholipid synthesis through deleting *NEM1* can partially alleviate these phenotypes. Therefore, the anaphase defects in *ole1-20 mga2Δ* are likely caused by membrane rigidity and insufficient phospholipid synthesis. Our study uncovers fundamental link between lipid unsaturation and the dynamics of nuclear membrane during mitosis.

S4-13 Impact of lipid composition on mitochondrial function and cell fate decisions

Markus Keller

Institute of Human Genetics, Medical University of Innsbruck, Austria

Lipid diversity is substantially shaped by fatty acid availability that varies across cell types, tissues, and organisms, posing challenges to the lipid metabolic network to maintain functional homeostasis.
In this study we demonstrate that the mitochondrial respiratory chain remains functionally stable, even when subjected to considerable changes in membrane lipid composition. Despite substantial modulation of mitochondrial cardiolipin compositions, key parameters of oxidative phosphorylation were preserved. This suggests that mechanisms have evolved that efficiently buffer against variations in the lipid environment.
However, lipid diversity is impacting ferroptosis—a form of cell death driven by iron and lipid peroxidation. In contrast to the stability observed in oxidative phosphorylation, susceptibility to ferroptosis was significantly influenced by modifications of the lipid composition. Interestingly, in Barth syndrome, an inherited disorder affecting cardiolipin remodeling, cells exhibited an unexpected resistance to ferroptosis, despite elevated mitochondrial reactive oxygen species (ROS) levels. Conventional pro-ferroptotic factors, such as iron availability, were not responsible for this resistance. Instead, it was linked to the impaired formation of oligomeric structures in mitochondrial membrane transporter systems and reduced ER-mitochondria contact sites.
Thus, while stable membrane functions in varying lipid environments are essential, the regulation of lipid diversity remains a finely tuned process with profound implications for cell physiology and survival.

S5-01 10-second Classification of Lung Cancer Subtypes by Picosecond Infrared Laser Mass Spectrometry: Evaluation of Diagnostic Power Across Various Tissue Models

Lan Anna Ye[1], Michael Woolman[1], Francis Talbot[1], Alexa Fiorante, Yuki Sata[1], Hiroyuki Ogawa[1], Fumi Yokote[1], Nicholas Bernards[1], Michael Cabanero[1], Nhu-An Pham[1], Ming S Tsao[1], Benjamin H. Lok[1], Kazuhiro Yasufuku[1], Howard J. Ginsberg[2], and Arash Zarrine-Afsar [1]

[1]University Health Network, Toronto, ON
[2]Unity Health Toronto, St Michael's Hospital, Toronto, Ontario

Introduction: The diversity in prognosis, treatment and survival across lung cancer subtypes necessitates precise diagnosis. Leveraging the connection between lipid metabolism and cancer development, untargeted mass spectrometry profiling of tissue lipidome using various ambient ionization methods has become a promising approach for rapid determination of cancer types through comparing the acquired MS1 profile of a query specimen to that of a validated library of known lipid fingerprints. One such technique is Picosecond InfraRed Laser Mass Spectrometry (PIRL-MS). With just ~10-second sampling and analysis duration, we hypothesize that PIRL-MS may offer rapid precise classification of lung cancer subtypes.
Method: To evaluate the utility of PIRL-MS in rapid classification of different types of lung cancer we used a variety of tissue models including immortalized cell lines and xenografts thereof, patient derived xenografts (PDX) and primary patient tissue, subjecting them to 10-second PIRL-MS analysis. Tissue molecular content in the form of a gas plume was captured and profiled by a quadrupole Time of Flight (qToF) mass spectrometer (WATERS). PIRL-MS profiles were processed with multivariate Principal Component Analysis Linear Discriminate Analysis (PCA-LDA). The accuracy was determined using 20% leave out test (PCA-LDA model).
Results: Immortalized cell lines (n=3 technical replicates): A549 for adenocarcinoma (40 spectra), H460 for large cell carcinoma (40 spectra), H526 for small cell carcinoma (43 spectra), H2170 for squamous cell carcinoma (31 spectra) resulted in 100% accuracy from 20% cross-validation of the 4-component PCA-LDA model. Ten independent subcutaneous murine xenografts of same cell lines resulting in 190 adenocarcinoma, 194 large cell carcinoma, 193 small cell carcinoma and 190 squamous cell carcinoma spectra also performed with 100% accuracy using 20% cross-validation. Likewise, PDX (n=47 for adenocarcinoma with 703 spectra, n=3 large cell carcinoma with 63 spectra, n=14 small cell carcinoma with 191 spectra, n=17 squamous cell carcinoma with 297 spectra) exhibited a 98% accuracy. Primary patient tissue (n=48 for adenocarcinoma with 677 spectra, n=14 large cell carcinoma with 203 spectra, n=4 small cell carcinoma with 50 spectra, n=42 squamous cell carcinoma with 698 spectra) showed a 96% accuracy. Endobronchial ultrasound (EBUS) guided biopsies (n=24) were classifiable with a primary patient tissue PCA-LDA model (n=4 small cell carcinoma with 50 spectra and n=65 non-small cell carcinomas with 1035 spectra) with a diagnostic accuracy of 95%.

S5-02 10-Second Lipidomic Analysis with Picosecond Infrared Laser Mass Spectrometry to Diagnose Brain Cancer Types

Alexa Fiorante [1], Michael Woolman[1], David Munoz[2], Gelareh Zadeh[3,4], Sunit Das[2,4], Contributors to the Unity Health Brain Biobank[2], Taira Kiyota[5], Ahmed Aman[5], Howard Ginsberg[2,4] and Arash Zarrine-Afsar[1,3,4]

[1]Department of medical biophysics, University of Toronto
[2]Unity Health Toronto
[3]Princess Margaret Cancer Centre
[4]Department of Surgery, University of Toronto
[5]Ontario Institute for Cancer Research

Surveys of neurosurgical outcome data have suggested suitable extents (aggressiveness) of resection to improve survival for many brain cancer types. Here, personalization of the extent of resection relies on highly subjective intraoperative diagnoses wherein the depth of the diagnostic information depends on the level of experience of the pathologist. Picosecond InfraRed Laser Mass Spectrometry (PIRL-MS) uses picosecond bursts of mid-infrared laser radiation to extract, in a non-thermal manner, tissue lipids (1) present in cellular membranes, giving cells and nuclei their unique shapes (utilized in morphometric pathology with staining & microscopy) and (2) altered in tumorigenesis due to cross-talk with cellular signalling in metabolism (potentially also revealing cancer molecular subtypes in 10 seconds as shown) for real-time profiling of molecular content unique to each tumour type. Close to 1,300 frozen brain cancer specimens over 30 different classes of adult and pediatric cancers are being subjected PIRL-MS to build a comprehensive molecular signature library using supervised dimensionality reduction and methods. The sensitivity and specificity of the integrated morphometric and molecular diagnosis for pediatric brain cancers with 10-second PIRL-MS was > 96%. This classification could use as little as only 18 tissue lipids whose identities were determined using chromatography and tandem mass spectrometry. Greater than 90% sensitivity and specificity have been obtained for adult brain cancer classifications with 10-second PIRL-MS analysis utilizing 40 lipids spanning fatty acids, phospholipids and ceramides [This abstract has been previously published at 66th ICASS Conference held in Niagara Falls, CANADA on June 27th, 2024]

S5-03 Ceramide accumulation and neutral Ceramidase dysfunction associated with the progression of colorectal cancer

Law Shi Hui[†1], Ngui You Shan[†1], Liang-Yin Ke[1*]

[1]Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, Kaohsiung, Taiwan (R.O.C).

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of death in cancers. In Taiwan, it has remained the most frequent cancer for 15 consecutive years. Sphingolipid perturbations are commonly found in the colon of individuals with CRC. Lipidomic research revealed a correlation between disease progression and elevated ceramide levels in the patient's serum and colon. However, animal studies demonstrated that neutral ceramidase (nCDase), a ceramide-degrading enzyme, is highly expressed in cancerous tissue. There is considerable controversy regarding the role of nCDase in CRC, as inhibiting nCDase activity or expression levels has been found to increase ceramide levels and suppress tumor development. Therefore, our research aims to investigate the expression and functions of nCDase in CRC.
We hypothesized that nCDase is overexpressed yet malfunctioning in CRC. Colon tissues from stages I-IV CRC patients and healthy controls were obtained from the KMUH tissue bank (KMUHIRB-E(I)-20210127); stages I-IV referring cell lines were purchased for *in vitro* study. Total lipids were extracted from tissues or cells, then quantified and analyzed using ultrapure liquid chromatography/mass spectrometry ($UPLC/MS^E$). The mRNA and protein expression were measured using quantitative PCR and western blotting. RNA sequencing and peptide analysis were performed to explore protein structure.
Results revealed that d18:1/16:0 ceramide significantly increased in the later stages of CRC cells, indicating a positive correlation with malignancy. Upregulated of *CERS2*, *CERS6*, *DEGS1*, and *SMPD4* increased ceramide biosynthesis, while *nCDase* mRNA expression reduced significantly in the later stages of CRC tissues. The molecular weight of nCDase in cancer cells was 72 kDa, showing a shorter and less intact than the wild-type nCDase with 85.5 kDa. Upon analyzing the peptide and RNA sequence, a new isoform of nCDase was discovered, which lost from 498C to 587Q. Owing to its truncation, the catalytic domain is partially missing, and ceramide hydrolase activity may be inactive.
In conclusion, ceramide accumulation and ceramidase dysregulation were associated with CRC development. The newly identified nCDase lacks hydrolase activity, and further studies are necessary to understand the pathogenic roles of ceramide in CRC

S5-04 Characteristics of extracellular vesicle by gender and age in MAFLD patients

Tzu-Yi Chen (陳姿奕)[1], Tzu-Ching Kao[1], Sin-Tian Wang[1], Wei-Lun Huang[1,2] and Kung-Chia Young[1]

[1]Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
[2]Center of Applied Nanomedicine, National Cheng Kung University, Tainan, Taiwan.

Metabolic dysfunction-associated fatty liver disease (MAFLD), previously known as non-alcoholic fatty liver disease, includes a spectrum of liver conditions ranging from mild steatosis and steatohepatitis to cirrhosis, fibrosis, and hepatocellular carcinoma, all closely linked to metabolic dysfunction. Affecting around 30% of the global population, MAFLD is commonly associated with type 2 diabetes, obesity, and high-fat diets. Cellular senescence, which can be classified into replicative and stress-induced types, may contribute to the progression of MAFLD, though the precise mechanisms remain unclear. Extracellular vesicles (EVs), which are double-layered phospholipid nanoparticles secreted by cells, encapsulate various bioactive molecules such as DNA, RNA, proteins, and lipids. play a role in intercellular communication and potentially in disease progression, including MAFLD. This study aims to examine the differences in particle size and concentration of serum EVs in MAFLD patients, stratified by gender or age. A total of 57 non-MAFLD patients and 170 MAFLD patients were recruited in this study.

The MAFLD patients were divided into two main groups based on BMI (>23 and <23) and further stratified by gender or age. Nanoparticle tracking analysis (NTA) was conducted to assess the concentration and particle size of serum EVs. First, MAFLD patients had more EVs than did non-MAFLD patients and non-obese MAFLD patients had much more and much larger EVs than did non-MAFLD patients. In different gender groups, the values of waistline, GGT, creatinine, uric acid and Hb of male MAFLD patients were significantly higher than those of female MAFLD patients, while HDL-C of male MAFLD patients was significantly lower than that of female MAFLD patients. As the results of EVs measurement, in female group, the non-obese MAFLD patients had more and larger EVs than did non-MAFLD patients; in male group, there was no significant difference in concentration and size of serum EVs. In different age group, the value of HbA1c significantly increased with age. The results of EVs measurement showed that the particle size of EV in the serum of middle-aged MAFLD patients was larger than that of young MAFLD patients, especially in non-obese MAFLD patients. In conclusion, the MAFLD patients might increase the amount of serum EVs, especially in female or middle-aged non-obese MAFLD.

S5-05 Effect of electroacupuncture on the modification of lipid and lipoprotein levels in patients with ischemic stroke: a study for a single-blind randomized controlled trial

Arriagada Rios Sandra, MSc a,b, Ben-Arie Eyal, PhD a, Liao Yi Fang, MD c, Wen-Chao Ho, PhD c, Ming-Kuei Lu MD, PhD e, Sheng-Ta Tsai, MD e, Yu Chen Lee, MD, PhD a,c,f*

a Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan. b Department of Anatomy and Legal Medicine School of Medicine University of Chile. c Department of Acupuncture, China Medical University Hospital, Taichung, Taiwan.d Department of Public Health, China Medical University, Taichung, Taiwan.e Division of Parkinson's Disease and Movement Disorders, Department of Neurology, China Medical University Hospital, Taichung, Taiwan.f Chinese Medicine Research Center, China Medical University, China Medical University Taichung, Taiwan.* Correspondence: Yu Chen Lee, Graduate Institute of Acupuncture Science, China Medical University, 2 Yuh-Der Rd, Taichung City 40402, Taiwan (e-mail: d5167@mail.cmuh.org.tw).

Introduction: The use of electroacupuncture (EA) in post-ischemic stroke has been the subject of numerous studies. However, the effect of EA on cholesterol metabolites has not been thoroughly investigated. The inflammatory response in stroke has been associated with high serum cholesterol levels, low HDL-c levels, and high LDL-c levels. Early intervention with AD has been linked to an anti-inflammatory state that regenerates blood vessels and angiogenesis. This study aimed to evaluate the impact of EA on early ischemic stroke as a modulator of CHOL-t, HDL-c, and LDL-c in the blood, and its anti-inflammatory effect measured through individual levels and lipid indices before and after treatment with EA.

Materials and methods: A total of 14 patients with a first-time stroke diagnosis will be randomly assigned to one of two groups: an EA group and a sham EA group. All patients received the interventions three times a week for six sessions over two weeks. Patients were included immediately after the stroke, up to those admitted to the CMUH acupuncture department between 4 and 12 weeks. Outcome measurements included blood tests for total cholesterol, triglycerides, HDL with HDL-c cholesterol, LDL cholesterol, and LDL-c, along with the visual analog scale (VAS), the National Institutes of Health Stroke Scale (NIHSS), and the Barthel Scale. scale. index (BI)).

Results: 14 cases meet the inclusion standard. A total of 12 men of average age (67.5); and 2 middle-aged women (64.5 years), following the criteria of a single-blind randomized clinical trial, were separated into two groups of patients. A real AD group (5 cases) and a second sham or placebo group (5 cases). 2 patients did not complete all examinations. Our sample of patients has moderate, low HDL and high LDL levels; average blood lipid levels: (CHOL-t (169), HDL c (40.75), LDL c (107), TG (112)) compared to Taiwan's ACC and AHA indices, corresponding to our expectations of diagnosis before intervention with EA. At the time of admission, our group of patients had an index or ratio of moderate levels, compared with the indexes or ratio of Taiwan and AHA, ACC; The average levels of blood lipid index (ratio): CHOL-t/HDL (4), LDL/HDL (3), TG/HDL (3), LDL/BMI (5) correspond to moderate to high levels. Finally, from the levels recorded after completing the 6 sessions for two weeks, the acupuncture group managed to modulate the average of the differential formula: (initial time-final time) EA 1.3804 mg/dL compared to the Sham cases, which was from 0.08 to 0.833. mg/dL. The standard deviation obtained is 0.64, comparing what was observed before and after the intervention with EA.

Conclusions: This study will help determine the effect of EA on ischemic stroke recovery, focusing on metabolic changes in patients with early-stage stroke. EA treatment might modify risk indices (HDL-c), maintain or control (LDL- c), and generate localized reperfusion of the vascular areas involved in stroke. There is a correlation and degree of response to acupuncture according to time and morbidity factors, blood lipid levels, and the risk indices(ratio) that these represent.

S5-06 Evaluating Effects of Eicosapentaenoic Acid on the Liver Formation of Electronegative Low-Density Lipoprotein

Yi-An Chang 張椅銨 [1], Shih-Han Huang[1], Hua-Chen Chan[2]

[1]Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University.
[2]Department of Medical Laboratory Science, I-Shou University.

Cardiovascular disease (CVD) is the leading cause of death worldwide. Lowering plasma low-density lipoprotein (LDL) is current strategy for primary prevention of CVD. However, the residual risk remains a critical clinical challenge even when the plasma LDL concentration is lower than 70 mg/dL. Additionally, side effects pose another important clinical concern. Using liquid-chromatography, we separated human plasma LDL into five subfractions: L1-L5. Among these, L5 exhibited the highest electronegativity and atherogenic properties, contributing to vascular endothelial injury. L5 levels was increased in patients with high CVD risk. Chemical evidence revealed that L5 contains a high concentration of inflammatory lysophosphatidylcholine (LPC). Recently, we demonstrated that LPC induces apolipoprotein glycosylation by modulating the hypoxia-inducible factor 1 alpha (HIF-1α) pathway in the liver, resulting in an increased level of electronegative LDL.

Eicosapentaenoic acid (EPA), an omega-3 fatty acid, has been identified as having a protective effect on CVD prevention through multiple mechanisms, including inflammation inhibition, antioxidation, and lipid profiles improvement. *Apolipoprotein E* gene deficiency (*apoE*$^{-/-}$) has been implicated in lipid metabolic disorder associated with CVD. Building upon this knowledge, we aim to investigate the effects of EPA on reducing L5 electronegativity in the liver. To achieve this, we have designed two specific aims: *Aim 1*: Evaluate L5 levels in *apoE*$^{-/-}$ mice fed a high-fat diet with or without EPA. *Aim 2*: Analyze the lipidomics in plasma sample with ultra-performance liquid chromatography mass spectrometry. In this study, elevation of L5 was discovered in *apoE*$^{-/-}$ mice fed a high-fat diet (HFD) and L5% had a decreasing trend after EPA treatment. This current study aims to contribute to the advancement of knowledge regarding the beneficial effects of EPA in preventing CVD.

S5-07 Glutamine Deprivation Increases Ciliogenesis and Chemotherapy Resistance in Pancreatic Ductal Adenocarcinoma

Ming-Hui Chang and I-Chen Peng

Department of Life Sciences, National Cheng Kung University, Tainan City 701, Taiwan

Pancreatic ductal adenocarcinoma (PDAC) has a high mortality rate and poor prognosis because of its resistance to chemotherapy drugs. Primary cilia are small hair-like structures that have been shown to regulate cancer cells' resistance to chemotherapy drugs. Glutamine plays a critical role in supporting cancer cell growth and influencing drug sensitivities. However, the role of glutamine in ciliogenesis and chemotherapy resistance in PDAC, as well as the underlying mechanisms, remains uncertain. In this study, we found that primary cilia and the IC50 (half maximal inhibitory concentration) of cisplatin (CPT) were increased in PANC-1 cells surviving under glutamine deprivation (-QQ cells). Knockdown of intraflagellar transport 88 (IFT88) led to decreased cell survival rates of -QQ cells upon CPT treatment. Furthermore, the expression of glutamine synthetase (GS), glutaminase 1 (GLS1), and cluster of differentiation 36 (CD36) was enhanced, while that of acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS), and sterol regulatory element-binding protein 1 (SREBP1) was reduced in -QQ cells, indicating metabolic alterations in response to glutamine deprivation. These findings demonstrate the pivotal role of glutamine in modulating ciliogenesis and chemotherapy resistance in PDAC. Therefore, targeting glutamine and primary cilia in PDAC could potentially offer a novel therapeutic approach.

S5-08 Group IVF phospholipase A_2 (cPLA$_2\zeta$) regulates proresolving lipid production during acute skin wound healing

Yuki Nagasaki[1,2], Yoshitaka Taketomi[2], Yoshimi Miki[2,3],Ken Yasukawa[4], Kazuko Saeki[4], Takehiko Yokomizo[4], Toshiko Suzuki Yamamoto[5], Makoto Murakami[1,2]

[1]Division of Physiological Chemistry and Metabolism, Department of Biochemistry and Molecular Biology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[2]CDBIM, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[3]Division of Bioscience and Bioindustry, Graduate School of Technology Industrial and Social Science, Tokushima University, Tokushima, Japan
[4]Department of Biochemistry, Graduate School of Medicine, Juntendo University, Tokyo, Japan
[5]Department of Nutritional Science, Okayama Prefectural University, Okayama, Japan

Among the group IV cytosolic phospholipase A_2 (cPLA$_2$) family, group IVA cPLA$_2\alpha$ (PLA2G4A) acts as a key enzyme in arachidonic acid metabolism for eicosanoid generation, while group IVE cPLA$_2\varepsilon$ (PLA2G4E) contributes to the production of anti-inflammatory *N*-acylethanolamine. However, the biological functions of other cPLA$_2$ isoforms remained poorly understood. In this study, we show that group IVF cPLA$_2\zeta$ (PLA2G4F) is constitutively expressed in keratinocytes and regulates skin wound healing by mobilizing proresolving oxylipins. By 6 months of age, cPLA$_2\zeta$ knockout (KO) mice showed a notable increase in transepidermal water loss, an index of impaired skin barrier function, with elevated expression of keratinocyte differentiation and activation markers, compared to WT mice. Proliferation of primary keratinocytes was attenuated by cPLA$_2\zeta$ deletion, which was rescued by supplementation with culture supernatant of WT cells. An *in vitro* scratch-wound assay revealed that the wound closure rate in KO keratinocytes was significantly reduced compared to WT keratinocytes. Furthermore, in an *in vivo* wound healing model, KO mice showed a reduced migration of epidermal keratinocytes to the wound area during an early stage of the healing process. Skin lipidomics at this stage revealed decreases in several lysophospholipids and free fatty acids in KO mice. Importantly, while various proresolving oxylipins (primary products of 12/15- LOX and CYP450) were increased in WT skin at the early stage of healing process, this event was not observed in KO skin. These results suggest that cPLA$_2\zeta$ mobilizes skin lipids to regulate the proliferation, differentiation, activation and migration of epidermal keratinocytes and contributes to the physiological process of acute wound healing.

S5-09 IEX-1 is Crucial for LPAR2-mediated Radiation Mitigation and Regeneration

Derek Norman[1], Sue Chin Lee[1], Yoojin Shin[1], Bryan Kuo[1], Gábor Tigyi[1]

[1]Department of Physiology, University of Tennessee Health Science Center

Left unmitigated high-dose ionizing radiation causes DNA damage and ultimately death. Activation of the lysophosphatidic acid receptor subtype 2 (LPAR2) is one mechanism by which radiation damage can be mitigated. Additionally, the immediate early response gene X-1 (IEX-1) is induced by ionizing radiation and has been shown to promote cell survival via transregulatory signaling through ERK1/2 and Akt as well as resolution of γ-H2AX in the DNA damage response pathway (DDR). Thus, we hypothesized that IEX-1 may play a crucial role in LPAR2-mediated radiomitigation as well as in subsequent cellular regeneration.

Our laboratory has previously developed Radioprotectin-1 (RP-1), a potent LPAR2-specific agonist as a next generation to octadecyl thiophosphate (OTP)—a pan-agonist of LPAR. Here, we demonstrate that LPAR2 activation by RP-1 leads to prolonged and sustained ERK1/2 and Akt activation, as well as reduction in apoptosis in irradiated mouse embryonic fibroblasts (MEF). Further, RP-1 induces expression of IEX-1 in MEF, which is blocked by selective LPAR2 antagonism. We also show that RP-1 enhances survival of irradiated Lgr5$^+$ intestinal enteroids as well as Ki67$^+$ intestinal crypts from irradiated mice, denoting the role of LPAR2 in tissue regeneration.

Ultimately, our work demonstrates that RP-1 markedly improves overall survival outcome in mice exposed to both partial body irradiation (PBI) and total body irradiation (TBI)—where we found a significant increase in IEX-1 expression in the blood beyond that induced by TBI alone. Knockout of IEX-1 in both male and female mice leads to significant survival disadvantage upon TBI. However, IEX-1 KO only affected radiosensitivity in female mice exposed to PBI revealing a potential sex-dependent attenuation of mitigation in the males. Altogether, these data support the hypothesis that IEX-1 plays a critical role in LPAR2-mediated radioprotection and tissue regeneration.

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S5-10 Integrative Role of the Autotaxin-Lysophosphatidic acid Axis in Cancer Metastasis and Therapy Resistance

Gabor J. Tigyi[1,2], Sue Chin Lee[1], Mélanie A. Dacheux[1], Kuan-Hung Lin[1], Derek D. Norman[1], Junming Yue[2], Andrea Balogh[1,3], Hsinyu Lee[4], Zoltan Benyo[3],

[1]Departments of Physiology and
[2]Pathology, University of Tennessee Health Science Center, Memphis, Tennessee, USA
[3]Department of Translational Medicine, Semmelweis University, Budapest, Hungary
[4]Department of Life Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan

The development of therapy resistance and cancer metastasis (MET) significantly limits survival in most types of cancers. The lysophospholipase autotaxin (ATX, ENPP2), which generates lysophosphatidic acid (LPA), plays a central role in MET and the development of resistance to chemo-, radio- and immuno-therapy. ATX is the second most upregulated gene in therapy-resistant breast cancers. Similarly, ATX has been identified as a drug-resistance gene in ovarian cancer patients and is upregulated following radiation and chemotherapy. Because ATX plays a critical role in cancer progression and therapy resistance, understanding the molecular mechanisms responsible for its upregulation is of therapeutic significance. We recently discovered E2F7 as a transcription factor that upregulates ATX expression in both human and murine cells. However, E2F7 mediated ATX expression in murine cells is subjected to steric interference by Tp53. Specifically, we identified a Tp53 binding site in the first intron of murine *Enpp2* that is absent in human *ENPP2*. Binding of WT Tp53 disrupted the E2F7-mediated chromosomal looping and repressed *Enpp2* transcription exclusively in murine cells.

ATX expression is upregulated not only in carcinoma stem-like cells but also in cells of the tumor microenvironment (TME), playing an important role in MET. Previously, we demonstrated that pharmacological inhibition of ATX in stromal cells reduced the bone micrometastasis of MDA-BO2 breast cancer cells that lack ATX expression. However, MDA-BO2 inoculation caused LPA release from activated platelets, promoting tumor cell proliferation and secretion of IL-6 and IL-8. We further examined the contribution of ATX from other cell types in the TME to MET. We found that ATX from alveolar type II (ATII) pneumocytes and CD11b+ myeloid cells, but not cancer associated fibroblasts, promoted the metastasis of B16-F10 melanoma to the lungs. We show that targeted KO of ATX in ATII cells and CD11b+ myeloid cells reduced the metastatic burden of melanoma by over 30% and 50%, respectively. CD11b+ KO mice showed a 50% decrease of lung neutrophils. These mice had ~50% reduction in circulating ATX and reduction in 20:4 LPA. We found that activation of the LPAR5 receptor inhibits T cell receptor activation and cytotoxic CD8 T cell response. Importantly, our data from the global With LPAR5KO and CD11b+xATX KO mice suggest that ATX-LPA signaling axis modulate both innate and adaptive tumor immunity.

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S5-11 Investigating a Novel Therapeutic Approach Targeting Ceramide for the Treatment of Alzheimer's Disease

Yi-Wen Huang 黃怡文 [1], Chieh-Ju Wen[1], Liang-Yin Ke[1]

[1]Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University

Alzheimer's disease (AD) accounts for 60-70% of 46.8 million people with dementia worldwide. Given the scarcity of AD medications, discovering more effective therapies is crucial for preventing the disease from progressing. One of the most fundamental challenges in medication research is the lack of an animal model accurately representing the preclinical stage of human disease progression. Although transgenic mice with *APP/PSEN1/MAPT* mutations (3xTg-AD) exhibit β amyloid (Aβ) and tau protein abnormality, the lipid metabolism may exhibit characteristics distinct from humans. AD pathogenesis involves apolipoprotein E (apoE) polymorphism and the alteration of lipid metabolism. Notably, ceramide, which triggers Aβ deposition and tau phosphorylation, is elevated in the brain and plasma of AD patients. Therefore, we aimed to explore the lipid metabolism in the 3xTg-AD mouse model of AD.

Three-month-old 3xTg-AD mice and C57BL/6 mice were sacrificed (n=5 each). Plasma and brain tissues were collected to determine lipid metabolites, metabolic profiles, and other pathological findings. Lipids were extracted using the Folch procedure, and untargeted lipidomic profiling was evaluated using ultra-performance liquid chromatography-mass spectrometry. Enzymes responsible for sphingolipid metabolism were tested, including mRNA quantitative polymerase chain reaction, western blotting, and immunohistochemistry staining.

Results showed that C18:1 ceramide was the most abundant species and was highly elevated in the brains of 3xTg-AD mice, as compared to C57BL/6 mice. In contrast, sphingomyelin and the downstream metabolites sphingosine and sphingosine-1-phosphate were decreased. The ceramide accumulation, Aβ deposition, and tumor necrosis factor-α (TNF-α) were enhanced in 3xTg-AD mice and positively correlated. Additionally, the neutral ceramidase (nCDase) responsible for hydrolyzing ceramide was downregulated in 3xTg-AD, both mRNA and protein expression levels.

In conclusion, downregulated nCDase in 3xTg-AD may lead to sphingolipids alteration and ceramide accumulation. This finding was positively correlated with Aβ deposition and inflammation. We hope this research can provide new insight into potential therapeutics for AD.

S5-12 Investigation of the effect of ceramide synthase inhibition on Niemann-Pick disease type C.

Hana Hirose[1], Miaki Uzu[1], Hiroyuki Nakamura[1]

[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University

Purpose: Niemann-Pick disease type C (NPC) is an autosomal recessive genetic disorder characterized by central nervous system involvement. The main cause of the disease is intracellular accumulation of cholesterol due to mutations in the NPC1 protein, which is responsible for intracellular cholesterol transport. Therefore, the creation of a new therapeutic target is desired. Ceramide (Cer), a sphingolipid, also accumulates secondarily in NPC. Ceramide synthase (CerS) is an enzyme that has 1-6 isoforms and produces Cer with different chain lengths. In this study, we focused on CerS as a novel therapeutic target for NPC and performed a detailed analysis.
Methods and Results: We examined whether siRNA knockdown of CerS1-6 in dermal fibroblasts (NPC cells) derived from NPC patients reduces cholesterol accumulation. In this study, Filipin, a cholesterol staining reagent, was used as an indicator to evaluate cholesterol accumulation. The results showed that NPC-enhanced Filipin fluorescence was attenuated by knockdown of CerS2, 3, and 5, and that cholesterol accumulation was reduced. In NPC cells, late endosomes/lysosomes accumulate along with the accumulation of cholesterol. Therefore, we examined intracellular acidic fraction accumulation using LysoTracker. As a result of the examination, knockdown of CerS5 reduced the accumulation of acidic fractions. This suggested that the accumulation of late endosomes/lysosomes as well as cholesterol was also reduced. We further analyzed the mechanism by which cholesterol accumulation is reduced by CerS5 knockdown. In this study, we investigated two possible mechanisms of reduced cholesterol accumulation: decreased low-density lipoprotein (LDL) uptake and improved cholesterol transport to the endoplasmic reticulum. The results showed that knockdown of CerS5 in NPC cells did not alter low-density lipoprotein (LDL) uptake. On the other hand, knockdown of CerS5 suppressed activation of sterol regulatory element binding proteins 2 (SREBP2) and restored cholesterol ester levels, indicating that cholesterol levels in the ER are increased.
Discussion: Our results suggest that CerS knockdown in NPC cells contributes to improved cholesterol accumulation in NPC cells via improved cholesterol transport from late endosomes/lysosomes to the ER. This indicates that CerS may be a potential new therapeutic target for NPC cells.

S5-13 Lipid coated graphitic carbon nitride-drug conjugated nanocarriers for liver cancer therapy

Sk Jahir Abbas[1], Shiraz Mehmood[1], Yao-Ching Hung[2], Wen-Lung Ma[*1, 3]

[1]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan
[2]Department of Obstetrics and Gynaecology, Asia University Hospital, Taichung, Taiwan
[3]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan

Lipid based nanoparticles have been most widely studied for cancer-related applications due to their biomimetic properties with high drug encapsulation efficiency and controlled drug release 1. Although there are some limitations including stability and aggregation. To overcome those biological barriers, lipid polymer hybrid nanoparticles have been developed and used extensively in clinical research. 2 In this study, our aim is to establish a lipid- polymer and drug based nanocarriers (LPNs) system consisting of the semiconductor graphitic carbon nitride and anticancer drug cisplatin. The rational design of the biodegradable polymer-based nanocarriers LPNs systems can be controllable particle size, high encapsulation efficiency of drug loading, stability and controlled release. The LPNs can be used as a trimode combinatorial chemotherapy, PTT and PDT in hepatocellular carcinoma (HCC) treatment.

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S5-14 Lipid droplet size and metabolic regulation in hepatocytes at different stages of MAFLD

Hsuan-Ya Liao[1], Sin-Tian Wang[1], Hung Wen Tsai[3] and Kung-Chia Young[1,2]

[1]Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University.
[2]Department of Pathology, National Cheng Kung University.
[3]Institute of Basic Medicine, College of Medicine, National Cheng Kung University.

Background and aims:
Metabolic-associated fatty liver disease (MAFLD) is characterized by the accumulation of lipid droplets (LDs) in the liver. In previous studies, different sizes of LDs were presented in various stages of MAFLD patient liver tissue by histological staining, but their metabolic and pathogenic meaning is still unknown.
Method:
First, the steatohepatitis grade, fibrosis grade, and lipid percentage of 133 MAFLD patient's liver tissue were examined by a pathologist. Among these, LDs in 65 patients were photographed using the raster scan method and quantified using ImageJ. Secondly, the primary human hepatocytes (PHH) were treated with oleic acid (OA) or TMP-153 to induce large and small LD accumulation. Metabolic and pathogenic regulation was then assessed through transcriptomic analysis, qRT-PCR, western blotting, and lipid quantification.
Results:
The lipid percentage of 133 MAFLD patients has a significant increase from steatohepatitis grade 0 to grade 1(p-value <0.0001), and a substantial decrease from grade 1 to 2 (p-value= 0.0449) and from grade 2 to 3 (p-value=0.0124). Based on LD quantification results, from grade 0 to 1, the mean LD diameter was increased in the context of constant mean LD count. From grade 1 to 2, the mean LD diameter change was decreased in the context of increased LD count. From immunofluorescence staining of PHH, PLIN2 colocalized significantly more with large LDs in the OA group than smaller LDs in the TMP-153 group, while PLIN3 only colocalized with smaller LDs. Transcriptomic analysis of PHH also indicated gene expression of PLIN3 in TMP-153 group is significantly higher and had more genes overlapped with the microvesicular steatosis gene list than OA group, indicating TMP-153 group with smaller LDs may be more related to steatohepatitis and other more serious stages in MAFLD progression. PHH transcriptomic analysis in combined with results from online databases indicated that PI3K-AKT, AGE-RAGE, relaxin, and other related signaling pathways might differ in metabolic regulation from simple steatosis (grade 0) to steatohepatitis (>grade 1).
Conclusion:
The change in LD size may indicate the metabolic and pathogenic condition at different stages in the progression of MAFLD.

Short Talks

S5-15 Lipidomics: Gut Microbes and Cancer Diagnosis

Richard Cheng-Chih Hsu

Department of Chemistry, National Taiwan University

The intricate relationship between gut microbiota and cancer has garnered significant attention in recent years. Lipidomics, the comprehensive study of lipid profiles, provides an innovative avenue to explore the metabolic interactions between gut microbes and host health, particularly in the context of cancer diagnosis. This talk focuses on how gut microbiota modulate host lipid metabolism and the subsequent alterations in lipid profiles that may serve as biomarkers for cancer detection. By integrating lipidomics with microbial research, novel insights into cancer pathophysiology can be gained, offering a non-invasive, susceptible diagnostic tool. Furthermore, this approach could uncover lipid-mediated signaling pathways that contribute to cancer development and progression, potentially opening new avenues for therapeutic intervention. Understanding the lipidomic signatures associated with specific microbiota compositions or cancer patients may revolutionize early diagnosis and precision medicine.

S5-16 Lysophosphatidylcholine impairs the mitochondria homeostasis leading to trophoblast dysfunction in Gestational Diabetes Mellitus.

Shao-Chi Hung[1], Ting-Chieh Lai[1], Hua-Chen Chan[2], Liang-Yin Ke[1]

[1] Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung 807378, Taiwan;
[2] Department of Medical Laboratory Science, College of Medicine, I-Shou University, Kaohsiung 824005, Taiwan

Gestational diabetes mellitus (GDM) is a common pregnancy disorder linked to preeclampsia and macrosomia. In addition, GDM is associated with postpartum type 2 diabetes and cardiovascular disease. GDM prevalence is steadily increasing to 14.7%. GDM is characterized by dyslipidemia both during pregnancy and after postpartum. Placental trophoblast lipid accumulation impairs mitochondrial function. However, the exact lipids that impact the placental trophoblast and the underlying process have yet to be fully understood. GDM and normal pregnancy patients from Kaohsiung Medical University Hospital were recruited. The placenta and cord blood were collected at birth. Confocal and electron microscopy revealed the placenta and mitochondria structure. The low-density lipoprotein in cord blood was analyzed using liquid chromatography with mass spectrometry (LC/MS^E). In addition, *in vitro* experiments were conducted on choriocarcinoma cell lines (JEG-3) to investigate the mechanism of trophoblast mitochondrial function and morphology after lysophosphatidylcholine (LPC) treatment. Results showed that the structure of the placenta was modified along with mitochondria dysfunction in GDM. This malfunction was characterized by elevated syncytial knots, chorangiosis, and LOX-1 (lectin-liked oxidized LDL receptor-1) overexpression; GDM placenta also demonstrated a decrease in mass and an increase in the fusion of mitochondria. GDM cord blood LDL showed a significant increase in LPC. PCA analysis identified LPC 16:0 as the most crucial component in LC/MS^E. *In vitro* study indicates that LPC increases the production of reactive oxygen species (ROS) in mitochondria and leads to defects in mitochondria, including decreased electron transport chain and disruption of morphological homeostasis through the hypoxia-induced factor 1 alpha (HIF-1α) signaling pathway. Overall, LPC was pivotal in GDM, contributing to the impaired function of trophoblasts and mitochondria. We anticipate this finding will help us comprehend the mechanism of GDM and serve as a new therapeutic strategy in the future.

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S5-17 Monitoring and finding natural inhibitors for sphingomyelin synthase an enzyme associated with fatty liver disease

Siddabasave Gowda B. Gowda[1,2], Punith M. Sundaraswamy[1], Jayashankar Jayaprakash[1], Yusuke Minami[3], Divyavani Gowda[2], Shu-Ping Hui[2]

[1]Graduate School of Global Food Resources, Hokkaido University.
[2]Faculty of Health Sciences, Hokkaido University
[2]Graduate School of Health Sciences, Hokkaido University

Worldwide the number of cases associated with metabolic dysfunction-associated fatty liver disease (MAFLD) are rising drastically due to various factors including lifestyle and diet. Lipids are the essential nutrients which maintain several functions from membrane structure to cell signaling. Altered lipid metabolism is the key cause of MAFLD. Accumulation of a sphingolipid metabolite sphingomyelin is frequently observed in MAFLD as well as cancer. Previous studies demonstrated that inhibition of sphingomyelin synthase (SMS) could be a potential target for the control of MAFLD. However, there are limited SMS inhibitors reported for which are less potent and toxic. In this research we established a sensitive liquid chromatography/mass spectrometry-based method for monitoring the SMS activity using HeLa cells. Further, validated the method by in-vitro and in-silico studies with a natural inhibitor ginkgolic acid, which showed IC_{50} of 5.5 µM and 3.6 µM for SMS1 and SMS2 enzymes. Binding energies for GA with SMS1 and SMS2 are −6.6 kcal mol^{-1} and −8.2 kcal mol^{-1} respectively.

The developed method was applied to screen the seaweed extracts for potential SMS inhibitor discovery. The results showed that seaweed hijiki hexane extracts as strong inhibition for both SMSs. We further identified and characterized the active compound of hijiki as fatty acid (FA 22:1), which is responsible for SMS inhibition. The FA 22:1 showed IC_{50} of 4.5 µM and 6 µM for SMS1 and SMS2. Overall, an effort was made to discover food-derived SMS inhibitors for MAFLD control. Further investigations are in progress to reveal the inhibitory action using in-vitro and in-vivo models.

S5-18 Novel acetal-type lysoplasmalogen, a secondary product of group IIF phospholipase A_2, promotes skin wound healing

Yoshimi Miki[1], Niki Hirabayashi[2], Asuka Tamai[2], Akira Shigenaga[3], Makoto Murakami[4,5], Kei Yamamoto[1]

[1]Graduate School of Technology, Industrial and Social Sciences, Tokushima University
[2]Graduate School of Sciences and Technology for Innovation, Tokushima University
[3]Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University
[4]Graduate School of Medicine, The University of Tokyo
[5]AMED-CREST

We have reported that lysoplasmalogen (P-LPE), a unique lysophospholipid that is preferentially produced by group IIF secreted phospholipase A_2 ($sPLA_2$-IIF) in the suprabasal epidermis, regulates skin barrier formation and skin inflammation. In this study, we attempted to elucidate the physiological significance of the $sPLA_2$- IIF/P-LPE pathway in skin wound healing. We found that $sPLA_2$-IIF-deficient (*Pla2g2f*$^{-/-}$) mice exhibited a delay in re-epithelialization and wound closure after skin punching. Lipidomic analysis of the wound exudates showed that the production of P-LPE as well as LPE, EPA and DHA was $sPLA_2$-IIF-dependent. However, topical application of these lipids over the wounds of *Pla2g2f*$^{-/-}$ mice did not improve the wound closure, suggesting the contribution of other metabolite(s). We noticed that P-LPE is structurally unstable under acidic conditions and expected to be non-enzymatically isomerized to a more stable acetal-form (A-LPE) in a physiological acidic skin microenvironment. Topical application of chemically synthesized A-LPE (*trans*) to *Pla2g2f*$^{-/-}$ mice restored the delayed wound closure, whereas application of P-LPE or A-LPE (*cis*) failed to do so. Furthermore, histological analysis of the skin and *in vitro* scratch assay revealed that A-LPE (*trans*) enhanced the migration of epidermal keratinocytes. Finally, topical administration of A-LPE (*trans*) accelerated wound healing even in the skins of wild-type C57BL/6 mice and diabetic *db/db* mice. These results suggest that P LPE produced in skin wound exudate is converted to A-LPE under physiological acidic conditions of the skin and then improves wound healing. This novel lipid we have identified here would be a potential therapeutic agent to accelerate wound healing, particularly intractable wounds such as diabetic ulcers.

S5-19 Optimization of Curcumin and Gentamicin-Loaded Liposome Using a Box-Behnken Experimental Design

Adi Yugatama[1,2], Chien-Ming Hsieh[1,3]

[1]School of Pharmacy, College of Pharmacy, Taipei Medical University.
[2]Department of Pharmacy, Sebelas Maret University.
[3]Ph.D. Program in Drug Discovery and Development Industry, College of Pharmacy, Taipei Medical University

Curcumin (Cur), a bioactive compound extracted from Curcuma longa, is recognized for its antimicrobial properties. The combination of curcumin and gentamicin (Gen) has demonstrated synergistic antimicrobial effects against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus* by reducing their minimum inhibitory concentrations (MIC). However, the implementation of a dual-drug codelivery system encounters several challenges. Integration of hydrophobic drugs can modify liposome bilayer properties, thereby impacting drug penetration and in vivo release. Additionally, achieving optimal encapsulation efficiency for both drugs is crucial. Herein, we aimed to prepare and optimize curcumin and gentamicin-loaded liposomes (CurGen-Lip) with potential implications for antibacterial treatment.

Liposomes were synthesized utilizing a microfluidic approach and optimized via a three-factor, three-level Box-Behnken design (BBD), with curcumin concentration (X_1), lipid concentration (X_2), and total flow rate (X_3) as independent variables. The observed dependent variables included particle size (Y_1), curcumin encapsulation efficiency (Y_2), and gentamicin encapsulation efficiency (Y_3). The optimized formulation (CurGen-Lip) was subsequently characterized for particle size, encapsulation efficiency, drug loading, and drug release profiles.

The current demonstration of Design of Experiments (DOE) carried out using the BBD can aid in expediting the optimization of the formulation. The CurGen-Lip formulation exhibited a particle size of 173.03 ± 0.75 nm and a polydispersity index (PDI) value of 0.121 ± 0.012. The encapsulation efficiency and drug loading of curcumin were determined to be 94.69% ± 0.08% and 2.27% ± 0.27%, respectively, while those of gentamicin were 95.06% ± 0.31% and 23.92% ± 0.74%, respectively. In vitro studies indicated that CurGen-Lip exhibited enhanced drug release kinetics compared to free-drug formulations. These findings suggest that the CurGen-Lip formulation holds promise as a delivery system for antibacterial treatment. However, further study will be necessary to substantiate the dual antimicrobial effect.

S5-20 Optimization of VLDL-mimicking Lipid-polymer hybrid nanoparticles (VMND) for hepatocellular carcinoma treatment

Shiraz Mehmood[1], Pei-Yin Liao[1], Sk Jahir Abbas[1], Wei-Chung Cheng[1], Wen-Lung Ma[1*]

[1]Graduate Institute of Biomedical Sciences, School of Medicine, China Medical University, Taichung, Taiwan.
[2]Ph.D. Program for Health Science and Industry, Center of Tumor Biology, School of Medicine, China Medical University, Taichung, Taiwan.

Except for curative surgical removal of tumor lesions, clinical management for advanced Hepatocellular carcinoma (HCC) using multi-kinase inhibitors, e.g., Lenvatinib, as 1st line targeted therapy. Yet, the marginal survival benefit of Lenvatinib definitely can be improved from a low response rate in patients. There are two main strategies to increase the response rate in drug design. One is precisely selecting patients with theragnostic markers, the other is improving drug distribution with targeted control-release formulation. Recently, our team has discovered HCC prognosis-related lipid metabolism events. 1. We found VLDLR overly expressed in tumor lesions, suggesting a VLDL addictive behavior of HCC. Just like the Helen of Troy welcoming the Trojan Horse. 2. There are also lipid metabolites favorable for good prognosis. Take advantage of those discoveries, the current proposal would like to translate them as one pill. The first step is to realize the idea of VLDL mimicking nanoparticle drug carrying Lenvatinib (VMND) as therapeutics. The second step is to test the possibility of lipidomic-based VMND drug design. For the first step, we have been practiced it with a proof-of-concept experiment performed by the team. Hence, this proposal would like to define the manufacture procedure for future scale-up. Lipid-polymer hybrid nanoparticle delivery systems have combined numerous advantages including the polymeric core and the biomimetic ability of the phospholipid shell into a single platform. VMND comprising a poly (lactic-co-glycolic acid) (PLGA) core and a lipid shell composed of 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, TPGS, and 1,2-disteroyl-sn-glycero-3- phosphoethanolamine N [succinyl-polyethylene glycol] (DSPE-PEG 2000) synthesized to improve the therapeutic potential of Lenvatinib through novel engineered delivery system VMND. With successfully establishment of lipid-polymer hybrid nanoparticle delivery systems in the lab, we're able to stabilized manufacture procedure in the team. For the second step, we will be optimizing the lipid loading capacity with add-in prognostic favorable lipid metabolites in the VMND manufacturing process. With the success in step one, the second step will be more detailed in defining lipid composition and formulation optimization.

Short Talks

S5-21 The emerging role of lipids as drivers of systemic inflammation and organ failure in advanced liver disease

Joan Clària

Biochemistry and Molecular Genetics Service, Hospital Clínic-DIBAPS, CIBERehd, Barcelona, Spain; European Foundation for the Study of Chronic Liver Failure, Barcelona, Spain and Department of Biomedical Sciences, University of Barcelona, Barcelona, Spain

Lipids are recognized not only as source of energy and essential cellular components involved in organelle homeostasis, but also in the regulation of cell signaling, immune responses and inter-organ communication. Patients with advanced liver cirrhosis are at risk of developing acute-on-chronic liver failure (ACLF), a syndrome associated with organ failure across the six major organ systems (liver, kidney, brain, coagulation, circulation, and respiration) resulting in high short-term mortality. The onset of ACLF development is closely associated with systemic inflammation and is frequently precipitated by recurrent infections since these patients are immunocompromised. In this study, we investigated the variations in the blood lipidome in a large cohort of 826 patients with advanced liver cirrhosis and their role in ACLF pathophysiology, especially their contribution to the systemic hyperinflammatory response. Untargeted lipidomics analysis revealed that the circulating lipid landscape associated with advanced cirrhosis was characterized by a generalized lipid suppression, which was more manifest in patients with ACLF. In these patients, we identified a specific cholesteryl ester (CE) fingerprint with enough power to discriminate patients with ACLF from those without. Consistent with reduced CE levels, lower high-density lipoprotein (HDL) levels were observed in patients with ACLF together with reduced levels of lecithin-cholesterol acyl transferase LCAT, the enzyme that mediates the transfer of fatty acids to CEs on the surface of HDL. Further analysis of HDL particle size by nuclear magnetic resonance (NMR) spectroscopy showed very low number of small HDL particles, which has been linked to inadequate or insufficient resolution, chronic inflammation, excessive tissue damage, and dysregulation of organ homeostasis. Indeed, the amount of small HDL particles inversely correlated with elevated concentrations of interleukin (IL)-6, IL-8, eotaxin and MIP-1beta, and higher disease severity. Furthermore, CE circulating in patients with ACLF had enriched composition of arachidonic acid, the omega 6-polyunsaturated fatty acid (PUFA) precursor of pro-inflammatory lipid mediators together with reduced content of the omega-3-PUFA eicosapentaenoic acid, which serves as substrate for the biosynthesis of pro-resolving lipid mediators. Together, these findings uncover a characteristic lipidome composition that favors a pro-inflammatory environment in the systemic circulation of patients with advanced cirrhosis, putting these patients at risk of developing ACLF.

S6-01 Deleting autotaxin in LysM+ myeloid cells impairs innate tumor immunity in models of metastatic melanoma

Sue Chin Lee, Mélanie A. Dacheux, Derek D. Norman, Yoojin Shin, Gábor J. Tigyi

Department of Physiology, University of Tennessee Health Science Center

Autotaxin (ATX) is a lysophospholipase D that generates lysophosphatidic acid (LPA) and regulates cancer metastasis, therapeutic resistance, and tumor immunity. We found that myeloid cells in human melanoma biopsies abundantly express ATX and investigated its role in modulating innate tumor immunity using two models of melanoma metastasis - spontaneous and experimental. Targeted knockout of ATX in LysM+ myeloid cells in mice (LysM-KO) reduced both spontaneous and experimental B16-F10 melanoma metastases by ≥50%. Immunoprofiling revealed differences in M2-like alveolar macrophages, neutrophils and regulatory T cells in the metastatic lungs of LysM-WT versus LysM-KO that are model-dependent. These differences extend systemically, with LysM-KO mice bearing experimental metastasis having fewer neutrophils in the spleen than LysM-WT mice. Our results show that 1) LysM+ myeloid cells are important source of ATX/LPA that promote melanoma metastasis by altering innate tumor immunity, and 2) intratumor and systemic immune profiles vary dynamically during disease progression and are model-dependent.

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Portions of this work were previously presented as part of a talk given by Dr. Mélanie A. Dacheux at the UTHSC Cancer Research in Progress Seminar Series.

S6-02 Diosgenin down-regulates cyclooxygenase-2 and promotes tissue repair in acute and chronic hepatic injury

Izumi Tsukayama, Keisuke Toda, Yuki Kawakami, Yoshitaka Takahashi, Toshiko Suzuki-Yamamoto

Department of Nutrition Science, Okayama Prefectural University.

Overproduced prostaglandin (PG) E_2 from arachidonic acid by transiently induced cyclooxygenase (COX)-2 leads to acute or chronic inflammation. Regulation of COX-2 expression is one of the keys to preventing and ameliorating inflammation.

Diosgenin is a natural steroidal saponin abundantly in the rhizomes of yam and is reported to have several effects on several diseases. In human non-small-cell lung carcinoma A549 cells, we have reported that diosgenin down-regulates COX-2 expression by translocating nuclear factor-kappa B (NF-κB) through glucocorticoid receptor (GR), which is blocked by the GR antagonist RU4861). The effect of diosgenin on COX-2 expression was confirmed in lipopolysaccharide (LPS)-induced acute hepatic injury in mice. In the LPS-induced acute inflammation, COX-2 which was found to be highly expressed in macrophages of the hepatic sinusoids and vascular endothelial cells was down-regulated by an intraperitoneal administration of diosgenin specifically in macrophages1). Subsequently, in methionine and choline-deficient (MCD) diet-induced metabolic dysfunction-associated steatohepatitis (MASH)-like chronic hepatic injury in mice, diosgenin (i.p.) down-regulated Ptgs2 (COX-2) in non-parenchymal cells in the liver and reduced the serum levels of ALT and AST. Histochemical analysis showed that diosgenin treatment reduced oil red-stained lipid droplets and picrosirius red-stained collagen fibers and the NAFLD activity score (NAS).

These results indicate that diosgenin down-regulates COX-2 expression through GR, and regulates COX-2 expression specifically in a macrophage-specific manner in liver inflammation. Furthermore, in MASH-like chronic hepatic injury, it was shown to reduce lipid accumulation and fibrosis ameliorating the chronic inflammation. Diosgenin is therefore expected to cell-selective COX-2 down-regulation, resulting in an anti-inflammatory effect without side effects.

1) Tsukayama, I. et al., Prostaglandins Other Lipid Mediat., 2021.

S6-03 Investigate the Immunomodulatory Role of Butyrate-induced Nuclear PD-L1 in Breast Cancer

Chee-Yang Tan[1,2], Chiao Lo5, Yi-Hsiu Weng[1,2], Jhen-Wei Ruan[1,2,4], Ming-Yang Wang[5,6], Pai-Sheng Chen[1,2,3,4]

[1]Institue of Basic Medical Sciences, College of medicine, National Cheng Kung University, Tainan, Taiwan.
[2]Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
[3]Breast Medical Center, National Cheng Kung University Hospital, Tainan 701, Taiwan.
[4]Research Center for Medical Laboratory Biotechnology, National Cheng Kung University, Tainan, Taiwan.
[5]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan.
[6]Department of Surgical Oncology, National Taiwan University Cancer Center, Taipei, Taiwan.

Short-chain fatty acids (SCFAs) have been reported to enhance the therapeutic effect of immunotherapy by activating immune cells, yet the direct impact on the cancer cell is rarely discussed. Among the SCFAs, butyrate treatment consistently promoted the expression of PD-L1, a well-known immune checkpoint protein that facilitates immune evasion, across various breast cancer cell models. Screening of PD-L1 upstream regulators revealed that Akt and ERK signaling, previously identified as downstream mediators of the butyrate receptor, are activated by butyrate stimulation. Further evaluation using small molecule inhibitors demonstrated that Akt signaling plays a post-transcriptional regulatory role. Additionally, as a known histone deacetylase (HDAC) inhibitor, butyrate treatment enhanced histone acetylation in breast cancer cells, indicating that chromatin relaxation may contribute to the transcriptional induction of butyrate-induced PD-L1. However, fatty acids could contribute to histone acetylation via fatty acid metabolism by providing carbon source for acetyl-CoA, the substrate of histone acetylation. Following treatment with fatty acids of varying carbon lengths showed that butyrate optimally enhances histone acetylation, thereby excluding the possibility that fatty acid metabolism, rather than HDAC inhibition, is responsible for the observed histone acetylation. Unexpectedly, in our breast cancer models, butyrate-induced PD-L1 was primarily localized in the nuclear fraction rather than on the cell membrane. Contrary to the typical immunosuppressive role of PD-L1, butyrate did not suppress but enhanced T-cell-mediated cytotoxicity. Therefore, transcriptomic analysis was further conducted, revealing that cytokine signaling was one of the potential target pathways regulated by nuclear PD-L1. Taken together, we discovered butyrate-nuclear PD-L1 induction via Akt and histone acetylation-directed chromatin relaxation, which contributes to cytokine signaling and immune modulation.

S6-04 Modulation of astrocyte activation by Sphingomyelin

Ryo Kadowaki[1], Hiroyuki Nakamura[1]

[1] Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University, JAPAN

Background: Astrocytes constitute about 20~40% of glial cells, making up the central nervous system (CNS). Although astrocytes usually maintain homeostasis in the CNS, they change phenotype to the activated state in neurodegenerative disease conditions by pro-inflammatory cytokines, such as IL-1α and TNF-α. Under an activated state, astrocytes release inflammatory cytokines, which induce neuronal injury. Sphingolipids are bioactive lipids that act as signal and/or structural molecules. Previous reports have indicated that they are also associated with astrocyte activation. Here, we evaluated astrocyte activity by changing sphingomyelin (SM), the most abundant sphingolipid in mammalian cells, levels in HASTR/ci35, a conditionally immortalized human astrocyte.

Methods: The astrocyte activation marker's mRNA expression levels were analyzed by qPCR, and protein expression levels were detected by Western blotting.

Results and Discussion: Increasing endogenous SM levels by inhibiting neutral sphingomyelinase and the external addition of SM increased protein and mRNA expression levels of astrocyte activation markers in IL-1α/TNF-α treated-HASTR/ci35. On the other hand, the reduction of intercellular SM levels by the knockdown of sphingomyelin synthase or ceramide transport protein (CERT) decreased protein and mRNA expression levels of astrocyte activation markers in IL-1α/TNF-α-treated cells. Next, we estimated the impact of CERT knockdown on the NF-κB pathway, which is predominantly involved in astrocyte activation, to elucidate how reducing intracellular SM levels suppresses astrocyte activation. We found that CERT knockdown did not alter IκBa phosphorylation, degradation, and p65 nuclear translocation. Thus, we next evaluated p65 post-transcriptional modification, such as phosphorylation and acetylation. CERT knockdown-mediated SM reduction did not change phospho-p65 (Ser 536) protein expression levels but significantly reduced acetyl-p65 (Lys 310) protein expression levels. Given these results, we finally examined the effects of CERT knockdown on HDAC1 and HDAC3, which regulate acetyl-p65 protein expression levels. Interestingly, protein expression of HDAC1 and HDAC3 was significantly increased by CERT knockdown. These results suggest that reducing intracellular SM levels suppresses astrocyte activation by inhibiting the NF-κB pathway via the induction of HDAC1 and HDAC3 protein expression.

Short Talks

S6-05 Oncogene-Induced Senescence Elicits a Marked Lipid Metabolic Shift and Change in Cellular Morphology

Stephen Ruiz[1,2], Chris Horozsko[1], Daniel Heller[1]

[1]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center.
[2]BCMB Program, Weill Cornell Medical College, Cornell University.

Oncogene-induced senescence (OIS) is a cell-state defined by permanent growtharrest coupled to a secretory program that may stimulate a pro-inflammatory phenotype. It is triggered upon sustained oncogenic signaling and antiproliferative activity caused by an activating mutation of an oncogene or inactivity of a tumor-suppressor gene. It has been postulated that senescence may prove as tumor suppressive. While senescent cells are stuck in a growth-arrested state, their metabolic activity remains dynamic. Interestingly, little is still known about the metabolic requirements of senescent cells and how they sustain the production of the senescence-associated secretory phenotype (SASP). In this work, we explored the metabolic alterations that occur with the entry of cells into an oncogene-induced senescent state. Using biochemical, genetic, and optical tools, including a nanotube-based sensor for endo-lysosomal lipids and electron microscopy, we found that upon sustained activation of oncogenic RAS signaling and induction of senescence, OIS cells encounter a marked shift in its cellular morphology, lipid droplet accumulation and marked endo-lysosomal lipid accumulation. Additionally, lipidomic analyses of more than 150 lipid species suggest that OIS cells trigger an increase in lyso-phospholipid species and contained less total saturated/unsaturated phospholipids, plasmalogen, and ether phospholipids. Taken together, this work demonstrates that OIS cells elicit a distinct metabolic state and specifically re-shaping lipid metabolism.

S6-06 Regulation of replicative senescence by bioactive lipids in primary human hepatocytes

Sin-Tian Wang[1], Wei-Chia Chen[1], Ching-Ya Chang[1] and Kung-Chia Young[1,2]

[1]Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Tainan, Taiwan.
[2]Institute of Basic Medicine Science, National Cheng Kung University, Tainan, Taiwan.

Metabolic-dysfunction associated fatty liver disease (MAFLD) is an age-related disease, and progress from steatosis, hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. However, the molecular mechanism of disease development and hepatic aging progress on lipid-metabolic dysregulation are not fully understood. In this study, the replicative senescence model was established by cultivated primary human hepatocytes (PHH) and monitored the senescent phenotypes and lipid changes in different passages (P3~P13). Three stages were classified in cultivated PHH model, stage 1 (Young): the P3 and P5 cell were rarely displayed senescent phenotypes; stage 2 (Early-senescence): the P7 and P9 cells upregulated cycles arrest biomarkers and accumulated lipids; stage 3 (Late-enescence): the P11 and P13 cells were exhibited telomere shortening, Lamin B1 loss, induction of DNA damage foci, increment of ROS level. Moreover, it was reconfirmed that the accumulation of lipids and upregulation of senescent biomarkers in 50-62 weeks old mice. Lipidomic analysis showed that the percentage of glycerolipids was decreased in early-senescent cells, but other lipids were increased in early- or late-senescent cells, such as lysoglycerophospholipid, sterol lipid and sphingolipids. In free fatty acid quantification, some C20~C22 unsaturated fatty acid were increased in early- and late-senescent PHH. Furthermore, manipulation of lipid remodeling, especially cytosolic free fatty acid was regulated senescent progression in PHH with chemical inhibitor, such as TMP-153, a SOAT inhibitor blocking cholesteryl ester forming and pyrrophenone, a cPLA2 inhibitor blocking fatty acid releasing from glycerophospholipids. Finally, the PHH treated with C20~C22 unsaturated fatty acids were display senescent phenotypes. In conclusion, the very-long-chain fatty acid in lipid metabolic modulation between different lipids might promote/delay cellular senescence in hepatocyte.

S6-07 Resolvins Regulate Neutrophil Extracellular Traps: Implications for COVID-19, Microbial Infections and Coagulopathies

Nan Chiang and Charles Nicholas Serhan

Center for Experimental Therapeutics and Reperfusion Injury, Mass General Brigham and Harvard Medical School, Boston, MA 02115, USA

The specialized pro-resolving mediators (SPMs) are a potent superfamily of molecules that are stereoselective, anti-inflammatory, pro-resolving and organ-protective. These include the resolvins, protectins and maresins as well as the newly identified thirteen-series (T-series) resolvins (RvTs). The T-series resolvins are biosynthesized from precursor n-3 docosapentaenoic acid (DPA). Since excessive neutrophil extracellular traps (NETs) are recognized as a source of collateral tissue damage in bacterial and viral infections including SARS-CoV-2 infections, we tested whether stereochemically-defined synthetic RvTs and D-series resolvins (RvDs) can regulate leukocyte NET formation, i.e., NETosis. Using microfluidic devices capturing NETs with human whole blood, the four RvTs (RvT1-RvT4; 2.5 nM each) and RvD2 potently reduced NETosis [Chiang et al., *Blood*; 139:1222]. With IL-1beta-stimulated human neutrophils, each RvTs (RvT1-RvT4) and RvDs (RvD1-RvD5) dose- and time-dependently decreased NETosis. At 1 and 10 nM, RvT1 and RvD2 were the most potent in the RvT and RvD families, respectively. RvD2 limited NETs in a receptor (DRV2/GPR18)-dependent manner. In murine *E. coli* and *S. aureus* infections, D-series resolvins lower bacterial burden and antibiotic requirement [Chiang et al., *Nature*; 484:524-8]. In *S. aureus* infections, RvT1, RvT2, RvT3 and RvT4, 50 ng each in combination, also limited exudate neutrophil infiltration and bacterial burden. In addition, exudates from RvT-treated mice reduced NETs by >60% compared to vehicle.

Since macrophages play an essential role in clearing NETs, we investigated whether resolvins enhance NET clearance by human macrophages. At 10 nM, RvD2 and the four RvTs each enhanced NET uptake by human macrophages. RvT2 was the most potent of the four RvTs, giving >50% increase in macrophage phagocytosis of NETs. RvT-stimulated NET clearance by macrophages involved signaling via the cAMP-PKA-AMPK axis. RvT2 also enhanced NET clearance by mouse macrophages *in vivo*. Taken together, these results provide evidence for new resolution mechanisms of RvTs, i.e., limiting NETosis and enhancing NET clearance by macrophages. Thus, monitoring NETosis and its regulation by D-series and T-series resolvins as well as other SPMs may be useful in long COVID and other NET-associated infections, coagulopathies and pathologies.

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Short Talks

S6-08 Role of the group IIF phospholipase A_2/lysoplasmalogen pathway in atopic dermatitis

Kei Yamamoto[1], Niki Hirabayashi[2], Makoto Murakami[3,4], Yoshimi Miki[1]

[1]Graduate School of Technology, Industrial and Social Sciences, Tokushima University
[2]Graduate School of Sciences and Technology for Innovation, Tokushima University
[3]Graduate School of Medicine, The University of Tokyo
[4]AMED-CREST

Keratinocytes play an important role in skin barrier function, and disturbances in the proliferation and differentiation of these cells lead to impaired barrier function and various types of skin diseases. We have reported that lysoplasmalogen (P-LPE), a primary metabolite of group IIF secreted phospholipase A_2 ($sPLA_2$-IIF) that is localized in the suprabasal epidermis, activates keratinocytes and contributes to exacerbation of Th1-driven contact dermatitis and Th17-driven psoriasis. In this study, we examined the physiological significance of the $sPLA_2$-IIF/P-LPE pathway in Th2-driven atopic dermatitis. Application of an MC903-induced atopic dermatitis model resulted in significant reductions in serum IgE and epidermal thickening as well as a marked decrease in the frequency of scratching behavior in *Pla2g2f*$^{-/-}$ mice compared to control mice. Comprehensive lipidomics of the stratum corneum showed that P-LPE was the only lysophospholipid whose level was changed in correlation with the phenotype of *Pla2g2f*$^{-/-}$ mice. Treatment of the skin with P-LPE restored the defective scratching behavior in *Pla2g2f*$^{-/-}$ mice, while forcible degradation of P-LPE by topical application of recombinant lysophospholipase D from *Thermocrispum*, a lysoplasmalogen-specific hydrolase, prevented scratching behavior in wild-type mice. The increase in IL-4-positive T cells was attenuated in the skin of *Pla2g2f*$^{-/-}$ mice. Moreover, the expression of IL-4 receptor (IL-4Rα) was also decreased in the spinal dorsal root ganglia of the deficient mice. Thus, the scratching behavior associated with atopic dermatitis may be caused by corporation of the $sPLA_2$-IIF/ P-LPE pathway with the IL-4/IL-4Rα pathway. Finally, to examine the relationship between P-LPE and human skin pathology, lipidomics was performed using lipids extracted from the stratum corneum of human atopic dermatitis patients. A significant increase in P-LPE was observed in patient skins compared to healthy subjects. These results indicate that $sPLA_2$-IIF contributes to the Th2 response, where P-LPE aggravates the itch behavior, in atopic dermatitis, raising the possibility that the $sPLA_2$-IIF/P-LPE pathway is a novel drug target for atopic dermatitis.

Short Talks

S6-09 $sPLA_2$-III recruits eosinophils by driving the paracrine LPA- eotaxin-2 axis in chitin-induced peritonitis

Kyohei Horikiri[1,2], Yoshitaka Taketomi[2], Kenji Kondo[1], Tatsuya Yamasoba[1], Makoto Murakami[2,3]

[1]Department of Otolaryngology and Head and Neck Surgery, Graduate School of Medicine, The University of Tokyo
[2]Laboratory of Microenvironmental and Metabolic Health Sciences, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo
[3]AMED-CREST, Japan Agency for Medical Research and Development

Introduction: Although the recruitment and functions of eosinophils are regulated by various lipid mediators, the molecular mechanisms underlying this process remain incompletely understood. Among the phospholipase A_2 (PLA_2) family, secretory PLA_2s ($sPLA_2$s) control various biological responses by mobilizing lipid mediators in extracellular tissue microenvironments. Herein, we uncovered a new mechanism of eosinophil control by $sPLA_2$.
Methods and Results: Expression analysis of a full set of $sPLA_2$s in bone marrow-derived immune cells *ex vivo* and those recruited in chitin-induced peritonitis, a type-2 innate immune response, *in vivo* revealed that eosinophils highly express and secrete $sPLA_2$-III. Recruitment of eosinophils into the peritoneal cavity was reduced in $sPLA_2$-III-deficient (*Pla2g3*$^{-/-}$) mice compared with wild-type (*Pla2g3*$^{+/+}$) mice. Lipidomics of peritoneal exudates demonstrated that chitin-induced release of lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and lysophosphatidic acid (LPA), but not polyunsaturated fatty acid-derived oxylipins, from dying cells was markedly reduced by $sPLA_2$-III deficiency. The reduced eosinophil recruitment in *Pla2g3*$^{-/-}$ mice was restored by supplementation with these lysophospholipids, with LPA showing the strongest effect. Administration of an autotaxin inhibitor or an $LPA_{1/3}$ antagonist to *Pla2g3*$^{+/+}$ mice reduced eosinophil infiltration to a level similar to that in *Pla2g3*$^{-/-}$ mice. Moreover, eosinophil infiltration was markedly impaired in LPA_1-deficient mice or in chimeric mice transferred with LPA_1-deficient bone marrow cells, implying that LPA is a major lipid mediator that acts downstream of $sPLA_2$-III. Single-cell RNA-sequencing of peritoneal cells revealed that autotaxin was expressed in mesothelial cells and fibroblasts and LPA_1 in macrophages expressing eotaxin-2 (*CCL24*), an eosinophil-chemotactic factor. Importantly, the level of eotaxin-2 in the peritoneal exudate was reduced by $sPLA_2$-III deficiency and depletion of macrophages with clodronate liposomes impaired eosinophil infiltration, suggesting that $sPLA_2$-III-driven macrophage LPA_1 signaling is linked to eotaxin 2-dependent eosinophil migration.
Discussion: In the chitin-induced peritonitis, $sPLA_2$-III secreted from eosinophils mobilizes LPC and LPE from phospholipids in dying cells. The LPC and LPE are converted by stromal-derived autotaxin to LPA, which then acts on a subpopulation of macrophages expressing LPA1 receptor, thereby promoting eotaxin-2 production and amplifying eosinophil recruitment.

Short Talks

S6-10 Targeting lipid peroxidation in protection against muscle wasting

Katsuhiko Funai[1]

[1]Department of Nutrition & Integrative Physiology, University of Utah

Lipid hydroperoxides (LOOH), a class of lipid reactive oxygen species, appears to promote disuse-induced skeletal muscle atrophy. Genetic (GPx4 overexpression or LPCAT3 knockout) or pharmacological (N-acetylcarnosine) suppression is sufficient to ameliorate muscle atrophy, and to a greater extent muscle weakness, induced by physical inactivity. We now report that LOOH scavenging similarly protects against muscle wasting induced by aging and lung cancer cachexia, having more robust effects on sustaining force-generating capacity than muscle mass. The precise mechanism by which LOOH contributes to muscle dysfunction is unclear, but we have recently developed a novel mass spectrometry discovery platform to detect proteins that are post-translationally modified by reactive lipid carbonyls (CAPDD or carbonylated proteomics by DNPH derivatization). We hypothesize that carbonylated proteins are targeted for degradation leading to impaired proteostasis, contributing to muscle wasting.

Note

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